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VALIDATION AND DEVELOPMENT FOR THE ESTIMATION OF PIRFENIDONE IN MARKETED FORMULATION BY RP-HPLC

Krishna Satya A[1*], Sunitha N[2]
1.Associate Professor,Department of Biotechnology , Acharya Nagarjuna University, Guntur, Andhra Pradesh
2.Research scholar, Department of Biotechnology , Acharya Nagarjuna University, Guntur, Andhra Pradesh
Address for Correspondence
Dr . A Krishna Satya
Associate Professor Department of Biotechnology Acharya Nagarjuna University Guntur, Andhra Pradesh
Ph No: 9490639577
Mail ID: akrishnasatya78@gmail.com

ABSTRACT:

The RP-HPLC method was proved to be simple, precise accurate and sensitive from the results of the validation and it is suitable method for the estimation of pirfenidone in its pharmaceutical dosage forms.

A RP-HPLC method was developed using the mobile phase of 50:48.5:1.5 %v/v methanol and water and tri etylamine. The run time of the developed method was six minutes, which reduces the solvent usage. The chromatographic conditions use ambient temperature which can yield accurate, precise results in the range of 25-35 ºC. The results of the validation parameters showed that the method is accurate and precise. Finally, it can be concluded that the methods for quantitation of pirfenidone by RP-HPLC Method in its pharmaceutical dosage forms can be applied for the routine analysis because of simplicity, accuracy, and preciseness.

KEY WORDS: Pirfenidone, tri ethylamine, methanol, RP-HPLC

INTRODUCTION:

Pirfenidone is a novelantifibrotic drug approved for mild to moderatye idiopathic pulmonary fibrosis(IPF) 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[1-22].

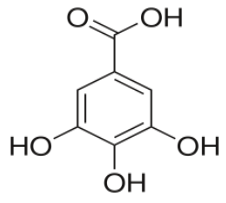

Fig 1: Structure of Pirfenidone

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MATERIALS AND METHODS:

Chemicals and Reagents:

Drug sample:

- Pirfenidone was obtained from Yarrow Chem products, Mumbai, India.

Chemicals:

- HPLC grade methanol-Merck life science Pvt Ltd
- HPLC Water

METHOD DEVELOPMENT

Selection of Mobile Phase: Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard and sample solution was run in different mobile phases. From the study, Methanol was preferred over acetonitrile because it gives sharper peak and less cost than Acetonitrile.From the various mobile phases, methanol : water : Triethylamine (50: 48.5:1.5 %v/v).

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 1000µg/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)

RESULTS AND DISCUSSION

The λmax of pirfenidone was found to be 315 nm

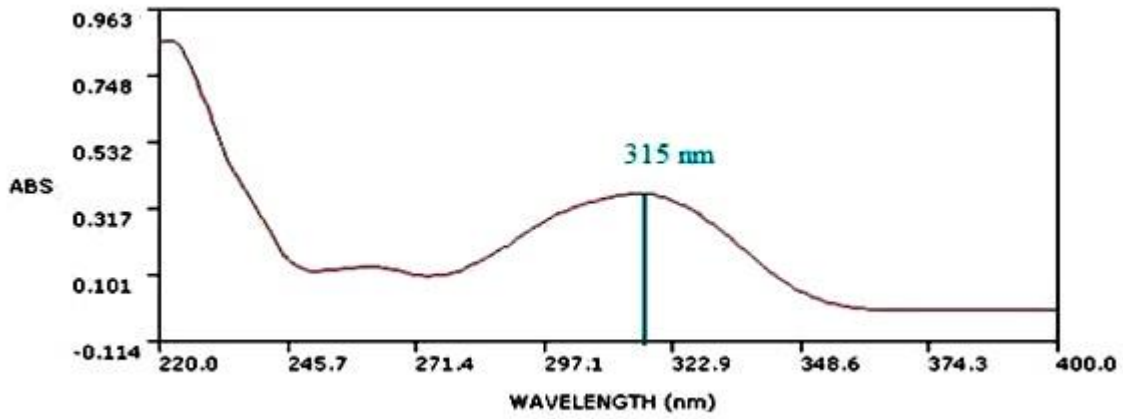

Figure 2.Spectrum of pirfenidone

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Chromatographic conditions:

Table 1: Optimized chromatographic conditions:

loloo	Flowrosil C18 column with 5 µm (Dimension)
Mobile phase	Methanol:water:triethylamine(50:48.5:1.5)%v/v
Flow rate	1ml/min
Temperature	Ambient
Run time	7min
Detection wavelength	315 nm
Injection volume	10µl
Mode of operation	Isocratic elution

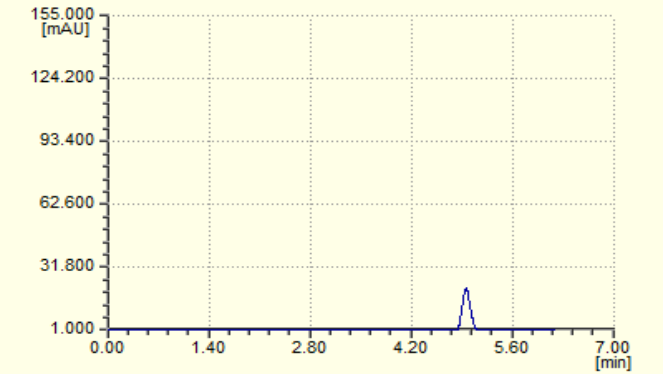

Figure no 3. Optimized Chromatogram for Pirfenidone

Table 2: Chromatogram results for optimized method

Mobile phase	Methanol:water:tri ethylamine(50:48.5:1.5)%v/v)
Stationary phase	Flowrosil C18 column with 5 µm (Dimension)
Wavelength	312nm
Run time	7min
Flow rate	1ml/min
Injection volume	10µg/ml
Mode of operation	Isocratic elution
Temperature	Ambient

Calibration curve

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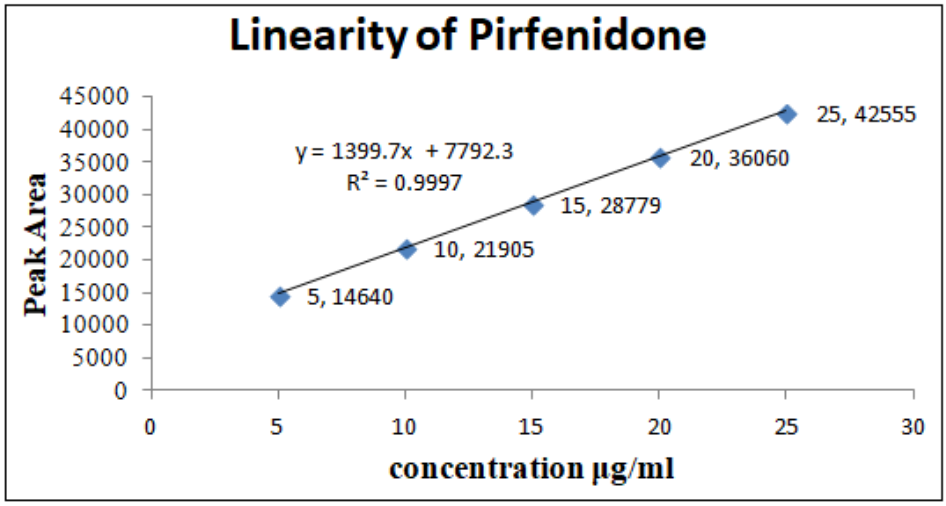

Figure no 4.Calibration curve for Pirfenidone

Assay

Table 3: Assay Results

S.No	Concentration (µg/ml)	Standard peak area (Mean±SD), n=3	Test peak area (Mean±SD), n=3	Amount of Pirfenidone found in mg (n=3)	Mean %recovery of Pirfenidone
1	**20µg/ml**	**36060**	**35560**	**19.848**	**99.24**

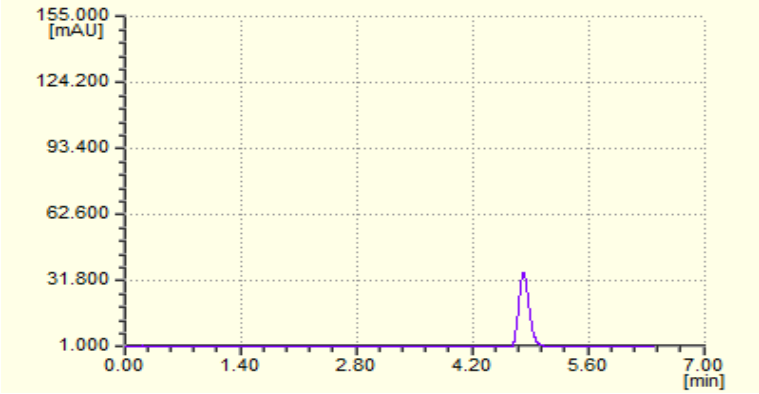

Figure 5: Chromatogram for 20 (µg/ml) Assay

Table 4: Chromatogram results for 20 (µg/ml) Assay

S.No	Drug Name	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	Pirfenidone	4.82	35560	5110.60	1.72

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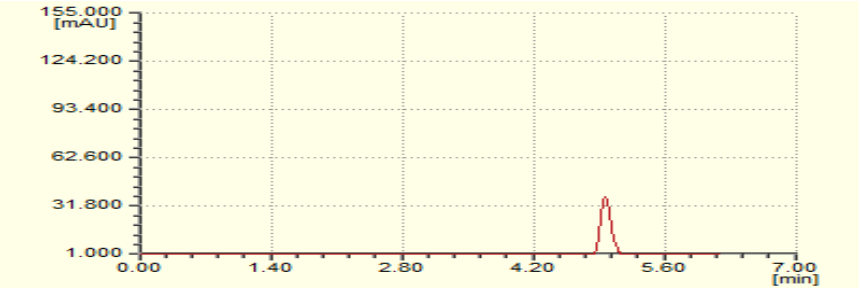

Figure 6: Chromatogram for (20 µg/ml) Standard

Table 5: Chromatogram results for (20µg/ml) standard

S.No	Drug Name	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	Pirfenidone	4.9	36060	5900.78	1.85

Validation

Specificity

Specificity of the HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities, other markers. A volume of 20µL of individual ingredients and other marker solutions were injected and the chromatogram was recorded. Peaks of other marker were not found at retention time of 4.97 min. Hence, the proposed method was specific for Pirfenidone.

Blank:

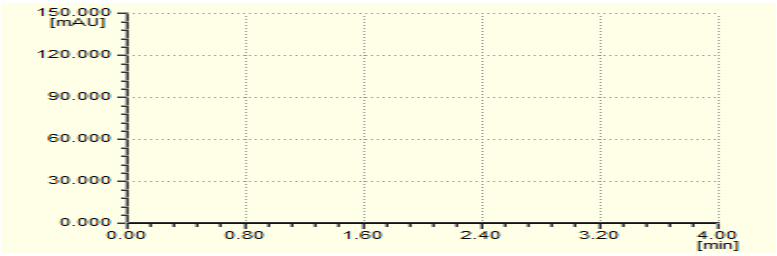

Figure 7.Blank injection chromatogram for specificity

Standard:

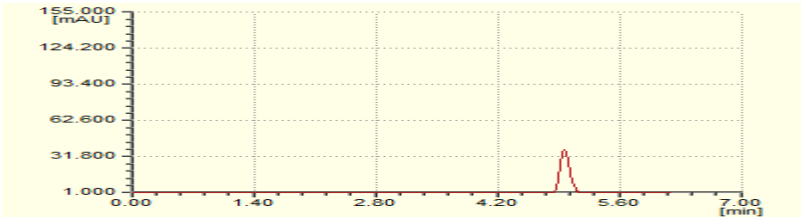

Figure 8.Chromatogram of Standard Pirfenidone

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Sample:

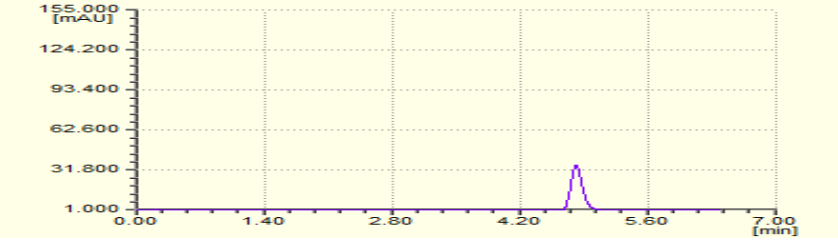

Figure 9.Chromatogram of Test Pirfenidone

Linearity

Linearity for this method was found to be in between 5-25µg/ml

Linearity 5 µg/ml

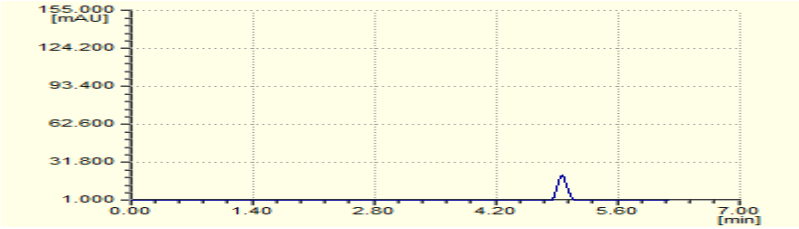

Figure 10: Linearity (5µg/ml) Chromatogram

Linearity 10 µg/ml

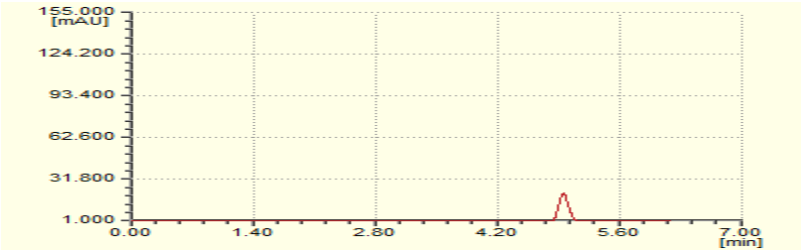

Figure 11: Linearity (10µg/ml) Chromatogram

Linearity 15µg/ml

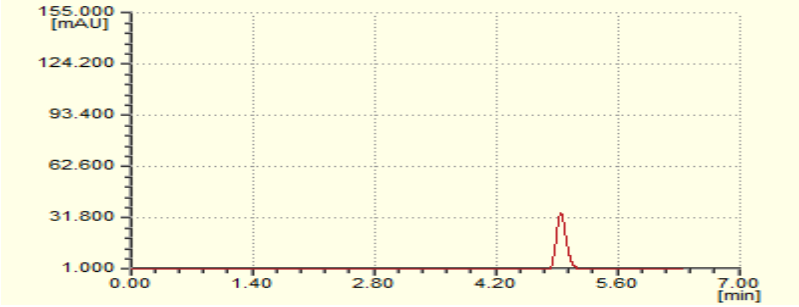

Figure 12: Linearity (15µg/ml) Chromatogram

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Linearity 20 µg/ml

Table 6: Chromatogram results for Linearity (20µg/ml)

S.No	Drug Name	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	Pirfenidone	4.9	36060	5900.78	1.85

Table 7: Chromatogram results for Linearity (25µg/ml)

S.No	Drug Name	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	Pirfenidone	4.9	42555	5566.84	1.78

Accuracy

- **Accuracy 50% Recovery level**

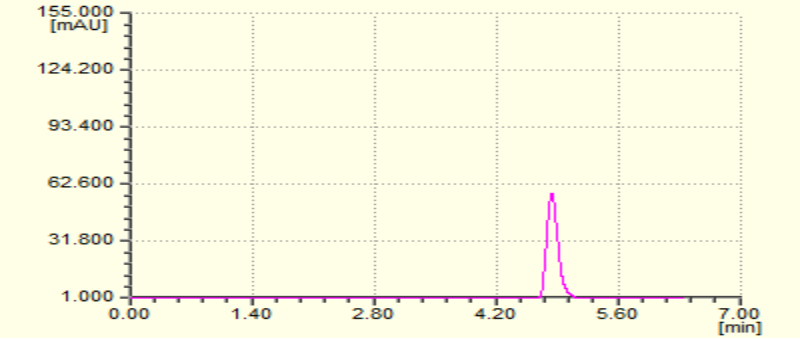

Figure 13: Chromatogram for Accuracy 50% recovery level

- **Accuracy 100% Recovery level**

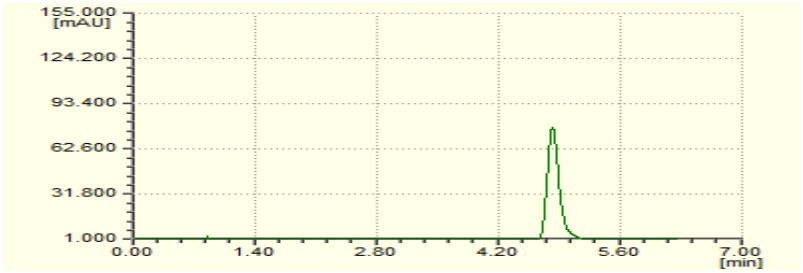

Figure 14: Chromatogram for Accuracy 100% recovery level Table

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❖ **Accuracy 150% Recovery level**

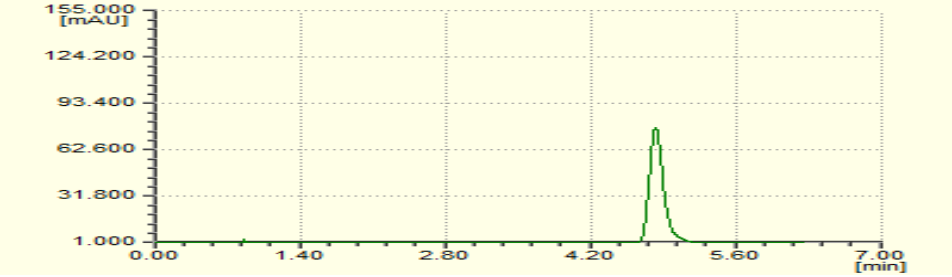

Figure 15 : Chromatogram for Accuracy 150% recovery level

Table 8: Accuracy Results

Recovery Level	Concentration(µg/ml)			Peak Area n=3	%Mean Recovery
	Test (Initial amount)	Standard (Amount added)	Predicted Concentration		
50%	20µg/ml	10µg/ml	30µg/ml	57520	101%
100%	20µg/ml	20µg/ml	40µg/ml	71376	100%
150%	20µg/ml	30µg/ml	50µg/ml	84516	98%

Precision

Precision was done with the 20µg/ml (n=6) concentration.

❖ **Repeatability(Morning)**

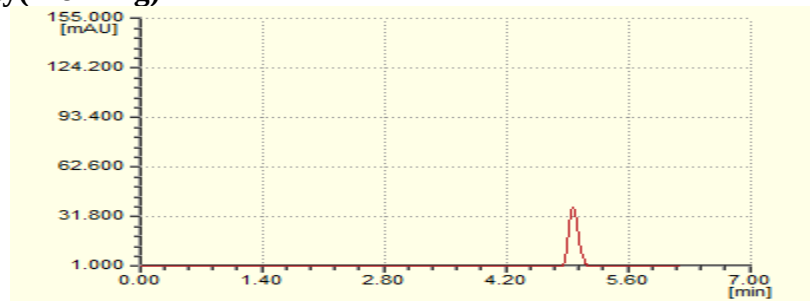

Figure 16: Precision Chromatogram of Standard Pirfenidone at 20 µg/ml

Repeatability (Evening)

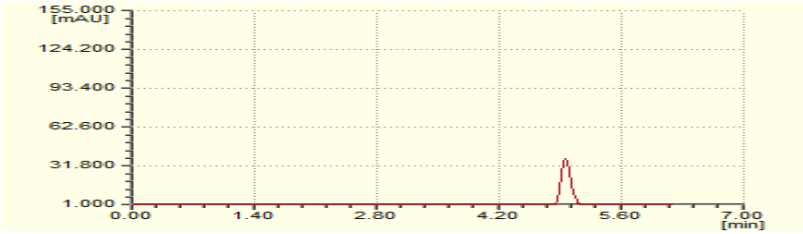

Figure 17: Precision Chromatogram of Standard Pirfenidone at 20 µg/ml

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Reproducibility (Day 1)

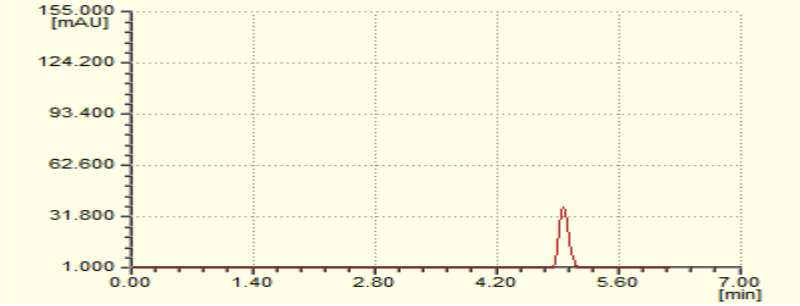

Figure 18: Precision Chromatogram of Standard Pirfenidone at 20 µg/ml

Table 9 :Chromatogram results for Reproducibility

Conc.20µg/ml Spikes	repeatability Day 1	Retention time(min)	Theoretical plates	Tailing factor
1	36060	4.98	5900	1.78
2	36012	4.94	5869	1.85
3	36105	4.95	5741	1.68
4	35987	4.95	5966	1.91
5	35899	4.97	5768	1.89
6	36014	4.98	5988	1.75

Reproducibility (Day 2)

Table 10: Chromatogram results for Reproducibility

Conc.20µg/ml Spikes	repeatability Day 2	Retention time(min)	Theoretical plates	Tailing factor
1	36040	4.98	5900	1.78
2	36561	4.94	5869	1.85
3	36211	4.95	5741	1.68
4	35990	4.95	5966	1.91
5	35856	4.97	5768	1.89
6	36111	4.98	5988	1.75

Robustness:

Six replicates were taken and study for Robustness was performed at a concentration of 20µg/ml by deliberate change in flow rate.

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Flow rate 0.8 ml/min

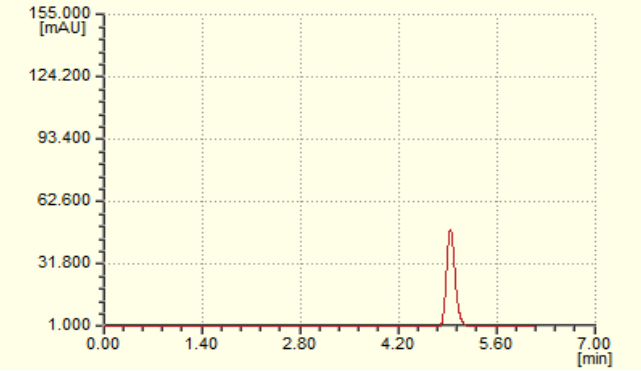

Figure 19: Chromatogram for Robustness (Flow rate 0.8 ml/min)

Table 11. Robustness Results

Robustness		Flow rate 0.8ml/min		Flow rate 1.2ml/min	
Retention time = 4.97		**Retention time**	**Peak area**	**Retention time**	**Peak area**
Absorbance at 20µg/ml	**S1**	4.98	36060	4.92	36561
	S2	4.96	36115	4.91	36772
	S3	4.96	36899	4.93	35754
	S4	4.98	35879	4.93	36112
	S5	4.99	36174	4.92	36453
	S6	4.98	36172	4.91	36528
Mean (n=6)		4.97	36216.5	4.975	36294.5
Standard deviation (SD)		0.01549193	351.65139	0.01378405	369.274288
RSD		0.00311709	0.0097097	0.00277066	0.01017439
%RSD		0.31%	0.97%	0.27%	1.01%

Ruggedness

- **Analyst-1**

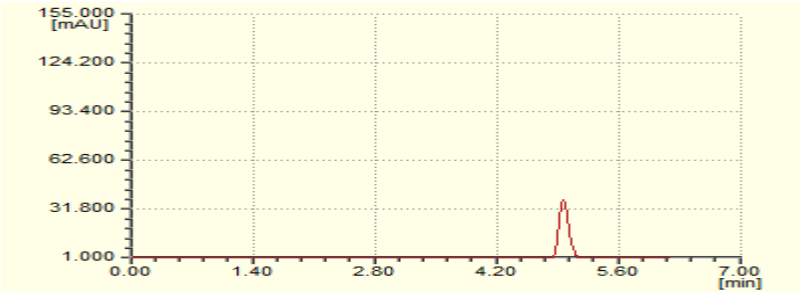

Figure No 20 . Chromatogram for Ruggedness

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❖ **Analyst-2**

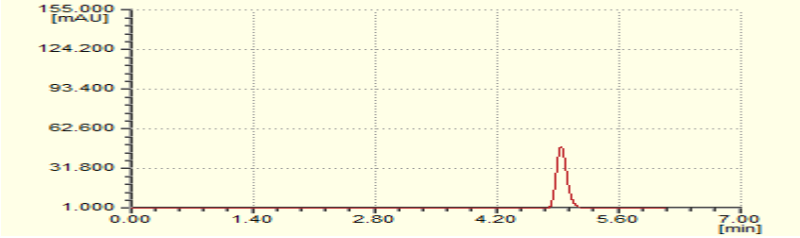

Figure No 21: Chromatogram for Ruggedness

Table 12: Ruggedness Results

Ruggdness		Analyst 1		Analyst 2	
		Retention time	**Peak area**	**Retention time**	**Peak area**
Absorbance at 20µg/ml	**S1**	4.95	36060	4.92	36151
	S2	4.94	36012	4.94	37015
	S3	4.94	36105	4.93	36001
	S4	4.97	36211	4.92	36060
	S5	4.96	35899	4.93	36200
	S6	4.95	36014	4.92	36001
Mean(n=6)		4.951666667	36050.17	4.92666667	36238
Standard deviation		0.01169045	157.7857	0.00816497	389.040872
RSD		0.00236091	0.00437	0.0016573	0.01073572
%RSD		0.23%	0.43%	0.16%	1.07%

Limit of detection and Limit of quantification

Table13: LOD & LOQ

Limit of Detection	Limit of Quantification
0.05	0.15

CONCLUSION

Literature survey indicates that the methods for the determination of pirfenidone by RP-HPLC method were time consuming and costlier. So the present work aimed for the development of sensitive, economical and simpler methods for the estimation of pirfenidone in its pharmaceutical dosage forms.

A RP-HPLC method was developed using the mobile phase of 50:48.5:1.5 %v/v methanol and water and tri etylamine. The run time of the developed method was six minutes, which reduces the solvent usage. The chromatographic conditions use ambient temperature which can yield accurate, precise results in the range of 25-35 ºC. The results of the validation parameters showed that the method is accurate and precise.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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