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Research Article

DEVELOPMENT OF CHROMATOGRAPHIC METHOD AND VALIDATION FOR ESTIMATION OF PIRFENIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The Pirfenidone (orphan drug) is a novel antifibrotic agent used in idiopathic pulmonary fibrosis. A new RP-HPLC method was developed and validated for the quantitative determination of Pirfenidone in tablet dosage form. Separation was achieved on C_{18} analytical column (250 × 4.6 mm, 5 µm) using Acn: Methanol: Water (65:15:20) as mobile phase with PDA detector. λ max was found to be at 317 nm. The flow rate was 1ml/min. standard curve was found to be linear over the concentration range of 5-25 µg/ml with r^2 of 0.9989. The % recovery was found to be within 98-100 % and RSD < 2%. The method was validated as per ICH guidelines for linearity, precision, accuracy, robustness. The method was successfully applied for determination of Pirfenidone in tablet dosage form. The develop method was simple, less time consuming and cost effective.

Key words: RP-HPLC, Pirfenidone, Validation, Assay.

INTRODUCTION

Pirfenidone (orphan drug) is first clinically used novel antifibrotic agent approved for idiopathic pulmonary fibrosis (IPF) in Japan (Pirespa), Europe (Esbriet) and India (pirfenair).[1] It is orally available pyridone derivative that has antifibrotic, anti-inflammatory and antioxidative actions.[1] Patients with IPF shows symptoms such as shortness of breath, Cough, destruction of healthy lung tissue and hampered daily physical activities.[2] The exact cause of IPF is unknown. The disease causes lungs to become scarred and stiffened, which may lead to difficulty in breathing. IPF usually occurs in people aged 50 to 70.[3] It is believed to occur due to inflammatory response from an unknown substance. Patients diagnosed with IPF experience progressive pulmonary insufficiency and most die of respiratory failure.[1] The estimated survival upon diagnosis is approximately 3 years. Treatment for IPF includes oxygen therapy, pulmonary rehabilitation and lung transplant. Pirfenidone is the only therapeutic agent for treatment of IPF. In animal experimental models, PFD has demonstrated and antifibrotic effect in several tissues, such as lung, liver and kidney. Pirfenidone / 5-methyl-1-phenyl-2-(1H)-pyridone is small heterocyclic molecule which on oral administration shows that it is rapidly metabolized to 5 – hydroxyl Pirfenidone and 5-Carboxy Pirfenidone. Major metabolite i.e. 5-Carboxy Pirfenidone is eliminated in the urine (>87%). Various HPLC and LCMS/MS methods are reported for Pirfenidone for biological fluids like plasma, serum, urine, but very few methods are reported for estimation of Pirfenidone in pharmaceutical dosage form.[3]

MATERIALS AND METHOD

Chemicals and reagents

Pirfenidone was provided as gift sample from Cipla, Vikroli. Purity of drug was evaluated by obtaining its IR spectra. No impurities were found and the drug was used for further study without any purification. HPLC grade methanol, water, Acn were obtained from research chem, Mumbai were of standard quality. Film coated tablets of Pirfenidone tablet IP 200 mg (Pirfenex ^{RX}, Cipla Ltd) were purchased from local market.

Instrumentation

The chromatographic technique was performed on shimadzu UV 1800 double beam UV-visible spectrophotometer with UV probe software and JASCO HPLC 4000 on Chrom NAV software on reverse phase inertsil C_{18} column (250 × 4.6 mm × 5µm) as stationary phase.

Chromatographic condition

Chromatographic separation was performed on inertsil C_{18} analytical column. Mobile phase consisting of Acn: Methanol: Water (65:15:20) at flow rate 1.0 ml /min with injection volume of 10 µl.

PREPARATION OF CALIBRATION CURVE FOR UV

Working standard stock solution (A1)

Accurately weigh 10 mg of Pirfenidone and transfer it to 10 ml volumetric flask, dissolved it in Acn and volume was made up to mark with Acn. (Concentration of 1000 µg/ml of Pirfenidone)

Working standard stock solution (A2)
Pipette out 1 ml of above stock solution (A1) and transfer it to 10 ml volumetric flask, and volume was made up to mark with Acn. (Concentration of 100 µg/ml of Pirfenidone)

Working standard solution
2 ml of standard stock solution (A2) was transferred to 10 ml volumetric flask and volume was made up to the mark with Acn. (Concentration of 20 µg/ml). Standard solution of 20 µg/ml was scanned between 400 to 200 nm. From the spectra, λ max of Pirfenidone was selected at 317 nm for method development.

SAMPLE PREPARATION

Standard stock solution
Accurately weigh 0.01 gm of Pirfenidone drug and transferred it in 10 ml volumetric flask. Make up the volume up to the mark with Acn to prepare a stock of concentration 1000 µg/ml. from this solution pipette out 1 ml and transferred to 10 ml volumetric flask and make up the volume upto the mark with diluent (Acn) to prepare a stock of concentration 100 µg/ml. from this solutions further dilutions were made to prepare a concentration of 5, 10, 15, 20, 25 µg/ml.

Method validation

Linearity
Linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was performed by diluting standard stock solution (100 µg/ml) to give final concentration in the range of 5µg/ml to 25 µg/ml. 10 µl of each concentration was injected and calibration curve was constructed by plotting peak area vs drug concentration. Correlation coefficient should not be less than 0.999.[4]

Accuracy
Accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Accuracy was performed in triplicates and compares the results. % recovery was performed by spiking known quantity of drug at 80,100,120% to a pre-quantified sample solution.[4]

From the result % recovery was calculated

1. Mean recovery should be in the range of 98-102%
2. The relative standard deviation should not be more than 2.0%

Precision
Precision of an analytical procedure is the closeness of agreement between a series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Precision is consider at three levels. Repeatability, intermediate precision and reproducibility. Prepare 6 different test solutions. Inject duplicate injection of each test solution. Pipette 1 ml of stock solution (100 µg/ml) in 10 ml volumetric flask and dilute with solvent in sufficient quantity and make volume up to the mark with solvent to get concentration of Pirfenidone 10 ppm.[4]

Robustness
Robustness of an analytical method, is the capacity to remain unaffected by small but deliberate variations to remain unaffected by small but deliberate change in the method parameters. Robustness is carried out by changing parameters like flow rate at 0.8ml/min and 1.2 ml/min, wavelength 312 nm and 322 nm, temperature 25˚c and 35˚c and mobile phase composition.[4]

Analysis of marketed tablet formulation
Accurately weigh 5 tablets of (Pirfenidone) and crushed to a fine powder. Accurately weigh and transfer a quantity of powder sample equivalent to 10 mg of Pirfenidone into a 10 ml volumetric flask. Add sufficient diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Filter the solution through 0.45 µm nylon filter. Pipette out 1 ml of above stock solution into 10 ml volumetric flask and dilute up to the mark with diluent for 10 µg/ml solution.[4]

RESULTS AND DISCUSSION

The present work was aimed to chromatographic method development and validation for estimation of Pirfenidone in pharmaceutical dosage form. A reverse phase chromatographic technique was developed to determine Pirfenidone at 317 nm on C_{18} column. (250 × 4.6 mm ×5 µm)

HPLC method development and optimization
In the literature survey of Pirfenidone there were many methods developed on RPHPLC using mobile phase such as Acn: water, methanol: water, Acn: buffer i.e. binary system. But, no suitable method were developed using tertiary system i.e. combination of Acn: methanol: water (65:15:20) on C_{18} column at flow rate 1 ml/min at detection wavelength 317 nm. Hence this mobile phase was chosen as the best chromatographic condition for the entire study.

Table no 1: Optimization conditions for HPLC

Equipment	HPLC JASCO – 4000
Column	C_{18}(250 × 4.6 mm, 5 µm)
Detector	PDA
Mobile phase	Acn: Methanol: Water (65 : 15 : 20)
Flow rate	1.0 ml / min
Wavelength	317 nm
Injection volume	10 µl
Column oven	30˚c
Run time	6 minutes

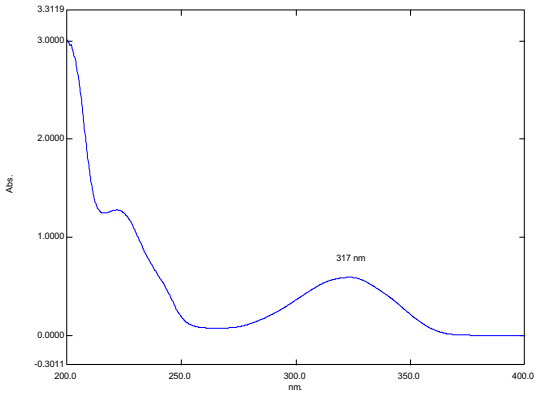

Figure no 1: UV spectra of Pirfenidone in acetonitrile

METHOD VALIDATION

Linearity
The calibration curve for Pirfenidone was linear over the concentration range 5-25 µg/ml. calibration curve was constructed by plotting the peak area vs the drug concentration. The regression equation for the calibration curve was found to be y = 17312x + 7782.8 with correlation coefficient of 0.9989 which is nearly equals to unity.

Table no 2: linearity data for Pirfenidone

Sr.no	Concentration	Peak area
1	0	0
2	5	98208
3	10	185887
4	15	272348
5	20	352721
6	25	435909
Mean		**2241478.8**
Correlation coefficient		**0.9989**
Slope		**7782.8**

R^2 = 0.9989
Linearity equation: y = 17312x + 7782.8
Acceptance criteria: correlation coefficient 'r' should not be less than 0.997

Observation: peak area is directly proportional to the concentration of analyte in the sample, therefore the proposed method is linear in the specified range.

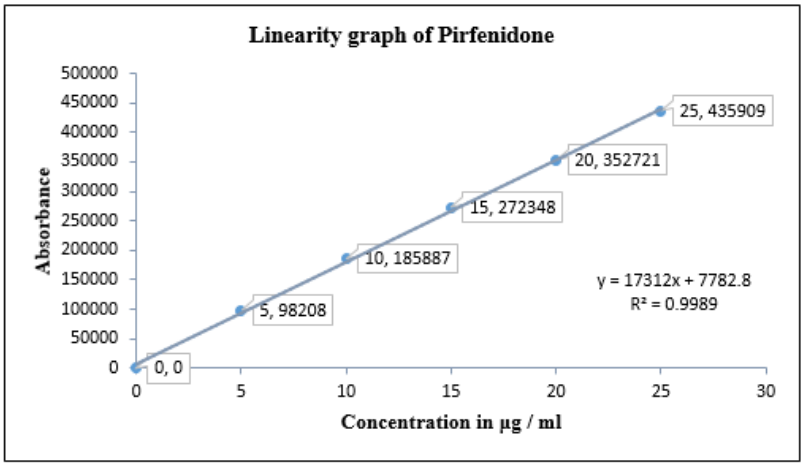

Figure no 2: linearity graph of Pirfenidone

Accuracy
Accuracy was performed in three different levels for Pirfenidone. Analysis was done in triplicate for each level. The % recovery is within 98-102%.

Table no 3: accuracy data for Pirfenidone

Concentration level (%)	Sample amount in µg / ml	Amount added in µg / ml	Peak area	Amount recover	% Recovery	% Mean recovery ± S.D	% RSD
80 %	10 10 10	8 8 8	325159 315879 319889	18.33 17.79 18.02	101.84 98.87 100.15	100.292347 ± 1.49	**1.48 %**
100 %	10 10 10	10 10 10	346242 347956 348523	19.40 19.64 19.68	97.75 98.24 98.41	98.13 ± 0.342	**0.34 %**
120 %	10 10 10	12 12 12	395535 392286 390896	22.39 22.21 22.12	101.80 100.95 100.59	101.118422 ± 0.62	**0.61 %**

Acceptance criteria: % recovery as per ICH guidelines should be within 98 – 102%
Observation: % recovery of Pirfenidone at 80 %, 100%, 120 % concentration level was found to be 100.29, 98.13, 101.11 respectively, which are within the limits.
Precision
The precision of the method was determined by system precision, method precision and intermediate precision. System precision was determine by injecting sample of 10 µg/ml for six different times. Method precision was determine by injecting 6 different samples of 10 µg/ml for six different times in a column. In intermediate precision, intraday and interday precision studies were carried out each at 3 different concentrations on different days and different analyst. % RSD for all precision studies was found to be less than 2%

Table 4: System and method precision

Sr.no	Concentration (µg/ml)	System precision	Method precision
1	10	183740	185887
2	10	183781	185202
3	10	183898	185246
4	10	183862	182452
5	10	183878	184524
6	10	183884	185321
Mean		**183840.5**	**184772**
S.D		**64.35**	**1216.39**
% RSD		**0.035**	**0.65**

Intermediate precision

Table no 5: Intraday precision of Pirfenidone

Sr.no	Concentration	Peak area	Mean absorbance ± S.D	RSD	% RSD
1	10	179519	179662.33 ± 497.72	0.00277	**0.277**
		179252			
		187274			
2	15	246620	246475 ± 171.97	0.000698	**0.069**
		246520			
		246285			
3	20	330023	330464.6 ± 644.63	0.001951	**0.198**
		330153			
		331218			

Acceptance criteria: % RSD should be less than 2.0 %
Observation: the relative standard deviation was found to be within 0.0006 – 0.00277 % for intraday precision. % RSD was found to be less than 2.0 %, which is within the limits.

Table no 6: Interday precision of Pirfenidone

Sr.no	Concentration	Absorbance	Mean absorbance ± S.D	RSD	% RSD
1	10	185517	186683 ± 1009.81	0.0054	**0.54**
		187258			
		187274			
2	15	250803	250803.33 ± 38.50	0.00015	**0.015**
		250765			
		250842			
3	20	335943	335844.66 ± 97.02	0.00028	**0.028**
		335842			
		335749			

Acceptance criteria: % RSD should be less than 2.0 %
Observation: the relative standard deviation was found to be within 0.00015 – 0.0054 % for interday precision. % RSD was found to be less than 2.0 % for interday precision. It was observe that % RSD was less than 2.0 % for intraday and interday precision, therefore the developed method is precise for its use.

Robustness

Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provide an indication of its reliability for routine analysis. The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changing from the original condition. The results obtained from assay of test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. % RSD was less than 2.0% indicating that the method is robust.

Table no 7: Robustness data for Pirfenidone

Sr.no	Parameters	Retention time	Peak area	NTP
Flowrate	**0.8 ml / min**	4.31	235038	5969
		4.32	235054	5968
		4.35	235079	5970
	Mean ± S.D	**4.31 ± 0.0060**	**235057 ± 20.66**	**5969 ± 1**
	% RSD	**0.139**	**0.0087**	**0.016**
	1.2 ml / min	2.9	158484	4359
		2.912	158490	4340
		2.92	158486	4365
	Mean ± S.D	**2.91 ± 0.010**	**158486.7 ± 3.05**	**4354.66 ± 13.05**
	% RSD	**0.345**	**0.0019**	**0.299**
Temperature	**25˚c**	3.510	189397	5245
		3.518	189384	5232
		3.522	189375	5254
	Mean ± S.D	**3.51 ± 0.0061**	**189385.3 ± 11.06**	**5243.66 ± 11.06**
	% RSD	**0.173**	**0.0058**	**0.210**
	35˚c	3.463	190208	4752
		3.478	190220	4760
		3.459	190214	4742
	Mean ± S.D	**3.4705 ± 0.010**	**190214 ± 6**	**4751.33 ± 9.018**
	% RSD	**0.305**	**0.003154**	**0.189**
Wavelength	**312 nm**	3.443	185642	4934
		3.449	185664	4952
		3.387	185687	4960
	Mean ± S.D	**3.446 ± 0.02**	**185664.3 ± 22.50**	**4948.6 ± 13.31**
	% RSD	**0.69**	**0.012**	**0.2690**
	322 nm	3.463	184669	5019
		3.468	184672	5021
		3.475	184664	5030
	Mean ± S.D	**3.465 ± 0.0060**	**184668.3 ± 4.04**	**5023.33 ± 5.85**
	% RSD	**0.173**	**0.0021**	**0.116**
Mobile phase composition	**70 ACN : 20 Methanol : 10 water**	3.267	311123	3441
		3.272	311160	3448
		3.264	311115	3455
	Mean ± S.D	**3.26 ± 0.004**	**311132.7 ± 24.00**	**3448 ± 7**
	% RSD	**0.123**	**0.007**	**0.23**
	60 ACN :20 Methanol: 20 water	3.537	193838	4821
		3.545	193848	4830
		3.534	193920	4885
	Mean ± S.D	**3.541 ± 0.005**	**193868.7 ± 44.73**	**4845.33 ± 34.64**
	% RSD	**0.16**	**0.023**	**0.71**

Acceptance criteria: % RSD should be less than 2.0 %
Observation: system suitability parameters was found to be within limit at all variable conditions for robustness study conducted with % RSD not more than 2.0 %.
Assay

Table no 8: Assay results for Pirfenidone

Sr.no	Concentration µg / ml	Sample area	Standard area
1	10	188074	185887
2	10	186854	188502
3	10	187798	187746
4	10	186814	188852
5	10	184912	188954
6	10	186207	189921
Average		187043.16	188533.5
Tablet average weight		291.7 mg	
Standard weight		10 mg	
Sample weight		14.5 mg	
Label amount		200 mg	
Standard purity		100.01 %	
Assay		99.80 %	

Observation: Amount of Pirfenidone present in taken dosage form was found to be 99.80 %

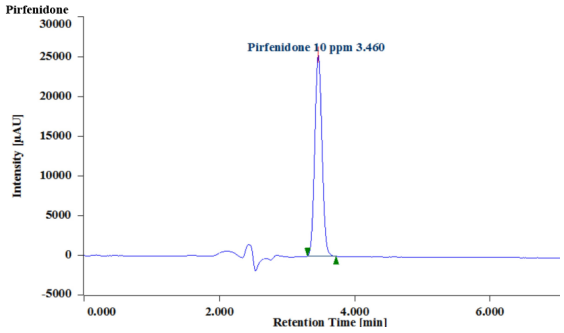

Figure no 3: chromatogram of sample of Pirfenidone

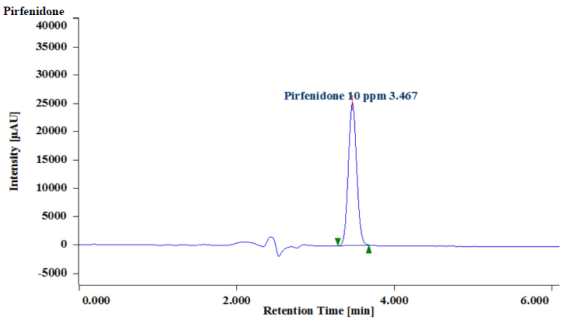

Figure no 4: chromatogram of standard of Pirfenidone

CONCLUSION

The Pirfenidone is an antifibrotic, anti-inflammatory and antioxidant agent having high solubility and high permeability. A simple, rapid, selective, precise and accurate HPLC method has been developed for the estimation of Pirfenidone in bulk drugs and its tablet dosage form. λ max of Pirfenidone in water was found to be 311 nm. λ max of Pirfenidone in ACN was found to be 317 nm. Acn was used as a diluent for sample preparation. The developed method was optimized to get reproducible results with minimum run time. The stationary phase was C_{18} (250 × 4.6 mm, 5 µm), mobile phase was Acn: methanol: water (65:15:20), flow rate (1.0 ml/min), injection volume (10 µl), PDA detection is at λ max 317 nm and run time was 6 minute. This method is validated for specificity (selectivity), linearity (range), Precision (System, method, intermediate), Accuracy (recovery), robustness, filter suitability which was found to be within the specified limit. Influence of acid, alkaline, oxidative, thermal and photolytic stress conditions on Pirfenidone was studied. Results indicated that Pirfenidone is unstable in solution form after suitable time period. The results of the developed method indicates that, this method may be used into routine analysis for the determination of assay in Pirfenidone tablet dosage form.

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