EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 8
552-558
Year: 2018

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

Snehal Ganpat Tekawade*, Dr. Ashok Pandurang Pingle, Rina Mohan Sonawane and Rutuja Prabhakar Sonare

Department of Pharmaceutical Chemistry, Maratha Vidya Prasarak Samaj's College of Pharmacy Nasik.

***Corresponding Author: Snehal Ganpat Tekawade**
Department of Pharmaceutical Chemistry, Maratha Vidya Prasarak Samaj's College of Pharmacy Nasik.

Article Received on 19/06/2018 **Article Revised on 09/072018** **Article Accepted on 29/07/2018**

ABSTRACT
The new RP-HPLC method was developed and validated for the quantitative determination of Pirfenidone, a novel antifibrotic agent used in idiopathic pulmonary fibrosis. The separation was achieved on GRACE C_{18} analytical column (250×4.6mm, 5µ) using Methanol: Water (90:10 v/v) as mobile phase with UV-3000-M detector. The flow rate was 0.9 ml/min. The method was validated as per ICH guidelines for precision, linearity, accuracy, robustness. The proposed method was found to be accurate, reproducible & consistent. The standard curve was linear over the concentration range 10-50 µg/ml with r^2 close to one (0.999). The % recovery was found to be within 99-100% and relative standard deviation <2%. The developed method was successfully applied for determination of Pirfenidone in the tablet. The simple, less time consuming and cost effective method was developed.

KEYWORDS: RP-HPLC, PIRFENIDONE, VALIDATION, ETC.

1. INTRODUCTION
Pirfenidone 5-methyl-1-phenyl-1,2-dihydropyridin-2-one (Fig.1) is a novel antifibrotic agent approved for mild to moderate Idiopathic pulmonary fibrosis (IPF) as orphan drug in Japan and Europe. It was initially approved in Japan and Europe followed by India and USA in 2010 and 2014 respectively.[1] Pirfenidone is the only drug which has been approved for the treatment of IPF. Patients with IPF suffer from shortness of breath, cough and hampered daily physical activities. Treatment for IPF consists of oxygen therapy, pulmonary rehabilitation and lung transplant. Pirfenidone is the only therapeutic agent available for the treatment of IPF.[2] IPF is a rare incurable disease, often fatal; which mostly affects geriatric patients causing fibrosing interstitial pneumonia of unknown etiology. 5-methyl-1-phenyl-1,2-dihydropyridin-2-one (fig. 1) is a small simple heterocyclic molecule with molecular mass of 185.22 daltons and can be administered orally.[3]

Literature survey revealed that HPLC and LC-MS/MS methods are reported for determination of Pirfenidone from biological fluids such as plasma, serum and urine but very few methods have been reported for the estimation of Pirfenidone in pharmaceutical dosage form.[4] Oral tablet formulation containing active Pirfenidone equivalent to 200 mg is available in the territorial markets of Japan, Taiwan, Korea and India whereas it is available in capsule formulation in the USA. Assay or monograph for Pirfenidone or its formulation is not available yet in any pharmacopoeia. Recently we have reported chromatographic method development and validation for estimation of Pirfenidone in pharmaceutical dosage form. It was learned that recently one report has published HPLC method for determination of Pirfenidone in tablet formulations. We are reporting a better assay including HPLC method for routine quality control of Pirfenidone formulations.

1.1 Structure

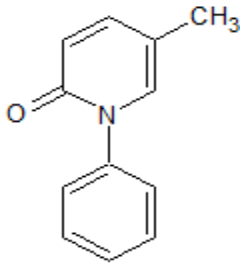

Fig. 1: Chemical structure of Pirfenidone.

2. MATERIALS AND METHOD
2.1 Chemical and reagents
Pirfenidone was purchased from Modern Science Apparatus Pvt. Ltd. Nashik, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities

were found. The drug was used without further purification.

HPLC-grade Methanol, Water and other reagents was obtained from Merck were of standard Quality. Film coated tablets of Pirfenidone Tablet IP 200mg (Pirfenex®, Cipla Ltd., Malpur, Dist. Solan 173205 India) were procured from local market.

2.2 Instrumentation

The chromatographic technique was performed on Shimadzu UV 2450 Double beam UV-Visible spectrophotometer with software UV probe, reversed phase Grace C18 column (250mm x 4.6ID, Particle size: 5 micron) as stationary phase, Ultrasonic cleaner, Wenser High Precision Balance PGB 100, Wenser Ultra Sonicator WUC- 4L, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

2.3 Chromatographic condition

Chromatographic separation was performed on Grace C18 analytical column. Isocratic mobile phase consisting of Methanol: Water (90:10 v/v) was delivered at flow rate 0.9ml/min. injection volume was 20µL.

2.4 Preparation of calibration standards for UV

Standard stock solution(A_1): An accurately weighed about 10.0mg quantity of Pirfenidone was transferred in a 10.0mL of volumetric flask, dissolved in a sufficient quantity of methanol and volume was made up to the mark with methanol. (Conc. 1000µg/ml of Pirfenidone).

Working stock solution (A_2): A 1.0mL of standard stock solution was transferred in 10.0mL of volumetric flask and volume was made up to the mark with methanol. (Conc. 100µg/ml of Pirfenidone).

Working standard solution: A 0.3mL of working stock solution (A_2) was transferred in 10.0mL of volumetric flask and volume was made up to the mark with methanol (Conc. 3µg/ml Pirfenidone). Then further dilution of drug like 6, 9, 12, 15µg/ml from stock solution was prepared in the same solvent.

Standard solution of 3-15µg/ml was scanned between 400nm to 200nm. Overlain spectra of the drug was recorded. From the spectra 224nm and 314 nm (λmax of Pirfenidone) were selected for the method development.

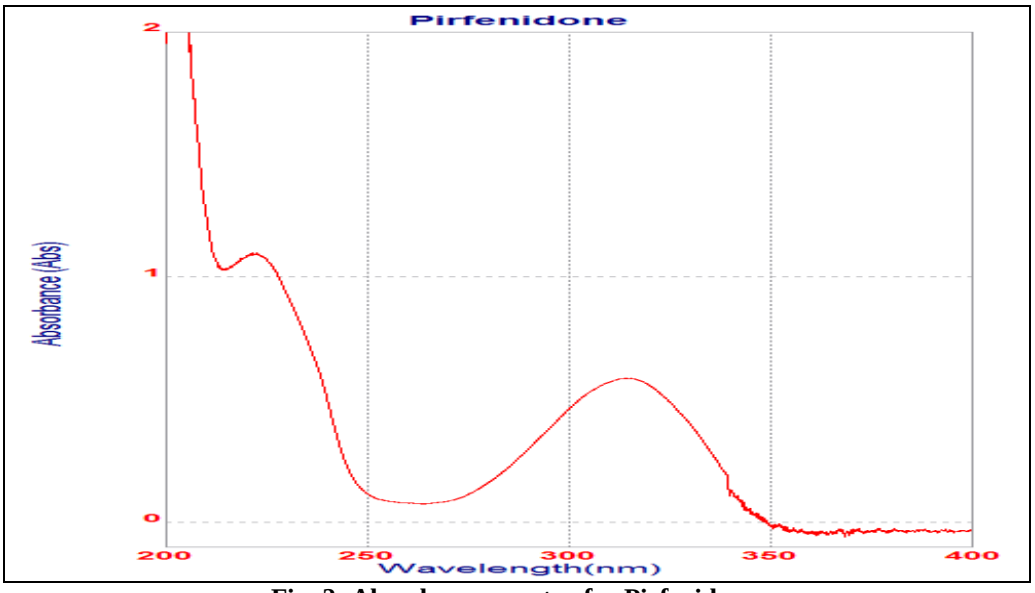

Fig. 2: Absorbance spectra for Pirfenidone.

The absorption maxima for Pirfenidone were found to be 224 nm and 314 nm.

2.5 Sample Preparation

A) Standard Stock Solution

Procedure

Accurately weighed 0.01 gm of pure drug (Pirfenidone) was dissolved in a 10 ml of methanol which gives 1000 ppm solution. From this solution the dilutions like 10, 20, 30, 40, 50 ppm were prepared.

B) Sample solution preparation
Procedure
Table no. 9: Dilution for sample preparation.

Sr. no.	Concentration (ppm)	Volume of stock solution (ml)	Volume made up to (ml)
1	10	0.1	10
2	20	0.2	10
3	30	0.3	10
4	40	0.4	10
5	50	0.5	10

2.6 Method Validation

A. Linearity

[5]Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range.[5]

Linearity was performed by diluting standard stock solution to give final concentration in the range of 10µg/ml to 50µg/ml for Pirfenidone. 20µL of each concentration injected and calibration curve was constructed by plotting the peak area versus the drug concentration. Correlation coefficient should not be less than 0.999.

B. Accuracy

[5]The accuracy of an analytical method is closeness of test results obtained by that method to the true value.[5] Accuracy is calculated from the test result as the percentage of analyte recovered by the assay. Accuracy was performed in triplicates and compares the results. % recovery was performed by spiked known quantity of drug at 50%, 100% and 150% to a pre-quantified sample solution and analyses sample. 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%

C. Precision

[5]The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.[5]

Prepare six different test solutions. Inject duplicate injection of each test solution. Pipette 0.3ml of stock solution in 10ml volumetric flask and dilute with solvent in sufficient quantity and make volume up to mark with solvent to get concentration of Pirfenidone 30ppm.

D. Robustness

[5]It is the measure of capacity of the method to remain unaffected by small but deliberate change in the method parameter and provide an indication of its reliability under normal usage.[5] The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. Carry out the following procedure individually by changing in wavelength 222nm and 226nm and flow rate 0.7 and 1.1ml/min.

E. Analysis of marketed tablet formulation (Assay)

Twenty tablets of each brand (Pirfenex) were purchased from the local market, weighed and crushed to a fine powder. Accurately weigh and transfer a quantity of powder sample equivalent to 10 mg of Pirfenidone into a 10ml clean volumetric flask, add sufficient diluents and sonicate to dissolve it completely and make volume up to the mark with the diluents. Filter the solution through 0.45µm membrane filter. Pipette out 0.3 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents for 30µg/ml solution.

3. RESULT AND DISCUSSION

The present work was aimed to Chromatographic method development and validation for estimation of Pirfenidone in pharmaceutical dosage form. A reversed-phase chromatographic technique was developed to determine Pirfenidone at 224 nm and Grace C18 column was adopted for the analysis.

HPLC method development and optimization: In the literature survey of Pirfenidone no suitable assay method was available for the determination of Pirfenidone using RP-HPLC. A mixture of Methanol: Water was selected as mobile phase. Initially the Pirfenidone drug sample solutions were analyzed using a mobile phase consisting of Methanol: Water (70:30, v/v) at a flow rate of 0.8 ml/min, 314 nm. Under these conditions, a broad peak was observed at 4.527 min. Then the mobile phase was changed to Methanol: Water (80:20 v/v), 314 nm and (80:20 v/v), 224 nm with a flow rate of 0.8 ml/min. and as a result retention time was 4.503 and 4.124min resp. So the flow rate was totally changed to 0.9 ml/min under which a sharp peak was eluted with good symmetry and retention time 3.644 ± 0.46 min. Hence a mobile phase containing Methanol: Water (90:10, v/v) with flow rate of 0.9 ml/min was chosen as the best chromatographic condition for the entire study.

Method validation

A. Linearity

The calibration curve for Pirfenidone was linear over the concentration range 10-50µg/ml. Calibration curve was constructed by plotting the peak area versus the drug

concentration. The data for the peak area of the drug corresponding to the concentration was treated by linear regression analysis (Table 1 and fig. 2) and the regression equation for the calibration curve was found to be y = 80476x + 40156 with correlation coefficient of 0.999 which is nearly equals to unity.

Table No. 1: Linearity of Pirfenidone.

Sr. no	Concentration (µg/ml)	Area
1	10	801572
2	20	1608874
3	30	2462546
4	40	3258815
5	50	4070415
Correlation coefficient	0.999	
Slope(m)	80476	
Intercept(c)	40156	

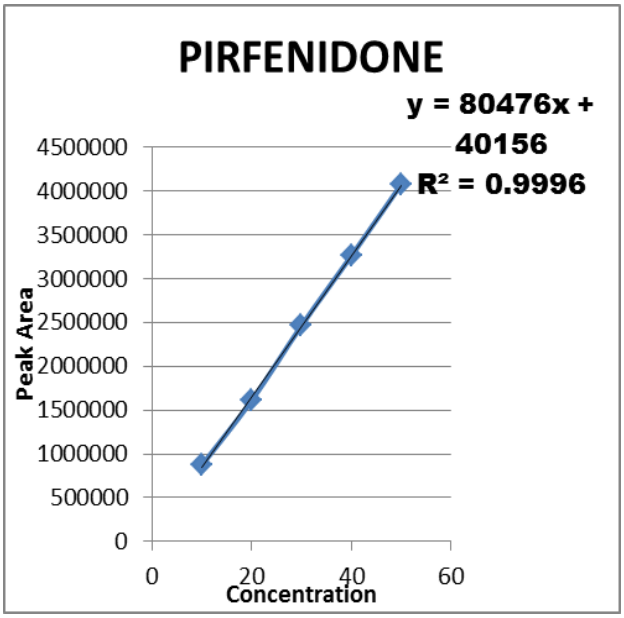

Fig. No. 3: Linearity of Pirfenidone.

B. Accuracy

Accuracy was performed in three different levels for Pirfenidone. Analysis was done in triplicate for each level. The resultant % RSD was in the range 0.17-0.27 (<2.0%) with recovery 99.67-99.89% (Table 2and3).

Table No. 2: Accuracy study of Pirfenidone.

Sr. no.	Concentration (ppm)	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	%RSD
1	10	871572	873887.6667	2389.651509	0.27345065	0.27345065
2	10	873746				
3	10	876345				
4	30	2462546	2467504.333	4410.424507	0.17874029	0.178740294
5	30	2468977				
6	30	2470990				
7	50	4070415	4066907	9431.680073	0.23191285	0.231912854
8	50	4056224				
9	50	4074082				

Accuracy results were expressed in terms of percent recovery.

Table No. 3: % Recovery of Pirfenidone.

Sr. no.	% Composition	Standard sample	Tablet sample	Area of Standard	Area of Sample	% Recovery
1	50% recovery	20 ppm	10 ppm	2462546	2457311	99.78741514
2	100% recovery	20 ppm	20 ppm	3258815	3252066	99.79290018
3	150% recovery	20 ppm	30 ppm	4070415	4057284	99.6774039

C. Precision

The precision of the method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was calculated by assaying three samples of each at three concentration levels (30µg/ml) on the same day. The inter-day precision was calculated by assaying three samples of each at three concentration levels (30µg/ml) on two different days. The % RSD in precision studies was found to be 0.16% (intra-day) and 0.21% (Interday) which is <2.0% (Table 4).

Table No. 4: Precision studies of Bosentan Monohydrate.

Intraday precision				
Concentration	**Area**		**Mean**	**%RSD**
	Morning	**Evening**		
30	2462546	2463290	2464910	0.16%
30	2468977	2462548		
30	2470990	2461111		
Interday precision				
Concentration	Area		Mean	%RSD
	Day 1	Day 2		
30	2462546	2459517	2547960	0.21%
30	2468977	2460944		
30	2470990	2457960		

D. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate change in method parameter 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 detection wavelength was set at 224nm, the ratio of Methanol: Water in the mobile phase was applied as 90:10 v/v. The flow rate was set at 0.7 and 1.1 ml/min (± 0.2 ml/min) and wavelength was set at 222 and 226 (±02). The results obtained (Table 5) from assay of the test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. The %SD value of assay determined for the same sample under original conditions and robustness conditions was less than 2.0% (0.21-0.18) indicating that the method is robust (Table 5).

Table No. 5: Robustness study of Pirfenidone.

Parameters	Variation	R.T.	Tailing factor	Area	%RSD
Flow rate	0.9 ml/min	3.635	8133	1608874	0.25682368
	0.7 ml/min	4.103	8509	1602328	
	1.1 ml/min	3.328	4914	1609956	
Wavelength	224 nm	3.635	8133	1608874	0.18036796
	222 nm	3.668	8117	1604797	
	226 nm	3.633	7907	1610409	

E. Analysis of Marketed Tablet Formulation (Assay)

The proposed method was applied for the determination of Pirfenidone in marketed formulations (tablets) (Figure 4) and the % recovery is found to be 99.8152% (Table 7). Accepted range is 98-102%.

Table No. 7: Assay Result of Pirfenidone.

Drug	Label claim (mg/tab)	Concentration taken	Area	Amount found (µg/ml)	% assay
Pirfenidone	200	30 ppm	2457995	199.62	99.8152

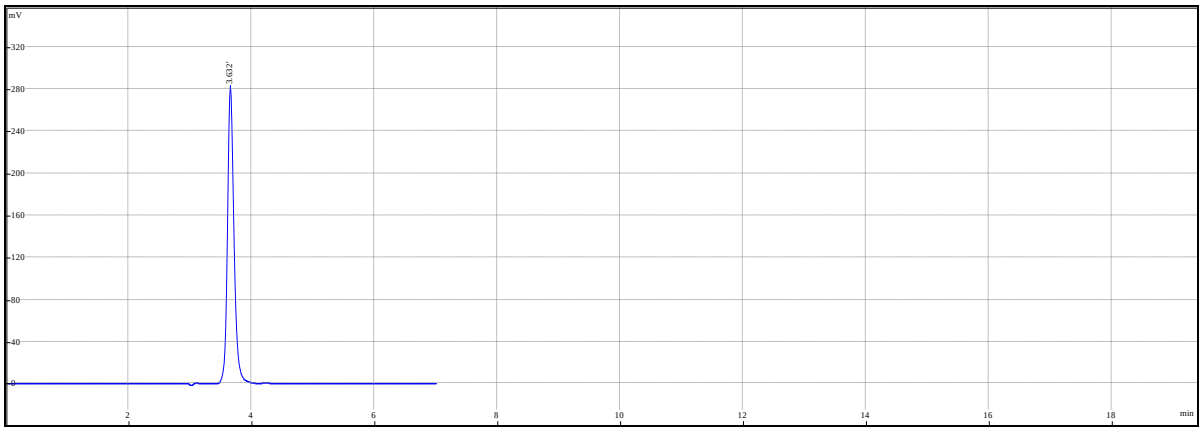

Fig. 4: Chromatogram of Assay.

4. CONCLUSION

In the present research work, a successful attempt was made to "Chromatographic method development and validation for estimation of Pirfenidone in pharmaceutical dosage form".

The method was developed by experimentation, based on literature survey and ascertained by statistical parameter of sampling. The simplicity, rapidity, reproducibility and economy of the proposed method completely fulfill the objective of this research work. In RP-HPLC method the analyte were resolved by using isocratic programme and mobile phase was used Methanol: Water (90:10 v/v), at a flow rate of 0.9 ml/min, on HPLC system containing UV-3000-M detector with Grace C18 (250 mm x4.61D, Particle size: 5 micron) column. The detection was carried out at 224 nm. The method gave the good resolution and suitable retention time. The results of analysis in the method were validated in terms of accuracy, precision, linearity, robustness.

The regression coefficient (r^2) for each analyte is not less than 0.999 which shows good linearity. The percent recovery was in the acceptable range. The % RSD was also less than 2% showing high degree of precision of the proposed method.

5. ACKNOWLEDGEMENT

I am grateful to all people who have helped and inspired me during my M. Pharmacy research work. It is a pleasure to convey my gratitude to them all in my humble acknowledgment. I am very much thankful to the principal, MVP Samaj's College of pharmacy, Nasik, for providing infrastructure and all the necessary facilities for this research work.

Once again I would like to thank all the people mentioned & those that I have unknowingly failed to mention but have been instrumental in the culmination of this project into a successful endeavor.

6. REFERENCES

1. https://www.drugbank.ca/drugs/ DB04951
2. Rubino C.M., Bhavnani S.M., Ambrose P.G., Pulmonary Pharmacology and Therapeutics, 2002; 22: 279.
3. Raghu G., Collard H.R., Egan J.J., Martinez F.J., Behr J., Brown K.K., et al. An official ATS/ERS/JRS/ALAT statement: Idiopathic pulmonary fibrosis: Evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med., 2011; 183: 788-824.
4. Parmar V.K., Desai S.B., Vaja T.; RP-HPLC and UV Spectrophotometric Methods for Estimation of Pirfenidone in Pharmaceutical Formulations, Indian J Pharm Sci., 2014; 76: 225-228.
5. ICH-Q2 (R1): Validation of Analytical Procedures: Text and Methodology, FDA, 1 March, 1995; 60: 11260.
6. Skoog D.A., Holler F.J., Crouch S.R.; Principle Of Instrumental Analysis, 6th edition, Thomson Publications; India, 2007; 1-3, 145-147, 180.
7. Mendham J., Denney R.C., Barnes J.D., Thomas M.; Vogel's Textbook of Quantitative Analysis, Pearson Education; Singapore, 2003; 8-9, 261-288.
8. Sharma B.K.; Instrumental Methods of Chemical Analysis, 25th edition; Goel Publication Co.; Meerut, 1983: 3,6.
9. Swartz M.E., Krull I.S.; Analytical Method Development and Validation, 2009: 26-27.
10. Snyder L.R., Kirkland J.J., Glajch L.J.; Practical HPLC Method Development, 2nd edition, John Wiley and sons, 1997: 1-20, 21-25.
11. Sethi P.D.; High Performance Liquid Chromatography; Quantitative Analysis of Pharmaceutical Formulations, 1st edition; CBS Publishers and Distributors Pvt. Ltd.; New Delhi, 2001: 3-11, 116-120.
12. Kasture A.V., Mahadik K.R., Wadoakar S.G.; More H.N.; Pharmaceutical Analysis vol-II, 19th edition; Nirali Prakashan, June 2010: 48-51.
13. Chatwal G.R., Anand S.K.; Instrumental Methods of Chemical Analysis, 5th edition; Himalaya publishing house, 2.624-2.625.
14. Willard H.H., Metritt L.L., Dean J.A., Settel F.A.; Instrumental Methods of Analysis, 7th ed.; CBS Publisher and Distributors, New Delhi, 1986: 513-530.
15. Jack C., Raymond P.H., Scott M.; Chromatography theory, 2002: 237-285.
16. USP-NF, Validation of Compendial Procedures, General Chapters, 2010; I: 734-736.
17. Thorat S.G., Tajne M.R., Padmane S.P., Ittadwar A.M.; Development and validation of simple, rapid and sensitive uv, hplc and hptlc methods for the estimation of Pirfenidone in tablet dosage form, Journal of Planar Chromatography, 2016; 61: 2978-2981.
18. Wang Y., Zhao X., Zhong J., Chen Y., Liu X., Wang G.; Simple determination of Pirfenidone in rat plasma via high-performance liquid chromatography, Biomed Chromatography; 2006; 20: 1375-379.
19. Shi S., Wu J., Shi S., Wu J., Zeng F.; Development and validation of an improved LC method for the simultaneous determination of pirfenidone and its carboxylic acid metabolite in human plasma, Chromatographia, 2009; 69: 459-463.
20. Tamilselvi N., Kurian D.S., Bioanalytical method development and validation of Pirfenidone by RP-HPLC method and its application to the determination of drug food interaction study in winster rats, IJPBR, 2012: 132-142.
21. Boadempudi S.B., Babur R., Reddy K.S; Development and substantiation of a RP-HPLC method for monitoring impurities in Pirfenidone drug substance, American journal of analytical chemistry, 2015; 6: 1019-1029.

22. Gayatri S.N, Biju V.M.; Assay of Pirfenidone by UV spectrophotometry, Journal of chemical and pharmaceutical research, 2016; 8(7): 36-41.
23. Ravishankar R., K. Anusha Rani, Ch. Devdasu, P. Srinivasa Babu; Development of a validated UV spectrophotometric method for the quantitative estimation of Pirfenidone in bulk and marketed tablet, International Journal of Pharmaceutical Research and Development, 2014; 6(09): 025-031.
24. Tong S., Wang X., Jiang H., Xuegu X., Pan Y., et. al.; Determination of pirfenidone in rat plasma by LC–MS-MS and its application to a pharmacokinetic study, Chromatographia, 2010; 71: 709-713.
25. Al Bayati M. A., Yan xie, F. Charles. Mohr, et.al.; Effect of Pirfenidone against vanadate induced kidney fibrosis in rats, Biochemical pharmacology, 2002; 62: 517-525.
26. Dosanjh A.; Pirfenidone anti-fibrotic agent with a potential therapeutic role in the management of transplantation patients, European journal of pharmacology, 2206; 536: 219-222.
27. Leonel Garcia, Ivan Hernandez, et.al.; Pirfenidone effectively reverses experimental liver fibrosis, Journal of hepatology, 2002; 37: 797-805.
28. Hanzhong Liu Peter Drew, Andrew C. Gaugler, et.al.; Pirfenidone inhibits lung allograft fibrosis through L-arginine-arginose pathway, American journal of transplantation, 2005; 5: 1256-1263.
29. James P. Kehrer, Solomon B. Margolin; Pirfenidone diminishes cyclophosphamide induced lung fibrosis in mice, Toxicology letters, 1997; 90: 125-132.
30. Goran Miric, Catherine Dallemagne, Zoltan Endre, et.al.; Reversal of cardiac and renal fibrosis by Pirfenidone and spironolactone in streptozotocin-diabetic rets, British journal of pharmacology, 2001; 133: 687-694.
31. Sonako Nagai, Kuniohamada, Michio Shigematsu et.al.; Open label compassionate use one year treatment with Pirfenidone to patients with chronic pulmonary fibrosis, Internal medicine, 2002; 41(12): 1118-1123.
32. Shaojun Shi, MD, Jianhong Wa Huating Chen, BS, et.al.; Single and multiple dise pharmacokinetics of Pirfenidone an antifibrotic agent in healthy Chinese volunteers, J. clin pharmacol, 2007; 47: 1268-1276.
33. Seiya Tada, Makota Nakamutu, Mnechika Enjoji et.al.; Pirfenidone inhibits dimethylnitrosamine induced hepatic fibrosis in rats, Clinical and experimental pharmacology; 2001; 28: 522-527.