



Research Article

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Assay of Pirfenidone by UV Spectrophotometry

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ABSTRACT

Pirfenidone is an Anti-fibrotic agent, effective in models of Pulmonary and lung fibrosis. It is available as oral and topical preparations. In this study, a rapid, reliable and simple assay method has been developed by using UV – Spectrophotometer. The assay of pirfenidone is based on measuring the absorbance at 220.80 nm wavelength. Pirfenex (A), Pirfetab(B), Fibridone (C) are the three different brands obtained from market for analysis. The concentration range was from 50 -3.125 ppm i.e. solutions of 50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm, 3.125 ppm of each brands were prepared and analysed. The % Assay is calculated and the squared correlation co- efficient for different brands were found to be 0.999 which are with in limit. Hence we can conclude that the method can be applied for fast, routine Quality control quantitative analysis of Pirfenidone formulation. The pirfenex(A) and fibridone(C) showed nearly same % Assay values of 101.26%, 101.00 %. The other brand i.e. pirfetab (B) showed its % Assay value as 100.18 %. All the brands % Assay values were found to be with in limit as per Indian Pharmacopeia.

Key words: Pirfenidone Assay, UV – Spectrophotometry, Pirfenex (A), Pirfetab (B) and Fibridone (C).

INTRODUCTION

The IUPAC name of Pirfenidone is 5-methyl-1-phenyl-2(1H)-pyridinone. Pirfenidone has well established Anti – fibrotic and Anti – inflammatory properties in various Invitro systems and animal models of fibrosis [1]. A number of cell based studies has shown that pirfenidone reduces fibroblast proliferations [2][3][4][5] inhibits TGF – β – Stimulated collagen production [6][7][8] and reduces the production of fibrogenic mediators such as TGF – β . Pirfenidone has also been shown to reduce production of inflammatory mediators such as TNF – α and IL - 1 β in both cultured cells and peripheral blood mono nuclear cells[8][9]. These activities are consistent with the broader Anti – fibrotic and Anti – inflammatory activities observed in animal models of fibrosis. It is a drug developed by several companies world-wide. In 2008, it was first approved in Japan for treatment of IPF after clinical trials, under trade name of Pirepsa by Shionogi & co. In October 2010, the Indian company Cipla launched it as Pirfenex. In 2011, it was approved for use in Europe, Canada (2012), United States (2014) under the same trade name Esbriet [10]. In 2014, it was approved in Mexico under trade name Ki – toscell LP indicated for pulmonary fibrosis and Liver fibrosis [11]. Literature survey reveals the determination of pirfenidone by RP – HPLC method as well as by combining RP – HPLC and UV – Spectrophotometry in pharmaceutical formulations [12][13][14]. The other method includes determination of pirfenidone and its acid metabolite by improved LC method [15]. The pharmacokinetic studies of pirfenidone in Rat plasma were carried out using LC – MS – MS method [16]. Compared to UV Spectrophotometry, HPLC, LC, LC – MS – MS methods are tedious and selective. They require expensive detectors and are also difficult to perform that might not be accessible in many laboratories. Hence the UV method is inherent, simple and very economical and can be considered as more convenient alteration technique. In this method cost free solvent water is being used for entire analysis.

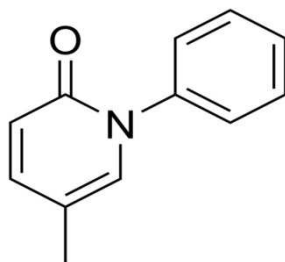


Figure 1: Molecular Structure of Pirfenidone (C₁₂H₁₁NO)

EXPERIMENTAL SECTION

Chemicals and reagents

The reference standard of pirfenidone was obtained from a reputed firm with certificate of analysis. The Brands of pirfenidone (A, B, C) were purchased from local pharmacy. The standard and samples were prepared using demineralised water.

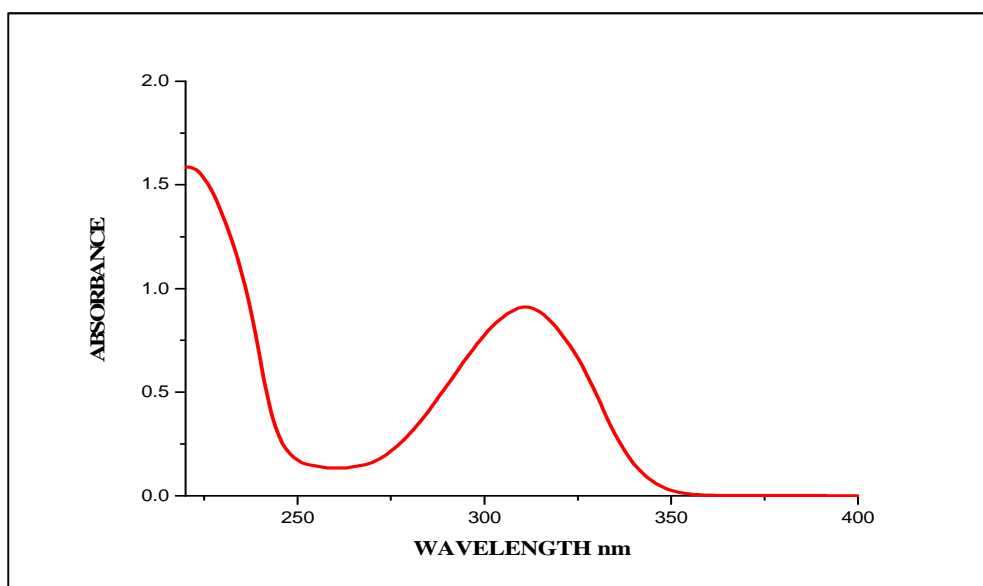
Instrumentation

A double beam UV – Spectrophotometer used was SHIMADZU UV – 2600. For weighing SHIMADZU AUX – 220 analytical balance was used and for Sonication of standard and sample solutions a digital Ultra sonicator was being used.

Selection of wavelength

About 50 ppm of Pirfenidone solution was accurately prepared in water. These solutions were scanned in the range of 200 – 400 nm UV regions. The wavelength of maximum absorbance was observed at 220.80 nm and this wavelength was adopted for absorbance measurement. The figure 2 represents the UV Spectrum of Pirfenidone.

Figure 2: UV Spectrum of Pirfenidone



Preparation of standard stock solution

Accurately weigh 5mg of standard and transfer it into a 100 ml beaker and add 20 ml of water. The standard solution is sonicated for five minutes to achieve good solubility of standard. The sonicated solution is then transferred to a 100 ml volumetric flask and then made up to mark with water.

Preparation of sample solution

The three different brands were obtained from a local pharmacy in trichy tamilnadu. The all the tablets of three brands i.e. A, B, C were labelled to contain pirfenidone 200 mg per tablet and has a shelf life of two years. 20 tablets each of three different brands of pirfenidone from marketed samples were accurately weighed and crushed uniformly with the help of mortar and pestle. By calculating the average weight equivalent to 5 mg of each brand of pirfenidone was transferred into three 100 ml volumetric flasks containing 10ml of water. The solutions were

sonicated for about 5 – 7 minutes and then filtered using Whatmann No 1 filter paper. The first 5 ml of solution is discarded and remaining amount of filtrate of three solutions was made up to mark with water.

Dilutions preparation

The dilutions of different brands of pirfenidone i.e. A, B, C were prepared from sample solution of each brand. Four different dilutions of 25 ppm, 12.5 ppm, 6.25 ppm, 3.125 ppm of each brand were prepared from 50 ppm sample solutions.

PROCEDURE

After preparation of standard and tablet solutions, strength of solution 50 ppm in 100ml, Absorbance of sample preparation, standard preparation and different dilutions (50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm, 3.125 ppm all in 100ml) in 1 cm cell at wavelength of maximum absorbance i.e. 220.80 nm using the blank solution was measured .calculate the quantity in mg of pirfenidone per tablet.

RESULTS AND DISCUSSION

The main purpose of study was to carryout easy, fast, less time consuming, cost – effective and accurate pharmaceutical assay for determination of pirfenidone in available brands. Among the three brands the brand A % Assay was found to be 101.26 %, brand B % Assay was found to be 100.18 % and the brand C was found to be 101.00 %. This method can be easily applied for regular analysis of pirfenidone. The method showed good linearity in the concentration range of 50 ppm – 3.125 ppm for all the three brands. The squared correlation co-efficient of three brands was found to be similar i.e. 0.999 and was with in specified limit. The table 1 shows Absorbance of three different brands. The table 2 shows % Assays of the three different brands and the table 3 represents the regression equation and squared correlation coefficient values. The absorbance was taken to calculate the % Assay, Regression equation to obtain regression line and to estimate further availability of drug. From figures 3, 4, 5 it is evident that the concentration is directly proportional to absorbance and the Beers – Lambert law is obeyed. Figure 6 depicts the bar graph for % assay of different brands. Thus the performed assay is beneficial to druggist, Pharmacists and health care professionals. The assay and linearity are with in Quality control range. As per I.P Pirfenidone tablets should contain not less than 98 % and not more than 105 %. The technique is well employed for analysis of three brands. All the results were analysed by using ORIGIN software.

Table 1: Absorbance of Pirfenidone

CONCENTRATION (ppm)	A	B	C
50	1.607	1.59	1.603
25	0.774	0.76	0.756
12.5	0.381	0.385	0.338
6.25	0.157	0.192	0.167
3.125	0.063	0.103	0.062

Table 2: % Assay of different brands

BRANDS	% ASSAY
A	101.26
B	100.18
C	101

Table 3: Regression Equation and Squared correlation co-efficient

Brands	R ²	Regression Equation
A	0.999	y = 0.0329x - 0.041
B	0.999	y = 0.031x - 0.009
C	0.999	y = 0.032x - 0.052

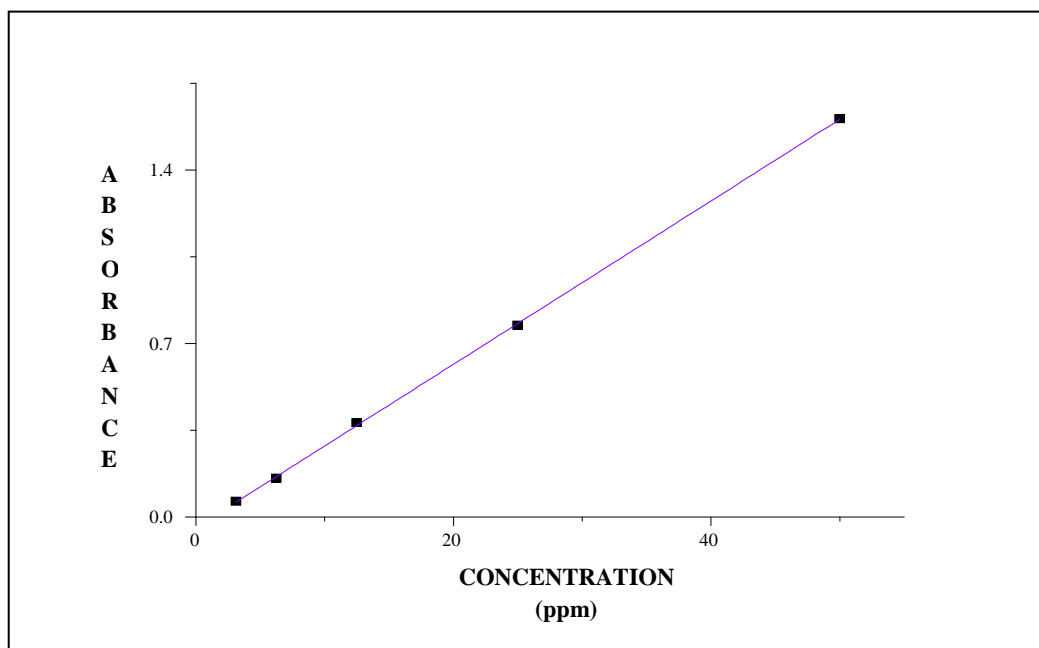


Figure 3: Linearity plot of A

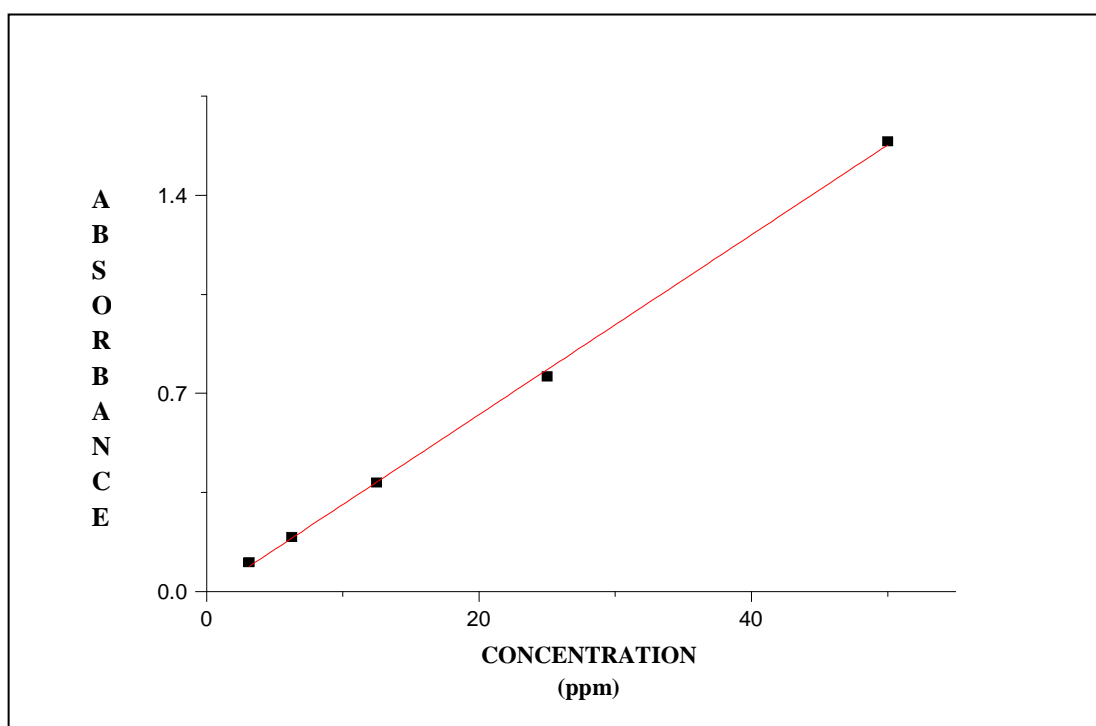


Figure 4: Linearity plot of B

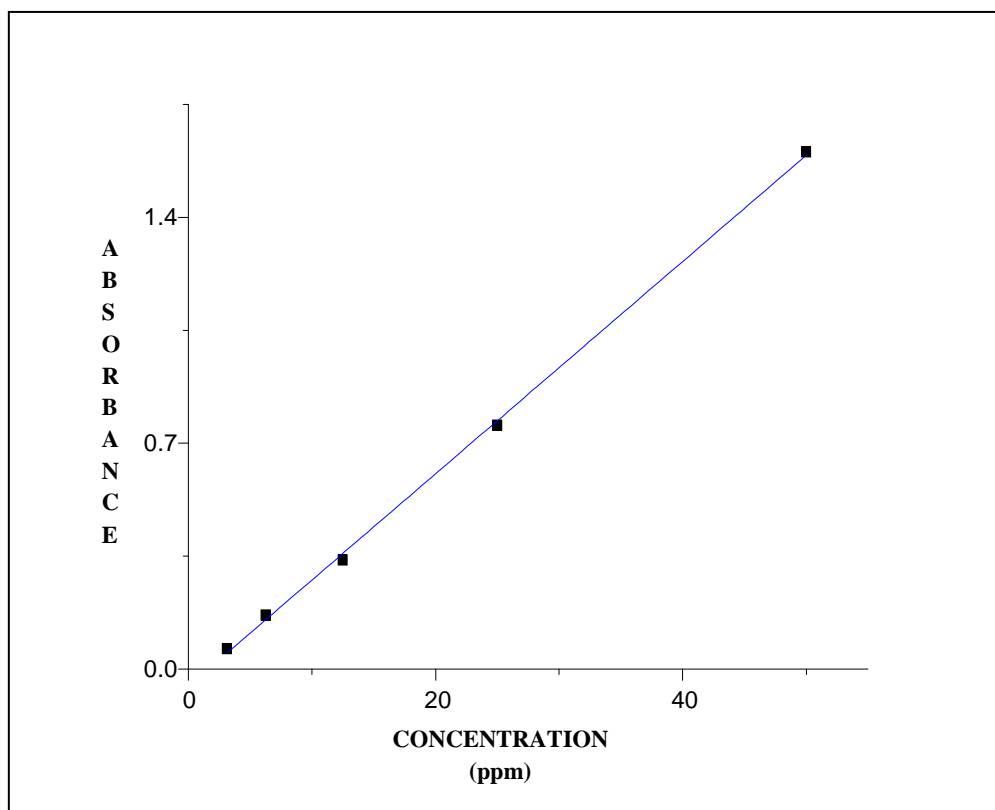


Figure 5: Linearity plot of C

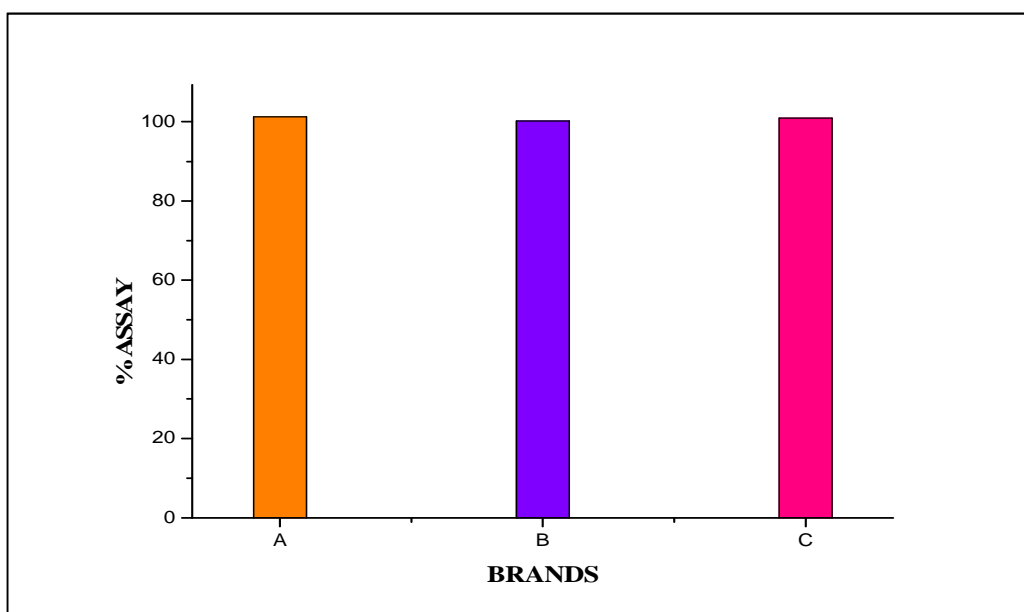


Figure 6: Graph for %Assay of different brands

CONCLUSION

It is concluded from above results and discussion that all the available brands of Pirfenidone are having results of assay and linearity within the specified quality control range. This method is simple, accurate and economical. The correlation coefficient value is also well within limit for all the three brands of Pirfenidone.

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