

Analytical Quality by Design (AQbD)–Based Development and Validation of a Stability-Indicating HPLC Method for Pirfenidone

Rushikesh Nagre*, Dr.Santosh Payghan, Prof. Amol Wadhve, Prof. Ashwini Pundkar

1. Research scholar, Dept. of Pharmaceutical Quality Assurance, Rajesh Bhiayya Tope College of Pharmacy, Chh.Sambhajinagar.
2. Principal. Dept. of Pharmaceutical Sciences, Rajesh Bhiayya Tope College of Pharmacy, Chh.Sambhajinagar.
3. Professor, Dept. of Pharmaceutical Sciences, Rajesh Bhiayya Tope College of Pharmacy, Chh.Sambhajinagar.
4. Professor, Dept. of Pharmaceutical Sciences, Rajesh Bhiayya Tope College of Pharmacy, Chh.Sambhajinagar.

Email id: uionyou65@gmail.com

ABSTRACT

Development of stability-indicating method by liquid chromatography are always a challenge for analytical researchers. A traditional way is by varying one chromatographic parameter at a time assessing the responses from each test. Recent international guidelines encourage the implementation of systematic approaches in analytical development, such as Analytical Quality by Design (AQbD). The aim of this paper was to develop, using the AQbD approach, an efficient and selective stability-indicating method by high performance liquid chromatography for nevirapine and its related substances. The Analytical Target Profile (ATP) and the Critical Method Attributes (CMA) were determined. Through the risk assessment studies, the high-risk analytical conditions were considered as Critical Method Parameters (CMP) and they were screened and optimized by Design of Experiment (DoE). The Method Operable Design Region (MODR) was defined using Monte Carlo Simulation. A control strategy was established by the definition of the system suitability of the analytical procedure.

Keywords: *AQbD, Pirfenidone, RP-HPLC, DoE, Method validation, Stability-indicating.*

INTRODUCTION

Pirfenidone is a novel antifibrotic drug approved for mild to moderate idiopathic pulmonary fibrosis as orphan drug in Japan and Europe. Pirfenidone is only drug which has been approved for the treatment of IPF. Pirfenidone is a small non-peptide molecule of low molecular weight (185.2 daltons) with the chemical name of 5-methyl-1-phenyl-2-(1H)-pyridone. Pirfenidone is an antifibrotic drug used in the treatment of idiopathic pulmonary fibrosis (IPF). Chemically, it is 5-methyl-1-phenyl-2-(1H)-pyridone. Due to its therapeutic importance, reliable analytical methods are essential for its quantification and stability assessment. Traditional analytical method development relies on trial-and-error, whereas Analytical Quality by Design (AQbD) provides a systematic, risk-based, and science-driven approach to ensure method robustness and lifecycle management. Analytical Quality by Design (AQbD) is a scientific, risk-based and systematic approach to analytical method development that focuses on predefined objectives, method understanding, and lifecycle management [2–4]. The concept of AQbD is derived from pharmaceutical Quality by Design (QbD) and is increasingly adopted in analytical method development to enhance method robustness and regulatory flexibility [5]. The API chosen to perform the science-based approach in the present work was nevirapine, used as part of combination therapy to treat HIV-1 infection [30]. Nevirapine 200 mg tablets are marketed in Brazil and other countries. Farmanguinhos (Fiocruz, Brazil) is the pharmaceutical industry responsible for supplying this medicine to the Brazilian population and as part of the registration process, the development of a stability-indicating method is necessary. Stability-indicating methods for analysis of nevirapine and its impurities have been described and almost all studies used high-performance liquid chromatography (HPLC) [31], [32], [33], [34]. However, different method conditions have been employed and no detailed and comprehensive study covering method development applying AQbD was identified in the literature, nor did any present information on measurement uncertainty to reduce the risk of false decision.

MATERIALS AND METHODS

Preparation of standard calibration curve:

The absorbance's of the standard solutions in water at 1-10 $\mu\text{g} / \text{ml}$ range was measured at 312 nm. Standard calibration curve was prepared by plotting maximum absorbance (λ_{max}) versus concentration. Linearity was studied using a regression equation.

Estimation of Pirfenidone in Marketed Formulation:

Preparation of Pirfenidone Test Solution

A stock solution (1000 $\mu\text{g} / \text{ml}$) was prepared by taking 20 tablets, crushed, powdered; weight equivalent to 1000 $\mu\text{g} / \text{ml}$ of standard pirfenidone solution was taken and dissolved in a 10 ml volumetric flask using Double distilled water. The solution sonicated for 20 min and placed undisturbed overnight, again sonicated for 15 minutes and filtered through Whatman filter paper. From the clear test stock solution, pipette out 1 ml and make up to 10 ml with Double distilled water (100 $\mu\text{g} / \text{ml}$). From that pipette out 1 ml and make up to 10 ml with Double distilled water to obtain a concentration of 10 $\mu\text{g} / \text{ml}$ and serial dilutions were prepared (1 $\mu\text{g} / \text{ml}$, 3 $\mu\text{g} / \text{ml}$, 5 $\mu\text{g} / \text{ml}$, 7 $\mu\text{g} / \text{ml}$, 9 $\mu\text{g} / \text{ml}$).

Method Validation

Linearity

Prepared in 6 spikes of 1-10 $\mu\text{g} / \text{ml}$ then analyze by spectrophotometrically. A graph of Concentration Vs Absorbance was plotted and correlation coefficient was calculated.

Accuracy (% Recovery)

The accuracy of the analytical method was assessed by determination of recovery for three concentrations corresponding to 50, 100 and 150 % of test solution concentration. For each concentration, three sets were prepared. The mean recovery of pirfenidone was reported.

Precision

Preparation of standard stock solution: About 10 mg of pirfenidone was taken in to 10 ml volumetric flask, and diluted up to 10 ml with diluent

A. Repeatability-

Repeatability expresses the precision under the same operating conditions over a short interval of time. It means the study was performed morning and evening for all concentrations (1-10 $\mu\text{g} / \text{ml}$ solution) 6 times and % RSD was calculated.

B. Intermediate precision-

Intermediate precision expresses within - laboratories variations: different days, different analysts, different equipment, etc. It means the study was performed different analyst (analyst -1 and analyst -2) for all concentrations (1 -10 $\mu\text{g} / \text{ml}$ solution) 6 times and % RSD was calculated.

C. Reproducibility-

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to the standardization of methodology). It means the study was performed today and tomorrow for all concentrations (1-10 $\mu\text{g} / \text{ml}$ solution) 6 times and % RSD was calculated. Robustness

The estimation of pirfenidone was performed at different environmental conditions (room temp at 29°C and elevated temp 35°C).

Ruggedness

The ruggedness is to expresses within laboratories variations: different days, different analysts, different equipment, etc

Detection Limit & Quantitation Limit

LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/S$ and $10\sigma/S$ respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

RP-HPLC Method

Preparation of Standard Stock Solution:

An accurately weighed quantity of (10 mg) pirfenidone was transferred to a 10mL volumetric flask, dissolved and diluted to the mark with Methanol : Water : Triethylamine (50:48.5:1.5 %v/v) to obtain standard stock solution of 1000 $\mu\text{g} / \text{ml}$.

Preparation of mobile phase:

Take 50 ml of methanol was transferred to 100 ml of volumetric flask then add 48.5 ml of double distilled water and add 1.5 ml of tri ethylamine then sonicate it for 1hr and filter the mobile phase (50:48.5:1.5 v/v).

Preparation of Pirfenidone test solution:

A stock solution (1000 µg/ml) was prepared by taking 20 tablets, weight equivalent to (1000 µg/ml) of standard Gallic acid solution was taken and dissolved in a 10 ml volumetric flask using Methanol : Water : Triethylamine (50 : 48.5:1.5 %v/v). The solution sonicated for 20 min and placed undisturbed overnight, again sonicated for 15 minutes and filtered through Whattman filter paper. From the clear test stock solution, pipette out 1 ml and make up to 10 ml with solvent (100 µg/mL), From that pipette out 0.5, 1, 1.5, 2 & 2.5 ml and make up to 10 ml with solvent to obtain a concentration of 525µg/ml.

Method validation

Specificity

Specificity include impurities, degradants, matrix, etc.

Linearity

Accurately weighed 10 mg of pirfenidone transfer to 10 ml of volumetric flask. Admade up the volume with Methanol : Water : Triethylamine and sonicate it for 10min (1000 µg /ml). These are prepared in 6 spikes of 5-25 µg / ml then analyze by Chromatography.

RESULT AND DISCUSSION

The λ_{max} of Pirfenidone was found to be 312 nm

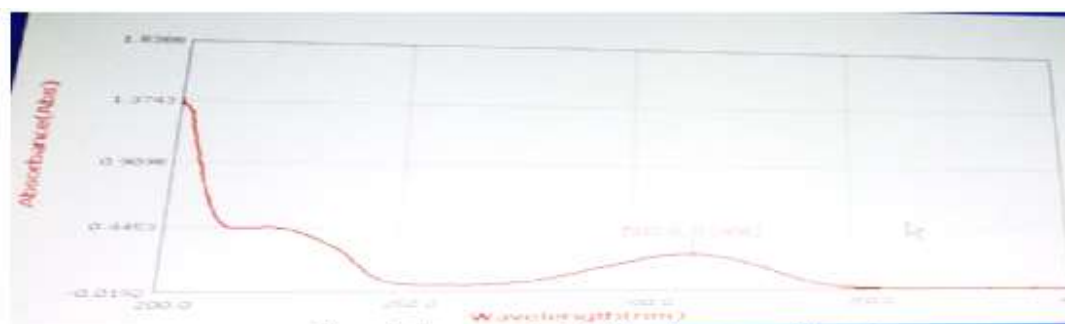


Figure 2: Spectrum of pirfenidone in water at 312 nm



Table 1: Standard Graph Values

S.no	Concentration(µg/ml)	Absorbance(nm)
1	1	0.0333
2	3	0.1156
3	5	0.1998
4	7	0.2880
5	9	0.3776
6	10	0.4113

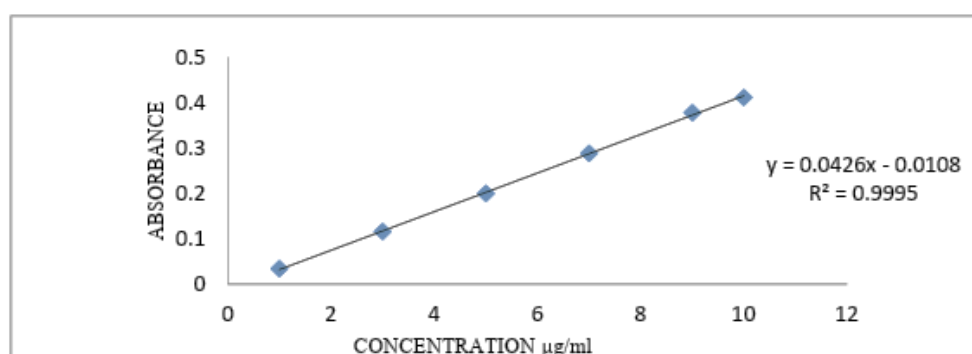
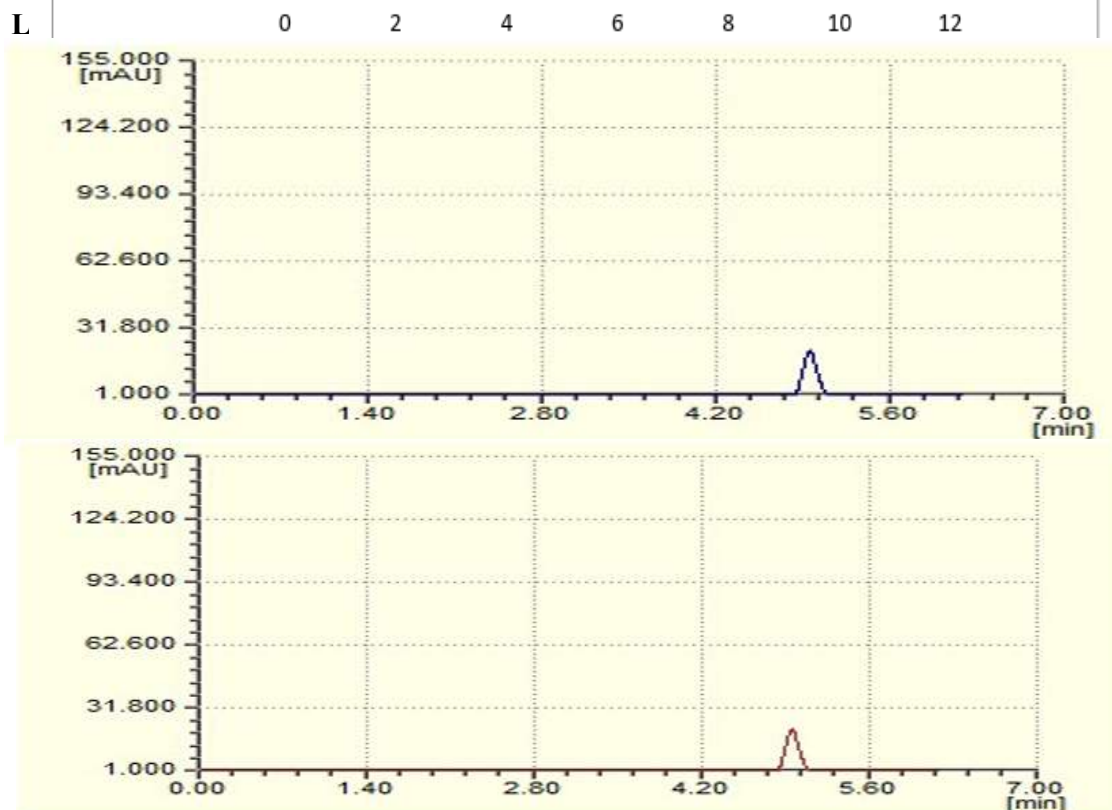
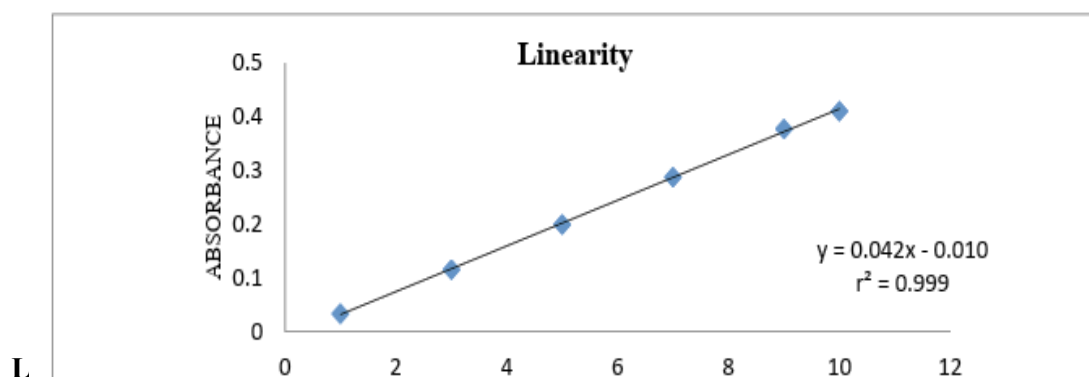


Figure 4: Calibration curve of Pirfenidone

Spectroscopic Method Validation of Pirfenidone

Table 3: Linearity values

S. No.	Conc. (µg/ml)	Absorbance (Mean, N=6)	Standard Deviation	%RSD
1	1	0.0333	0.0005	1.9
2	3	0.1156	0.0016	1.3
3	5	0.1998	0.0032	1.8
4	7	0.2880	0.0050	1.8
5	9	0.3776	0.0049	1.3
6	10	0.4113	0.0079	1.7



SUMMARY

Pirfenidone has been shown to have a significant effect on FVC decline and progression-free survival in Phase III clinical trials that enrolled patients with IPF with mild to moderately severe respiratory impairment. Treatment with antifibrotic agents has now entered the clinical arena, and treatment of IPF patients with pirfenidone is being perceived as a standard-of-care approach to disease management. Although many non-IPF forms of ILD can also lead to loss of lung function due to progressive fibrosis, there has been little clinical research to establish a potential role for antifibrotic agents in non-IPF ILD.

CONCLUSION

Pirfenidone, as compared with placebo, reduced disease progression, as reflected by lung function, exercise tolerance, and progression-free survival, in patients with idiopathic pulmonary fibrosis. Treatment was associated with an acceptable side-effect profile and fewer deaths. Idiopathic pulmonary fibrosis is a chronic, progressive, and fatal lung disease that is characterized by irreversible loss of lung function.¹ Although periods of transient clinical stability may be observed, continued progression of the disease is inevitable.² The prognosis is poor, with a 5-year survival rate that is similar to the rates for several cancers

BIBLIOGRAPHY

1. Ahn K. The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB Rep.* 2017;50:111–116. doi: 10.5483/BMBRep.2017.50.3.221. Dushenkov V., Graf B.L., Lila M.A. Botanical therapeutics in the modern world. *CUNY Acad. Works.* 2016;43:50–54.
2. Atanasov A.G., Zotchev S.B., Dirsch V.M., Supuran C.T. Natural products in drug discovery: Advances and opportunities. *Nat. Rev. Drug Discov.* 2021;20:200–216. doi: 10.1038/s41573-020-00114-z5. Azwanida N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med. Aromat Plants.* 2015;4:2167-0412.
3. Zhang T., Bai G., Han Y., Xu J., Gong S., Li Y., Zhang H., Liu C. The method of quality marker research and quality evaluation of traditional Chinese medicine based on drug properties and effect characteristics. *Phytomedicine.* 2018;44:204–211. doi: 10.1016/j.phymed.2018.02.009.
4. Sousa L.V.E., Gonçalves R., Menezes J.C., Ramos A. Analytical method lifecycle management in pharmaceutical industry: A review. *AAPS Pharm SciTech.* 2021;22:128. doi: 10.1208/s12249-021-01960-9.
5. Deidda R., Orlandini S., Hubert P., Hubert C. Risk-based approach for method development in pharmaceutical quality control context: A critical review. *J. Pharm. Biomed. Anal.* 2018;161:110–121. doi: 10.1016/j.jpba.2018.07.050. Jayagopal B., Shivashankar M. Analytical quality by design—A legitimate paradigm for pharmaceutical analytical method development and validation. [(accessed on 27 September 2022)]; *Mech. Mater. Sci. Eng. J.* 2017.
7. Rignall A. Analytical Procedure Lifecycle Management: Current Status and Opportunities. *Pharm. Technol.* 2018;42:18–23.
8. European Medicines Agency ICH Guideline Q9(R1) on Quality Risk Management. Step 2b, Amsterdam, The Netherlands. 2021.
9. Ramalinagm P., Shakir Basha S., Bhadraraya K., Beg S. Handbook of Analytical Quality by Design. Elsevier; Amsterdam, The Netherlands: 2021. Risk assessment and design space consideration in analytical quality by design; pp. 167–189.
10. United States Pharmacopeia . General Chapter, <1220> Analytical Procedure Lifecycle USP-NF. United States Pharmacopeia; Rockville, MD, USA: 2022
11. Elder D., Borman P. Improving Analytical Method Reliability Across the Entire Product Lifecycle Using QbD Approaches. *Pharmaceutical Outsourcing.* 2013. [(accessed on 7 August 2013)].
12. Hubert C., Lebrun P., Houari S., Ziemons E., Rozet E., Hubert P. Improvement of a stability-indicating method by Quality-by-Design versus Quality-by-Testing: A case of a learning process. *J. Pharm. Biomed. Anal.* 2014;88:401–409. doi: 10.1016/j.jpba.2013.09.026. [Tome T., Žigart N., Časar Z., Obreza A. Development and optimization of liquid chromatography analytical methods by using AQbD principles: Overview and recent advances. *Org. Process Res. Dev.* 2019;23:1784–1802. doi: 10.1021/acs.oprd.9b00238.
13. Wu C., Lee S.-L., Taylor C., Li J., Chan Y.-M., Agarwal R., Temple R., Throckmorton D., Tyner K. Scientific and regulatory approach to botanical drug development: A US FDA perspective. *J. Nat. Prod.* 2020;83:552–562. doi: 10.1021/acs.jnatprod.9b00949.
14. Das P., Maity A. Analytical quality by design (AQbD): A new horizon for robust analytics in pharmaceutical process and automation. *Int. J. Pharm. Drug Anal.* 2017;5:324–337.

15. European Medicines Agency ICH Guideline Q14 on Analytical Procedure Development Step 2b. Amsterdam, the Netherlands. 2022. [(accessed on 31 March 2022)]. .
16. Mishra V., Thakur S., Patil A., Shukla A. Quality by design (QbD) approaches in current pharmaceutical set-up. *Expert Opin. Drug Deliv.* 2018;15:737–758. doi: 10.1080/17425247.2018.1504768. Jain S. Quality by design (QBD): A comprehensive understanding of implementation and challenges in pharmaceuticals development. *Int. J. Pharm. Pharm. Sci.* 2014;6:29–35.