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Stability-Indicating RP-HPLC Method for Determination of Pirfenidone and force degradation in Pharmaceutical Formulations

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Abstract

Background:

Pirfenidone is an antifibrotic drug widely used in the treatment of idiopathic pulmonary fibrosis. Due to its therapeutic importance, the development of a reliable analytical method is essential for routine quality control, assay determination, and stability evaluation in pharmaceutical dosage forms.

Methods:

A rapid and economical reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative determination of pirfenidone in active pharmaceutical ingredient (API) and tablet formulations. Method validation was performed according to ICH Q2(R1) guidelines, and forced degradation studies were conducted following ICH Q1A(R2) recommendations to establish the stability-indicating capability of the method.

Results:

Chromatographic separation was achieved on an Inert Sustain C18 column (4.6 × 250 mm, 5 µm). The mobile phase consisted of Methanol and 0.1%OPA (90:10 v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out at 317 nm using a PDA detector. Pirfenidone eluted at approximately 3.8 minutes. The method showed good linearity over the concentration range of 5–50 µg/mL with a correlation coefficient of 0.999. The limits of detection (LOD) and quantification (LOQ) indicated sufficient sensitivity of the method. Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic conditions demonstrated that pirfenidone was well separated from its degradation products.

Conclusion:

The developed RP-HPLC method is simple, rapid, cost-effective, and stability-indicating. It is

suitable for routine quality control analysis and stability testing of pirfenidone in pharmaceutical formulations.

Keywords: Pirfenidone, Methanol, Validation, 0.1%OPA, Hplc Water.

Introduction:

Pirfenidone is an orally active antifibrotic and anti-inflammatory drug widely used in the treatment of idiopathic pulmonary fibrosis (IPF), a chronic and progressive interstitial lung disease characterized by excessive deposition of fibrotic tissue in the lungs. The drug exerts its therapeutic effect primarily by inhibiting the synthesis of transforming growth factor-β (TGF-β) and other profibrotic cytokines, thereby reducing fibroblast proliferation and collagen accumulation. Due to its clinical significance in slowing disease progression and improving patient outcomes, maintaining the quality, safety, and efficacy of pharmaceutical formulations containing pirfenidone is of paramount importance.

From an analytical standpoint, pirfenidone requires reliable and sensitive methods for its quantitative estimation in bulk drug substances and pharmaceutical dosage forms. The physicochemical properties of pirfenidone, including moderate solubility and potential susceptibility to degradation under various stress conditions such as light, heat, and oxidative environments, necessitate the development of robust analytical techniques capable of accurately determining the drug while differentiating it from possible degradation products.

Several analytical methods have been reported for the estimation of pirfenidone, including UV spectrophotometry and reverse-phase high-performance liquid chromatography (RP-HPLC). Among these techniques, RP-HPLC is widely preferred due to its high sensitivity, selectivity, reproducibility, and suitability for routine pharmaceutical analysis. Previously reported chromatographic methods for pirfenidone involve different stationary phases and mobile phase systems, leading to variations in retention time, resolution, and solvent consumption. However, some of these methods require relatively longer analysis times or higher volumes of organic solvents, which may not be ideal for routine quality control applications.e

In view of these considerations, the present study aims to develop and validate a simple, rapid, and cost-effective RP-HPLC method for the quantitative determination of pirfenidone in bulk drug and tablet dosage forms. The developed method is also subjected to forced degradation studies to assess its stability-indicating capability in accordance with the guidelines established by the International Council for Harmonization (ICH), ensuring that the method can effectively separate the intact drug from its potential degradation products.

METHODS AND MATERIALS

Chemicals and Reagents:

Pirfenidone was obtained aXs a reference standard of analytical grade. Methanol and OPA of HPLC grade were procured from Merck (Mumbai, India). HPLC-grade water was used throughout the analysis. All reagents were used as received without further purification.

Instrumentation

Chromatographic analysis was carried out using a Shimadzu LC-20AD high-performance liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array (PDA) detector. UV–Visible spectroscopic measurements were performed using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Sample weighing was conducted using an ATY224 analytical balance (Shimadzu, Kyoto, Japan). Sonication was performed using a SONICA 2200MH ultrasonicator (Soltec, Milan, Italy). A hot air oven (Vision Lab Equipment, Hyderabad, India) and a UV cabinet (Monoquartz, Chennai, India) were used for stress degradation studies.

RP-HPLC Chromatographic conditions

Chromatographic Conditions

Separation was achieved on an Inert Sustain C18 column (4.6 mm × 250 mm, 5 µm particle size). The mobile phase consisted of methanol and acetonitrile in the ratio of 90:10 (v/v), delivered at a flow rate optimized to achieve adequate resolution and peak symmetry. Detection was performed at a wavelength of 230 nm using a PDA detector. The injection volume was set at 20 µL, and the total run time was maintained at 5 minutes.

Preparation of Standard Solution

A standard stock solution was prepared by dissolving 10 mg of Pirfenidone in 10 mL of methanol to obtain a concentration of 1000 µg/ml. This solution was further diluted with the mobile phase to prepare working standard solutions within the required concentration range. All solutions were filtered through a 0.45 µm membrane filter prior to injection.

Preparation of Sample Solution

Twenty tablets were accurately weighed and finely powdered. An amount of tablet powder equivalent to 10 mg of Pirfenidone was transferred to a 10 mL volumetric flask, dissolved in methanol, and sonicated for 10 minutes to ensure complete extraction. The solution was then diluted to volume with the mobile phase and filtered through a 0.45 µm nylon membrane filter. Further dilutions were prepared as required for analysis.

RESULTS AND DISCUSSION

SYSTEM SUITABILITY:

Under optimized chromatographic conditions, Pirfenidone produced a sharp and symmetrical peak with consistent retention behavior. System suitability parameters, including retention time, theoretical plate count, and tailing factor, were found to be within acceptable limits, confirming adequate system performance for analysis. The system suitability results are summarized in Table 1, and a representative chromatogram is shown in Figure 1.

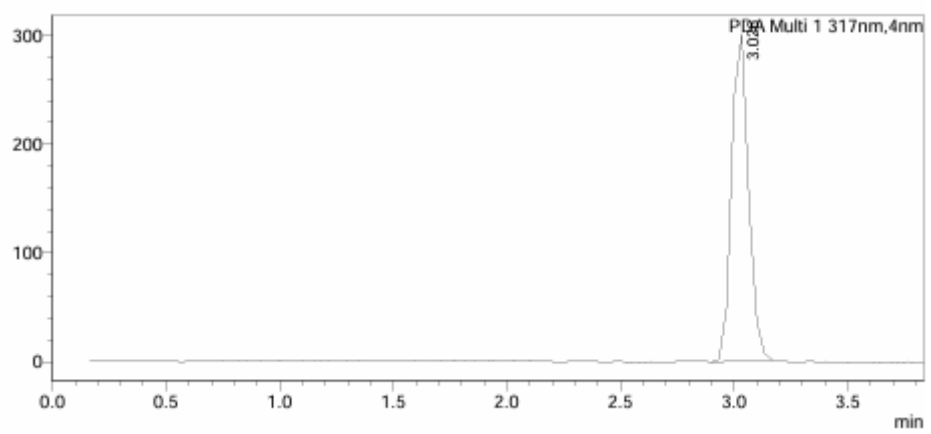

Figure 1: Chromatogram of system suitability

Table1: System requirements for the suggested HPLC technique

S.NO	PEAK AREA	RET TIME	PLATE COUNT	TAILING
1	1506888	3.02	3721	1.14
2	1502727	3.00	3639	1.20
3	1533242	3.06	3727	1.17
4	1509354	3.01	3621	1.17
5	1516467	3.02	3721	1.17
6	1518602	3.00	3639	1.20
Average	1514546.67	3.02	3678.00	1.18
STDEV	10908.46	0.02	49.78	0.02
% RSD	0.72	0.74	1.35	1.91
Limits	_	_	>2000	<2.0
% RSD	<2.0	<2.0	<2.0	<2.0

Linearity:

The linearity of the method was evaluated over the concentration range of 5–100 µg/mL. A calibration curve was constructed by plotting peak area against corresponding drug concentration. The method demonstrated a linear response across the studied range, with a correlation coefficient (R^2) of 0.999, indicating a strong linear relationship between concentration and detector response. Linearity data are presented in Table 2, and the calibration curve is illustrated in Figure 2.

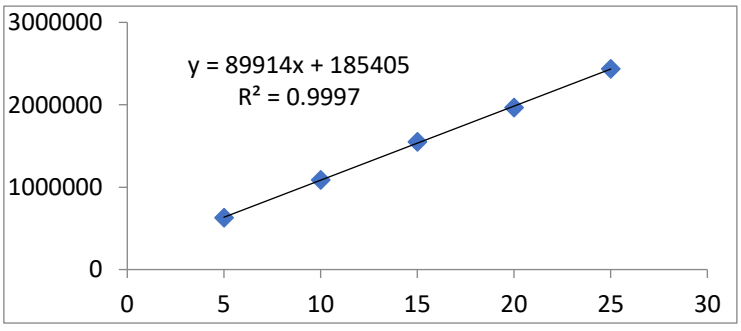

Figure 2: Pirfenidone linearity graph

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Table2: Linearity for Pirfenidone.

S.NO.	Level of linearity	Concentration(µg/ml)	Peak area
1.	50%	10	627975
2.	75%	20	1088941
3.	100%	30	1550106
4.	125%	40	1966516
5.	150%	50	2437038

Precision:

Method precision was assessed by repeated injection of a standard solution at 100% test concentration (500 µg/ml). The results showed low variability in peak area, with percentage relative standard deviation (%RSD) values below 2%, confirming the repeatability and reliability of the analytical method. Precision results are summarized in Table 3.

Table 3: Precision for proposed HPLC method

S.NO	Day 1	Day 2	Day 3
1.	1520526	1567885	1531237
2.	1565953	1534500	1530389
3.	1559220	1567341	1550317
4.	1533867	1560276	1544161
5.	1540857	1540265	1564360
6.	1536662	1531069	1521077
Average	1542847.50	1550222.67	1540256.83
STDEV	16870.35	16848.06	15773.46
RSD	1.09	1.09	1.02
Limits	% RSD: <2.0	% RSD: <2.0	% RSD: <2.0

ACCURACY:

The accuracy of the method was evaluated by recovery studies at three concentration levels (50%, 100%, and 150%) using tablet sample solutions. The percentage recovery values were found to be within the acceptable range of 98–102%, indicating that the method is accurate and free from interference by excipients present in the tablet formulation. The recovery results are presented in Table 4.

Table 4: Accuracy displaying the recovery percentage

S.NO	Levels	Concentration taken(µg/ml)	Concentration added (µg/ml)	Peak area	Concentration recoverd (µg/ml)	% recovery	Average Recovery
1	50%	15	10	2451618	10.2042	102.0423	99.95
2		15	10	2419953	9.8521	98.5206	
3		15	10	2426947	9.9298	99.2984	
4	100%	15	20	3546989	22.3867	111.9333	101.57
5		15	20	3040688	16.7557	83.7786	
6		15	20	3494027	21.7976	108.9881	
7	150%	15	30	4193365	29.5755	98.5849	99.07
8		15	30	4267428	30.3992	101.3306	
9		15	30	4158257	29.1850	97.2834	

LOD & LOQ:

The sensitivity of the method was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ values were found to be 1.50 µg/ml and 4.54 µg/ml respectively, demonstrating that the method is sufficiently sensitive for routine analysis. Representative chromatograms corresponding to LOD and LOQ are shown in Figures 3 and 4, and the calculated values are summarized in Table 5.

LOD and LOQ Chromatograms

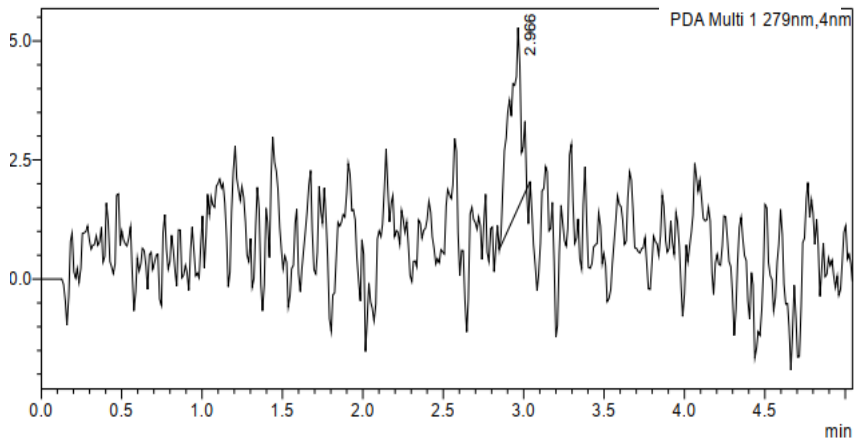

Figure 3: LOD chromatogram

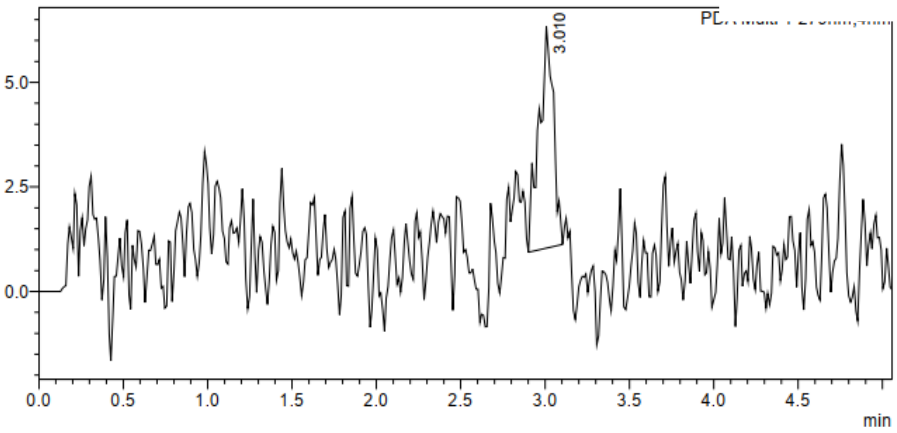

Figure 4: LOQ Chromatogram

Table 5: LOD and LOQ for the HPLC technique

Parameters	Slope from Linearity	SD of peak from system Suitability
	89914	185405
LOD =3.3xSD/Slope	0.400µg/ml	
LOQ = 10xSD/Slope	1.213µg/ml	

Robustness:

The robustness of the method was evaluated by making deliberate minor variations in chromatographic conditions. The observed results remained within acceptable limits,

indicating that small changes in analytical parameters did not significantly affect method performance, thereby confirming the robustness of the proposed RP-HPLC method.

Parameter	Condition	Condition	RT	Peak area	Theoritical plates	% Assay
Flow (ml/min) min±0.2 ml	Less flow	0.8ml/min	4.234	1800338	3707	109.95
	Optimized	1.0 ml/min	3.525	1550106	3446.00	94.67
	More flow	1.2 ml/min	3.069	1658106	3173	101.27
Temp (°C) min±5°C	Less temp	25	3.526	1382500	3421	84.44
	Optimized	30	3.525	1550106	3446.00	94.67
	More temp	35	3.482	1588251	3545	97.00
Wave length (nm) min±2 nm	Low WL	312nm	3.543	1538963	3490	93.99
	Optimized	317nm	3.525	1550106	3446.00	94.67
	High WL	322nm	3.552	1493321	3371	91.20
Mobile phase (ratio) min±10 ratio	Low	85:15	3.586	1493999	3490	91.25
	Optimized	90:10	3.620	1550106	3446.00	94.67
	High	95:05	3.482	8918618	3371	544.70

DEGRADATION STUDIES

1.Acid Degradation:

0.3 ml of the sample solution was transferred from the produced stock solution into a 10 ml volumetric flask.1 ml of 0.1N HCl was then added, and the mixture was heated to 50°C for 15 minutes before being cooled. One milli litre of 0.1N NaOH was added to this cooled solution to neutralise it and bring it up to the chromatogram is displayed in figure 5 after the mark with mobile phase was injected into HPLC and the peak responses were recorded.

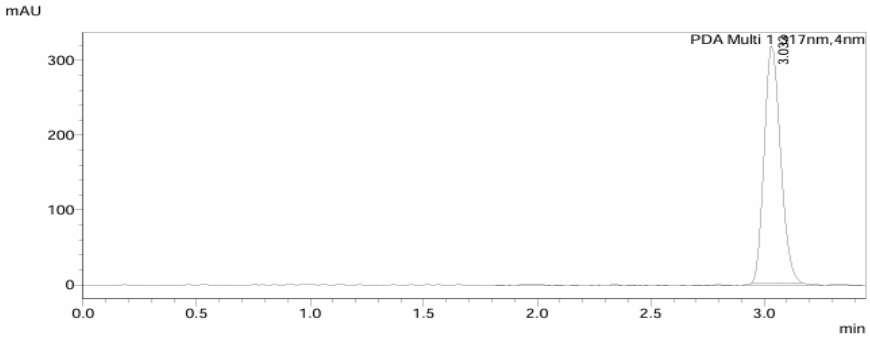

Figure 5 : Chromatogram Acid Degradation

2.Base degradation :

From the prepared stock solution, 0.3 ml of the sample solution was transferred to a 10 ml volumetric flask. 1 ml of 0.1N NaOH was then added, and the mixture was heated to 50°C for 15 minutes before being cooled. After adding 1 millilitre of 0.1N HCl to this cooled solution to neutralise it and top it off with mobile phase, the mixture was put into an HPLC, the peak responses were noted, and the chromatogram is displayed in figure 6.

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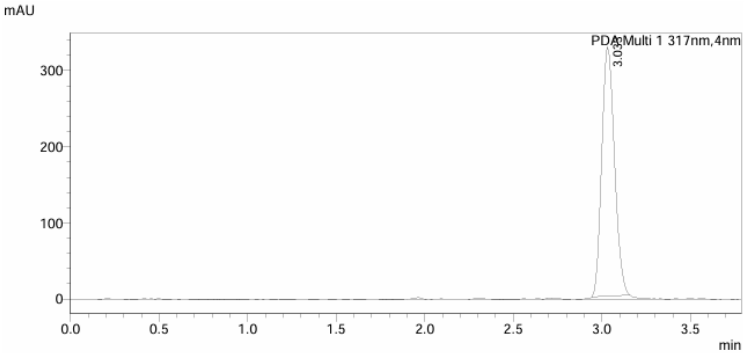

Figure 6 : chromatogram of Base Degradation

3. Peroxide Degradation:

0.3 ml of the sample solution was taken from the prepared dummy solution and placed in a 10 ml volumetric flask. 0.1 ml of 3% H2O2 was added to the flask and brought up to the volumetric flask's mark using mobile phase. The solution was then allowed to sit at room temperature for 12 hours before being injected into an HPLC, where the peak responses were noted and the chromatogram is displayed in figure 7.

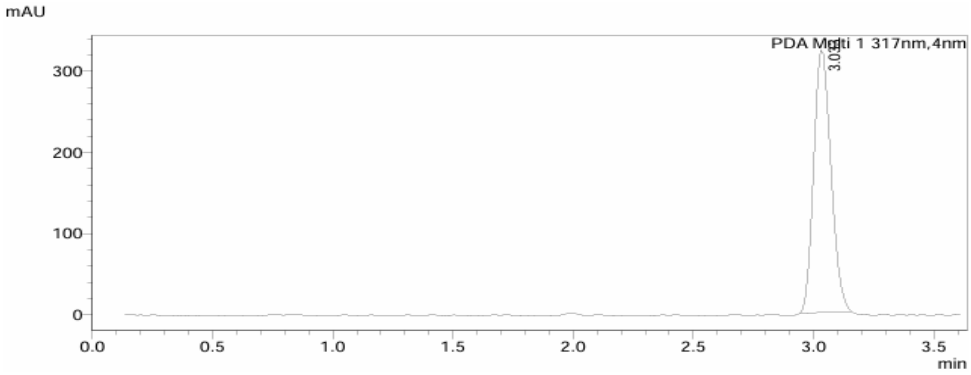

Figure 7: chromatogram of Peroxide Degradation

4. Thermal Degradation:

From the prepared stock solution, 0.3 ml of the sample solution was transferred to a 10 ml volumetric flask along with 1 milliliter of mobile phase. The mixture was then heated to 60°C for 10 minutes and allowed to cool. make up the difference with mobile phase, which was then injected into HPLC. The chromatogram is displayed in figure 8, and the peak responses were noted.

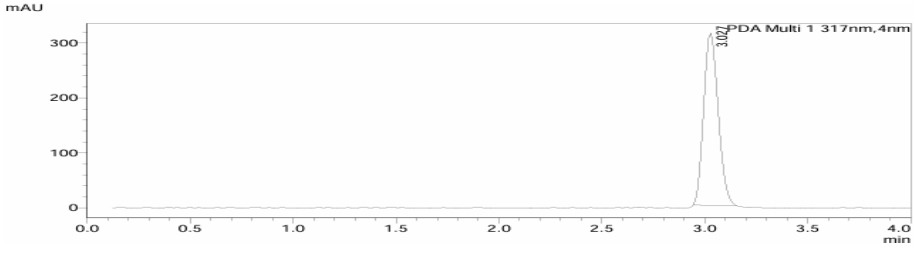

Figure 8 : Chromatogram of Thermal Degradation

Table 6: Degradation studies

S. No	Condition	Peak area	%assay	%degradation
1	Acid	1501645	91.712	8.288
2	Base	1533544	93.660	6.340
3	Peroxide	1522995	93.016	6.984
4	Thermal	1514227	92.481	7.519

DISCUSSION :

The present study describes the development and validation of a rapid and solvent-efficient RP-HPLC method for the quantitative estimation of pirfenidone in active pharmaceutical ingredient and tablet dosage forms. The method demonstrated satisfactory chromatographic performance, as evidenced by a well-resolved and symmetrical peak with consistent retention behavior. The observed retention time of approximately 3.0 minutes reflects efficient separation and contributes to reduced analysis time, which is advantageous for routine quality control laboratories.

Linearity of the method was established over the concentration range of 5–100 µg/mL with a strong correlation between analyte concentration and detector response (R^2 = 0.9997). This confirms that the method is capable of providing reliable quantitative results across a wide analytical range. Precision studies yielded %RSD values below 2%, indicating good repeatability and consistency of the chromatographic system. Accuracy assessment through recovery studies further demonstrated that excipients present in the tablet formulation did not interfere with analyte quantification, as recovery values remained within acceptable limits. Sensitivity of the proposed method was confirmed by the determined limits of detection and quantification. The obtained LOD and LOQ values indicate that the method is suitable for detecting and quantifying pirfenidone at relatively low concentrations, which is essential for stability studies and quality assessment. Robustness testing showed that minor variations in chromatographic conditions did not significantly affect analytical performance, highlighting the reliability of the method under normal laboratory conditions.

Forced degradation studies under acidic, alkaline, oxidative, and thermal stress conditions confirmed the stability-indicating capability of the method. The drug peak remained well resolved from degradation products in all stress conditions, demonstrating the specificity of the developed RP-HPLC method. Approximately 10% degradation observed under certain stress conditions further supports the suitability of the method for stability testing, as recommended by ICH guidelines.

When compared with previously reported RP-HPLC methods for pirfenidone analysis, the present method offers notable advantages, including shorter run time and reduced organic solvent consumption. Many earlier methods required longer analysis times or higher proportions of organic solvents, which may increase operational costs and environmental burden. In contrast, the proposed method provides a balanced approach by achieving rapid separation without compromising sensitivity or accuracy, making it well suited for routine quality control and stability-indicating applications.

LIMITATIONS OF THE STUDY :

Despite the successful development and validation of the proposed HPLC method, certain limitations should be acknowledged. The method was validated using laboratory-prepared samples and a limited number of commercial dosage forms; therefore, its applicability to a wider range of formulations was not extensively evaluated. Additionally, the method was developed for a single analyte under specific chromatographic conditions, and its performance with other structurally related compounds or combination products was not assessed. Forced degradation studies were performed under selected stress conditions; however, degradation behavior under long-term stability conditions was not investigated. Furthermore, robustness testing was limited to minor variations in chromatographic parameters, and the impact of variations in different column brands or instruments was not evaluated. Although the method demonstrated adequate sensitivity and reproducibility, further studies involving inter-laboratory validation could strengthen its reliability for broader regulatory and industrial applications.

S.No	Parameters	Results
1	Mobile phase	Methanol:0.1%OPA(90:10v/ v)
2	Wavelength detection	317nm
3	Calibration range(µg/ml)	5 – 25µg/ml
4	Regression equation	y = 89914x + 185405
5	Slope(m)	89914x
6	Intercept(c)	185405
7	Correlation coefficient (r^2)	0.999
8	Retention time	3.2 min
9	Systems Suitability	0.81%
10	LOD(µg/ml)	0.400µg/ml
11	LOQ(µg/ml)	1.213µg/ml
12	Accuracy	98.09 – 101.82%
13	Inter-day Precision	0.82 – 1.04
14	Intraday Precision	0.74 – 1.460
15	Robustness Flow rate	98.03-102.10%
16	Robustness Temperature	98.97-102.07%
17	Robustness Wavelength	98.99-102.20%
18	Assay	97.15
19	Acid degradation	8.288
20	Base degradation	6.340
21	H2O2 degradation	6.984
22	Thermal degradation	7.519

Conclusion:

The developed RP-HPLC method for the estimation of pirfenidone in API and tablet dosage forms was successfully validated according to ICH Q2(R1) Guidelines. The method exhibited excellent linearity in the concentration range of 5–100 µg/mL with satisfactory system suitability parameters. Accuracy, precision, and robustness studies showed %RSD values below 2%, indicating good reliability and reproducibility of the method. The LOD and LOQ were found to be 1.50 µg/mL and 4.54 µg/mL, respectively. Forced degradation studies demonstrated assay values within the acceptable range of 98–102%, confirming the stability-

indicating capability of the method. Therefore, the developed method is simple, rapid, economical, and suitable for routine quality control analysis of Pirfenidone in pharmaceutical formulations.

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Author contributions:

All authors made contributions design, acquisition of data and interpretation of data, agreed to submit to the current journal, gave final approval of the version to be published.

Ethical approvals:

In this study no experimental animals or human subjects were involved in this study.

Data availability:

All the data generated and analysed are included in this research article.

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