

## Analytical techniques for Pirfenidone and Terizidone: A review

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### Abstract

Pirfenidone is a broad-spectrum antifibrotic medicine. In 2014, it was approved for the treatment of the idiopathic pulmonary fibrosis (IPF) by the U.S. Food and Drug Administration. Terizidone is WHO categorized group IV anti TB drug. It is an antibiotic effective against *Mycobacterium tuberculosis* and also *M. avium* for the treatment of tuberculosis, both pulmonary and extra pulmonary. The fundamental aim of this article to assemble all reported analytical methods for qualitative and quantitative analysis of Pirfenidone and Terizidone in pharmaceutical formulation as well as in biological fluids. It consist of Spectroscopy, high performance liquid chromatography, High performance thin layer chromatography.

**Keywords:** Pirfenidone, Terizidone, Antifibrotic, Idiopathic pulmonary fibrosis, Analytical method.

### Introduction

Pirfenidone (5-methyl-N-phenyl-2-(1H)-pyridone, PFD) is a broad-spectrum antifibrotic medicine. In 2014, it was approved for the treatment of the idiopathic pulmonary fibrosis (IPF) by U.S. Food and Drug Administration.<sup>1</sup> PFD effect in organs such as lung, liver, and kidney. In a recent phase III multi-national clinical trial, pirfenidone has been shown to have beneficial effects for patients with various stages of idiopathic pulmonary fibrosis. It has a favorable benefit-risk profile and is generally well tolerated. However gastrointestinal events, photosensitivity reactions and rash are the most common adverse events.<sup>2</sup>

Idiopathic pulmonary fibrosis (IPF) is a devastating, progressive fibrotic lung disease with a median survival of 3–5yrs without proven effective therapy IPF develops from chronic epithelial cell injury and aberrant activation of progressive fibrosis. Therefore, the therapeutic strategy against IPF has shifted from corticosteroids and/or immune suppressants to antifibrotic agents, as reported in recent clinical trials.<sup>3</sup>

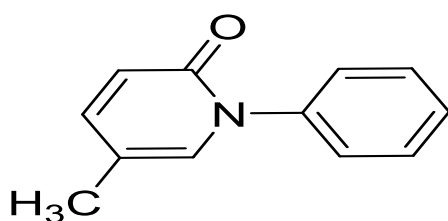
The antifibrotic activity of pirfenidone demonstrated in various organ fibrosis models is mirrored by the demonstration of pirfenidone inhibition of transforming growth factor beta (TGF- $\beta$ ) production. Thus, the inhibition of TGF- $\beta$  production is thought to be crucial to antifibrotic activity. In addition, pirfenidone does not normally inhibit either humoral or cellular immunity which be expected to rarely induce an immunosuppressive condition by its use. This characteristic can be an advantage of the treatment for IPF in clinical practice.<sup>4</sup>

Terizidone is WHO categorized group IV anti TB drug. It is an antibiotic effective against *Mycobacterium tuberculosis* and also *M. avium* for the treatment of tuberculosis, both pulmonary and extra pulmonary. It is classified as a second-line drug, i.e. its use is only considered if one or more first line drugs cannot be used. Terizidone is obtained by combining two molecules of Cycloserine and one molecule of terephthalaldehyde and is a broad spectrum antibiotic which greatly improved the disadvantages associated with Cycloserine.

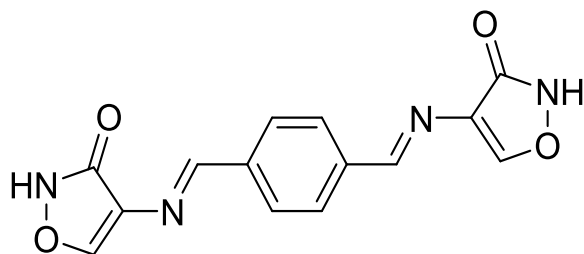
Its mode of action is similar to Cycloserine i.e. It acts by inhibiting cell wall synthesis by competitively inhibiting two enzymes, L-alanine racemase and D-alanine ligase, thereby impairing peptidoglycan formation necessary for bacterial cell wall synthesis.

Terizidone is completely and rapidly absorbed after oral administration. Maximum concentration in blood is achieved in 2 to 4 hrs. It was noted that the blood concentration of Terizidone was higher at all time intervals than the concentration attained in the blood after the same doses of Cycloserine. Terizidone intensifies the activating effect on ascending section of the reticular formation of brainstem and increase in overall reaction of brain but lower than cycloserine. Dizziness, slurred speech, headache and convulsions are amongst the few reported side effects. Others include tremors, insomnia, confusion, depression. The most dangerous side effects is suicidal tendency.<sup>5</sup>

The major goal of this review was to summarize the analytical techniques currently used for the determination of PFD and Terizidone. The primary aim is to emphasizing different methods which are employed for determination of PFD and Terizidone.



Pirfenidone



Terizidone

Qualitative and quantitative analytical techniques for PFD and Terizidone.

### Quantitative Technique Spectroscopy

The use of UV-VIS Spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years. The advantages of these methods are low time and labour consumption. The precision of these methods is also excellent. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.<sup>6</sup>

P. Ravisankar et al. studied the ultraviolet spectroscopic method for the estimation of pirfenidone from tablet formulation using double beam UV spectrophotometer. The lambda max was found to be 315 nm and obeys Beer's law in the concentration range of 2-10 µg/ml. The LOD and LOQ were found to be 0.00745 and 0.02259 µg/mL. The assay result was found to be 99.983±0.775.<sup>7</sup> Keshu Desai et al. Analyzed spectroscopic method for estimation of pirfenidone in tablet dosage form. This method was performed at 311 nm over the concentration range of 16-32 µg/mL and all the parameter was validated as per ICH.<sup>8</sup>

S. G. Thorat et al. studied the UV method for determination of pirfenidone. The UV spectroscopic method was performed at 311 nm using methanol. The linearity range was 10-60 µg/mL.<sup>9</sup> In the same way, K. Parmar et al performed the UV method for determination of Pirfenidone. This analysis was carried out at 317 nm methanol as solvent. The linearity range was 3-25 µg/mL. The method were accurate and precise with recoveries in the range of 98 and 102 % and relative standard deviation <2 %.<sup>10</sup>

Hemant K. Jain et al. developed two Spectrophotometric methods for estimation of Terizidone. The first method was based on AUC at the wavelength between 268-278 nm and second method was based on involved first order derivative spectrophotometry at wavelength 297 nm. 0.1 N NaOH was solvent for both the methods. The linearity for first method was in the concentration range from 4-12 µg/mL and for second method was 4-12 µg/mL. The accuracy of both methods was analyzed by recovery studies and % recovery values were found within acceptance criteria. Thus, proposed methods can be applied for routine analysis of Terizidone.<sup>11</sup>

Saurabh K. Khairnar et al. establish Spectroscopic method where as Terizidone was scanned in the range from

(400-200 nm) in 1 cm quartz in double beam UV spectrophotometer. The maximum wavelength was 273 nm. The linearity ranges was over the concentration of 4-12 µg/mL. LOD and LOQ was about 0.09741 mg/mL and 0.2937 mg/mL. The method was validated for several parameters like accuracy, precision as per ICH guidelines.<sup>12</sup>

### HPLC

The specificity of the HPLC method is excellent and simultaneously sufficient precision is also attainable. However, it has to be stated that the astonishing Specificity, Precision and accuracy are attainable only if wide-ranging system suitability tests are carried before the HPLC analysis. For the reason the expense to be paid for the high specificity, precision and accuracy is also high.<sup>13</sup>

P. Ravisankar et al. studied the separation of pirfenidone in tablet dosage form. This method was developed by using Welch C18 isocratic column (250 mm x 4.6 mm, 5 µm) with Shimadzu LC-20AT. The mobile phase was a mixture of acetonitrile: water (50:50 v/v) with apparent pH of 3.3. The flow rate was set at 1.0 mL/min and detection was carried out at 315 nm by using UV-spectro photometric detection. Linearity was established for Pirfenidone in the range of 2-10 µg/mL. The retention time of Pirfenidone peak was found at 3.863 minutes.<sup>14</sup> Ravichandra Babur et al. established RP-HPLC method for evaluation and quantification of impurities present in pirfenidone (PFD) drug substance. Separation was carried out with RX-C18 column (250 mm length, 4.6 mm inner diameter and 5.0 µm particle size, octadecylsilane chemically bonded to porous silica) by using 0.02 M KH<sub>2</sub>PO<sub>4</sub> buffer and acetonitrile as mobile phase using a simple gradient elution program. The flow rate was 1.0 mL/min and detection was carried out at 220 nm. The impurities were identified as 2-hydroxy-5-methylpyridine and Iodobenzene. The linearity range obtained was 0.017 to 0.380 µg/mL for 2-hydroxy-5-methylpyridine, 0.047 to 0.382 µg/mL for Pirfenidone and 0.030 to 0.99 µg/mL for Iodobenzene with the retention times of 3.248 min, 10.608 min and 24.241 min for 2-hydroxy-5-methylpyridine, Pirfenidone and Iodobenzene. The LOD and LOQ values were found to be 0.000005 mg/mL, 0.000017 mg/mL for 2-hydroxy-5-methylpyridine and 0.009 µg/mL, 0.030 µg/mL for Iodobenzene.<sup>15</sup>

Keshu Desai et al. studied the HPLC method where as the separation were carried out by Hypersil column with the methanol: water (80:20% v/v) as mobile phase. The detection was carried out 311 nm. Flow rate was 1 ml/min. Retention time was 3.952 min. Linearity was obtained in the concentration range of 2-12 µg/mL. LOD and LOQ were found to be 0.18.<sup>16</sup>

A Rajasekaran et al. developed RP-HPLC method for determination on pirfenidone in bulk drug. This method was achieved by using C18 column with 0.5% triethylamine as aqueous phase (pH adjusted to 4.5 with orthophosphoric acid) and organic phase as acetonitrile: methanol (90:10 %v/v) as mobile phase (55:45). The flow rate was 1 mL/min and detection was done at 315 nm with PDA detector. The linearity ranges was over the concentration range of 10 to 50

$\mu\text{g/mL}$  of pirfenidone. The retention time was 3.3 min. The correlation coefficient ( $R^2$ ) obtained for pirfenidone was 0.9998. The robustness study was performed by changing the pH, wavelength and flow rate by chemometric method.<sup>17</sup> V. K. Parmar et al. established the method for determination of pirfenidone. Chromatographic separation was carried out on phase C18 Zorbax Eclipse plus column and mobile phase was acetonitrile: water (35:65% v/v) and flow rate was 0.7 mL/min. Linearity range for pirfenidone was 0.2-5.0.<sup>18</sup> S. G. Thorat et al. established HPLC method and chromatographic condition were Eclipse XDB-C18 column (150 x 4.6 mm, 5  $\mu\text{m}$  with mobile phase consisting of phosphate buff: acetonitrile (pH 3.5) (72:28 v/v) at flow rate 1 mL/min. The linearity range for Pirfenidone was 5-70  $\mu\text{g/mL}$  for HPLC. These methods were accurate and precise with recoveries in the range of 98.2-102.32 and relative standard deviation < 2%.<sup>19</sup>

Xiaoquan Liu et al. studied the high-performance liquid chromatographic method for the determination of pirfenidone and its major metabolites in rat plasma. The analysis was carried out on C18 column (250 x 4.6 mm id., 5  $\mu\text{m}$ ) with the mobile phase of acetonitrile: water containing 0.2% acetic acid (23:77% v/v) at flow rate 1 mL/min. The detection was carried out at 310 nm. The calibration curves were linear over a concentration range from 0.15 to 76.67  $\mu\text{g/mL}$ .<sup>20</sup> N. Tamilselvi et al. established the bioanalytical RP-HPLC method for determination of Pirfenidone and its application to the determination of drug food interaction study in wister rats. The separation was achieved on C18 column using the mobile phase in gradient mode constituting of eluant A HPLC grade water (adjusted to pH 3.5) and eluant B 20% acetonitrile and 15% of methanol in the ratio of (60: 40% v/v) at a flow rate 1 mL/min. The separation was detected at 324 nm. The method was good linear between range of 50-250 ng/mL. The LOD and LOQ was 20 ng/mL and 12 ng/mL. The half lives ( $T_{1/2}$ ) of pirfenidone before and after food were calculated and found to be 2.732158 h and 4.009485 h. Area under the curve (AUC) of pirfenidone before food was calculated as 3060.95 ng $\cdot$ h mL<sup>-1</sup> and AUC was found to be 3053.07 ng/mL. Area under the curve AUC of pirfenidone after food was calculated as 2534.16 ng $\cdot$ h/mL and AUC was found to be 2510.64 ng/mL.<sup>21</sup>

S. V. Gandhi et al. studied the stability indicating reverse phase HPLC method for estimation of Terizidone. In

this study separation was carried out by HiQSil C8 column (250 mm x 4.6 mm, 5  $\mu\text{m}$ ) and mobile phase consist of Ammonium Acetate Buffer pH 3 (adjusted with glacial acetic acid) and methanol (60:40% v/v) at flow rate 1 mL/min. The detection was carried out at 264 nm and retention time of drug was 7.3  $\pm$  0.10 min. The linearity range was over the concentration range of 5-30  $\mu\text{g/mL}$ . The forced degradation study also carried out under various degradation conditions like acidic, basic, neutral hydrolysis, oxidation, dry heat and photolysis. The degradation of Terizidone was found under alkaline and oxidative condition.<sup>22</sup> Mwila Mulubwa et al. studied the HPLC-UV method for analysis of Terizidone in plasma. This drug was extracted from plasma by protein precipitation. The chromatographic separation was achieved by on Supelco Discovery HS C-18 (150 mm x 4.6 mm, 5  $\mu\text{m}$ ) reversed-phase column by using mobile phase as acetonitrile: water both containing 0.1% formic acid. Propanolol was used as an internal standard. The linearity range for Terizidone was between 3.125-200  $\mu\text{g/mL}$ .<sup>23</sup>

## Qualitative Techniques

### HPTLC

Thin layer chromatography is a popular technique for the analysis of a wide variety of organic and inorganic materials, because of its distinctive advantages such as minimal sample clean-up, wide choice of mobile phases, flexibility in sample distinction, high sample loading capacity and low cost. TLC is a powerful tool for screening unknown materials in bulk drugs (Szepesi and Nyiredy, 1996). It provides a relatively high degree of assertion that all probable components of the drug are separated. The high specificity of TLC has been exploited to quantitative analytical purpose using spot elution followed by spectrophotometric measurement.<sup>24</sup>

S. G. Thorat et al. established HPTLC method for estimation of pirfenidone. TLC was carried out by stationary phase precoated Silica Gel 60 F254 TLC Plate with Toluene: Methanol (80:20% v/v) as a mobile phase. The linearity range for Pirfenidone was 800-1600 ng/spot. The developed method were successfully applied for determination of Pirfenidone in tablets.<sup>25</sup>

**Table 1:** Representative Spectrophotometric methods for analysis of PFD and Terizidone

S. No	Compound	Method	Solvent	Lambda max	Linearity/LOD	Ref
1	PFD	Spectrophotometric Method	water, Acetonitrile buffer (50:50)	315	2-10 $\mu\text{g/mL}$ 0.00745	[7]
2	PFD	Spectrophotometric method	Methanol	311	16-32 $\mu\text{g/mL}$	[8]
3	PFD	Spectrophotometric method	Methanol	311	10-60 $\mu\text{g/mL}$	[9]
4	PFD	Spectrophotometric method	Methanol	317	3-25 $\mu\text{g/mL}$	[10]
5	Terizidone	Area under curve	0.1N NaOH	268-278	4-12 $\mu\text{g/mL}$	[11]
		First order derivative		297	4-12 $\mu\text{g/mL}$	
6	Terizidone	Spectrophotometric method	Methanol	273	4-12 $\mu\text{g/mL}$	[12]

**Table 2:** Reported analytical HPLC methods for determination of PFD and Terizidone

S. No	Name of drug	Column	Mobile Phase	Detector	Lambda max (nm)	Linearity/Retention time	Flow Rate	Ref
1	PFD	C18	Acetonitril: Water (50:50 v/v)	UV	315	2-10 Åµg/mL 3.863 min	1ml/min	[14]
2	PFD 2-hydroxy-5-methylpyridine. Iodobenzene	RX-C18	0.02 M KH <sub>2</sub> PO <sub>4</sub> buffer and acetonitrile	UV	220	0.017 to 0.380 µg/ml 0.047 to 0.382 µg/mL 3.248 min, 24.241 min	1ml/min	[15]
3	PFD	Hypersil column	Methanol: Water (80:20 v/v)	UV	311	of 2-12 µg/ml 3.952 min	1ml/min	[16]
4	PFD	C18	0.5% triethylamine (pH adjusted to 4.5 with orthophosphoric acid) Acetonitrile: Methanol (90:10)(55:45)	PDA	315	10 to 50 µg/ml 3.3 min	1ml/min	[17]
5	PFD	C18 Zorbax	Acetonitril: Water (35:65% v/v)			0.2-5.0.µg/ml 2.02±0.01 min	0.7ml/min	[18]
6	PFD	Eclipse XDB-C18	Phosphate buff: Acetonitrile (pH 3.5) (72:28 v/v)		311	5-70 µg/mL 5.562 min	1ml/min	[19]
7	Terizidone	HiQSil C8	Ammonium Acetate Buffer pH 3 (adjusted with glacial acetic acid) and methanol (60:40% v/v)	UV	264	5-30 µg/ml 7.3±0.10 min	1 ml/min	[22]

## Conclusion

Pirfenidone is a broad-spectrum antifibrotic medicine. In 2014, it was approved for the treatment of the idiopathic pulmonary fibrosis (IPF) by U.S. Food and Drug Administration. Terizidone is anti TB drug which is an antibiotic effective against Mycobacterium tuberculosis and also M. avium for the treatment of tuberculosis, both pulmonary and extra pulmonary. The published data explains that the HPLC technique is widely used for analysis of Pirfenidone but not more in case of Terizidone. Terizidone was analyzed more by Spectrophotometric technique. Pirfenidone and Terizidone also analyzed by HPTLC. There are various more technique which can be develop for analysis of pirfenidone and Terizidone like Electrophoresis, ELISA, RIA XRD and Hyphenated technique etc.

**Conflict of Interest:** None.

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