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ORIGINAL ARTICLE **OPEN ACCESS**

Pirfenidone: A Covid-19 Anti-Fibrotic Agent Quantification by Sensitive Analytical Techniques

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ABSTARCT

Pirfenidone is an anti-fibrotic (idiopathic pulmonary fibrosis) drug which is currently used to treat Covid-19 for curing lung infections. It works effectively by reducing lung fibrosis through down regulation of the production of growth factors and procollagens I and II. It was first approved by japan for the treatment of idiopathic fibrosis after clinical trials, in 2008. In the view of scarcity of research work related to this drug and the current scenario of Covid-19 virus on human lungs, we decided to work on pirfenidone which will help for the simple estimation. In the current paper specific methods were developed for quantification of pirfenidone by UV, HPLC and HPTLC. Ultra-Violet spectroscopy detection and quantification was done by using HPLC grade water as solvent. Linearity was constructed for the concentration range of 3-15µL for UV spectroscopy, 2-10 µg/ml for HPLC using methanol as diluent and 5-25µg/ml using methanol as diluent for HPTLC. The chromatographic system comprised of Shimadzu Prominance-i, LC-2030C HPLC system equipped with quaternary gradient pump and Shim-Pack GIST C18 (250X 4.6 mm, 5µm) column with PDA detector monitored at 310nm. HPTLC was performed on silica gel 60 F_{254} plates using mobile phase in the ratio of toluene and methanol 8:2 v/v. Analytical method validation was done according to ICH Q2 (R1) guidelines. System suitability, intraday precision and inter day precision calculations were performed and reported which were found to be within limits (%RSD<2%). The recovery studies were performed and amount recovered is found between 98.20-102.20%.

Keywords: *Pirfenidone, idiopathic pulmonary fibrosis, UV-spectroscopy, HPTLC, anti-fibrotic, ICH.*

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INTRODUCTION

Pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone), is a novel anti-fibrotic agent. It is first approved in Japan for the treatment of patients with idiopathic pulmonary fibrosis (IPF) in 2008. It was approved for use in the European Union in 2011, in Canada in 2012 and in the United States in october 2014. Pirfenidone could inhibit apoptosis, down regulate ACE receptors expression, decrease inflammation by several mechanisms and ameliorate oxidative stress and hence protect pneumocytes and other cells from COVID-19 invasion and cytokine storm simultaneously (1-3). Based on the pirfenidone mechanism of action and the known pathophysiology of COVID-19 it is confirmed that it has the potential for the treatment of COVID-19 patients. 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 USA. In the present paper we are reporting the best method for the quantification of pirfenidone by UV, HPLC and HPTLC (4-6).

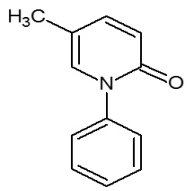

Figure 1: Chemical structure of pirfenidone

MATERIAL AND METHODS

Chemicals and Materials

HPLC grade water, HPLC grade acetonitrile, methanol, toluene, pirfenidone active pharmaceutical ingredient, pirfenidone tablets (FVP 200mg).

Instrumentation

Double beam Lab India made UV- spectrophotometric system was used which is equipped with UV-Win 5 software using 10 mm quartz cells with 1cm light path. The chromatographic HPLC system comprised of Shimadzu Prominance-i, LC-2030C equipped with quaternary gradient pump and shim-pack GIST C18 (250X 4.6 mm, 5µm) column along with PDA detector. The mobile phase consisted of HPLC grade water and acetonitrile in the ratio of (75:25 v/v) and pre-filtered samples were injected in to the column (automatic injector). HPTLC instrument consists of Aetron with sprayline software, twin trough glass chamber (10 cm × 10 cm) for TLC development and hamilton syringe (100 µL) for sample application where mixture of toluene- methanol (8:2 v/v) was used as mobile phase for the elution of TLC plate.

Preparation of standard stock solution:

Ten microgram of pirfenidone was weighed and transferred into 100 ml of volumetric flask and dissolved in 50 ml of methanol. The resultant solution was vertexed, sonicated, filtered and then made up to the mark with HPLC grade water to obtain 100 µg/ml solutions. From the stock solution further dilutions were made by using mobile phase and solvent to get the concentration solutions from 2-10 µg/ml for HPLC and 3-15 µg/ml for UV-spectrophotometric method and 5-24 µg/ml respectively for HPTLC method.

Preparation of sample stock solution:

Ten tablets of Pirfenidone were taken and crushed to make fine powder. Powder weight equivalent to 100 mg was taken into 100 ml of volumetric flask. To that 50 ml of methanol was added to dissolve the sample. The flask was kept in water bath shaker for 30 minutes to dissolve the sample along with excipients and made up to the mark using HPLC grade water. The resulting solution was filtered through 0.45µm nylon filter paper. Then the solution was kept for the sonication for 20 min for degassing purpose. The aliquots of solution was accurately diluted to get the concentrations of 2-10 µg/ml for HPLC and 3-15 µg/ml for UV-Spectrophotometric method and 5-24 µg/ml respectively for HPTLC method.

Development of spectrometric and chromatographic methods

UV-Spectrometric, HPLC and HPTLC methods were developed and validated in the present work. λ_{max} was determined as 310 nm by using water as solvent in UV-spectroscopy and validation was done for the concentrations of 3-15 µg/ml. Optimized HPLC conditions were fixed for a mixture of water and acetonitrile as mobile phase at 310 nm by using Shimadzu Prominance-i, LC-2030C. In present study HPLC system used was equipped with quaternary gradient pump and shim-pack GIST C18 (250X 4.6 mm, 5µm) column at 1.0 ml/min flow rate for 10 minutes run time by monitoring PDA detector at 310 nm. The linearity was constructed for 2-10 µg/ml concentrations respectively. HPTLC method was developed on Aetron system with spraylin TLC applicator software and the plates were analyzed by utilizing JUST TLC software. Twin trough glass chamber (10 cm × 10 cm) was used for TLC plate development and Hamilton Syringe (100 µL) for application of sample by using the mobile phase consisting of toluene- methanol (8:2 v/v). TLC plates used were silica gel 60 F_{254} plates. 8mm band was applied with 7mm band space in ascending order application at a rate of 5 sec μl^{-1}. 100 µL hamilton syringe was used for the purpose of sample application.

METHODOLOGY

Analytical method validation was performed according to ICH Q2 (R1) guidelines for the parameters of specificity, system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness (7-13).

Specificity:

Specificity is the ability of the analytical method to produce a response for the analyte in the presence of other components present in the solution; technically they can be like impurities, degradants or matrix. In this method the specificity is tested for the standard solution and blank and found no interference in the blank injection. Tailing factor and theoretical plates were taken into consideration.

System suitability:

System suitability was performed for the standard solution and confirmed the method suitability by taking tailing factor, theoretical plates, % RSD and retention time parameters into the consideration.

Linearity:

The linearity is validation parameter which confirms the ability of a method (within a given range) to obtain test results which will be directly proportional to the concentration of analyte in the sample. By giving different concentrations of sample solutions the regression value should be 0.999.

Limit of Detection (LOD):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The values were determined by calculating from slope and regression line by following the equation.

$$LOD = 3.3^* \sigma / S$$

Limit of Quantification (LOQ):

LOQ is the parameter which will explain about the detection and quantification of lowest amount. In the method the values of LOQ was determined from the following formula.

$$LOQ = 10^* \sigma / S$$

Precision:

Precision is an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogeneous sample under prescribed conditions. In the current study the % RSD for the sample solution was found below <2.0.

Accuracy:

Accuracy can be defined as the closeness of agreement between accepted reference value and the value found. In this study recovery was calculated by standard weighing method for 50%, 100% and 150%.

Robustness:

A robustness method was performed to confirm whether the method is capable of reproducibility during the deliberate changes taken place in the proposed method.

RESULTS AND DISCUSSION

The proposed methods by UV, HPLC and HPTLC were validated as per International Conference on Harmonization ICH Q2 (R1) guidelines for the parameters of system suitability, specificity, precision, accuracy and linearity and robustness. The linear concentration range for UV-spectroscopy was found between 3-15µg/ml, 2-10µg/ml for HPLC and 500-2500 ng/ml for HPTLC. System suitability, intraday precision and inter day precision calculations were performed and reported in table no.1 which were found to be within limits (%RSD<2%). The recovery studies were performed and amount recovered is found between 98.20-102.20%. It is confirmed that the proposed method is more sensitive, precise, accurate, cost effective and rapid one. Sensitivity of detection and solvents along with mobile phases used in the current study is proving that the proposed method is highly sensitive and suitable for commercial estimation of pirfenidone. All the validated parameters were showing best results, within acceptable limits and were procured in tables given.

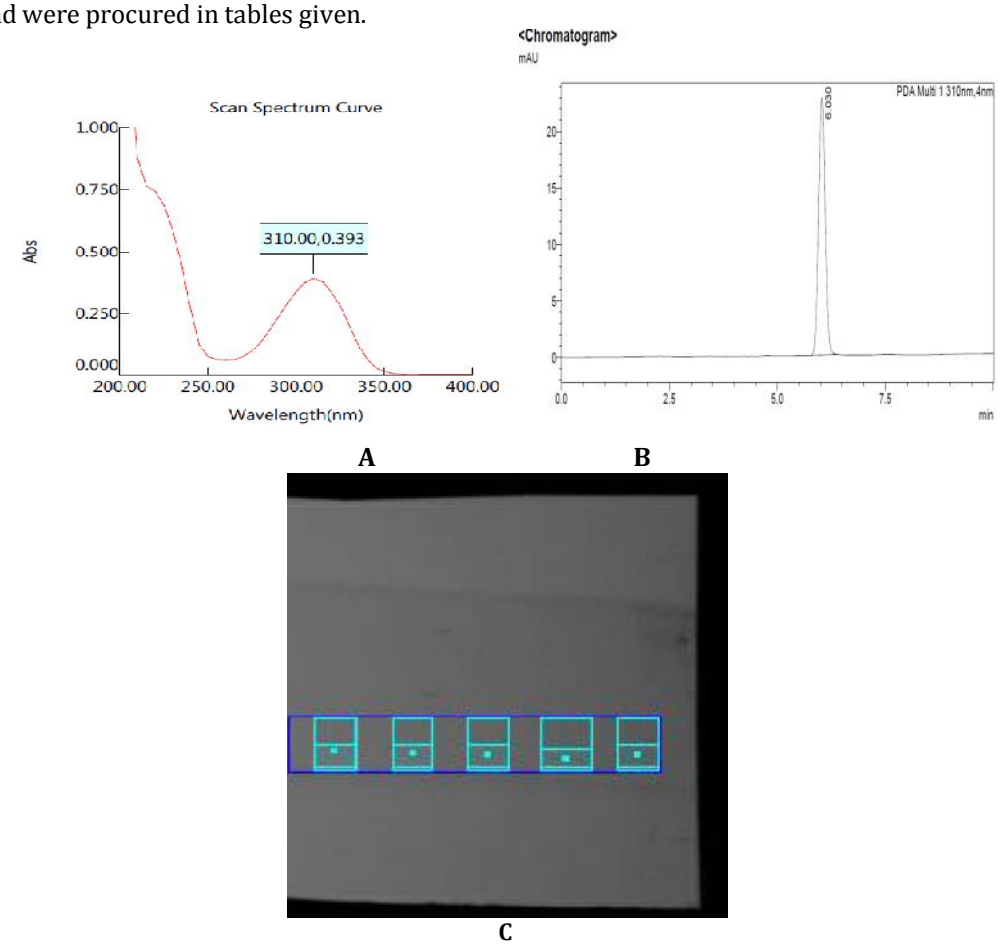

Figure 2: (A) UV spectra of pirfenidone; (B) Chromatogram of pirfenidone (C) Densitogram of pirfenidone

Table 2: Recovery studies for UV, HPLC and HPTLC method.

Method	Amount taken (µg/mL)	Amount added (µg/mL)	%Recovery
UV	9	6	100.07
		9	99.91
		12	99.47
HPLC	6	4	100.46
		6	101.24
		8	100.96
HPTLC	15	10	100.12
		15	99.96
		20	100.36

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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