Research Article

DEVELOPMENT AND VALIDATION OF NOVEL ISOCRATIC RP-HPLC METHOD FOR THE ESTIMATION OF PRULIFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A Novel Reversed phase High performance liquid chromatography method has been developed and validated for the estimation of Prulifloxacin. A simple, precise, specific, sensitive and accurate Reversed phase HPLC method was developed and validated as per the ICH guidelines for the quantitative determination of Prulifloxacin in bulk and pharmaceutical dosage forms. Isocratic chromatography was performed on Inertsil ODS C18 column (250mm ×4.6mmx 5 µm) at ambient temperature with phosphate buffer: Acetonitrile 30:70%v/v as a mobile phase, pH 3.5 adjusted with 0.1M ortho phosphoric acid at a flow rate is 1.0 ml/min. The eluent detection was carried out by using photodiode array detector at 278 nm. The retention time of Prulifloxacin was 2.764 min. The linearity was observed from 60-180µg/ml with r^2=0.999. The limit of detection and limit of quantitation were found to be 0.84µg/ml and 1.8µg/ml respectively. The Statistical analysis proves that the method was found to be simple, selective, reliable, accurate and reproducible for successfully used for routine quality control analysis of Prulifloxacin.

Keywords: Prulifloxacin (PFX), RP-HPLC and validation.

INTRODUCTION

Prulifloxacin[1,2] 7-{4-[(Z)-2, 3-Dihydroxy-2-butenyl]-1-piperazinyl}-6-fluoro-1-methyl-4-oxo-1H, 4H-[1, 3] thiazeto [3, 2-a] Quinoline-3-carboxylic acid cyclic carbonate is an orally active fluoroquinolone antibiotic and is a prodrug of ulifloxacin. Prulifloxacin belongs to fourth-generation fluroquinolones and has extensive Gram-negative coverage, good Gram-positive coverage and also possesses activity against anaerobes[3]. It is mainly used in the treatment of bronchitis exacerbation and lower urinary tract infection[4]. It shows the antibacterial activity by inhibiting DNA gyrase enzyme thus preventing DNA replication and synthesis[5, 6]. PFX is type-IV topoisomerase. However to our knowledge, no article related to RP-HPLC determination of prulifloxacin in bulk and pharmaceutical dosage forms. This paper describes the method development and validation of RP-HPLC method for the assay of prulifloxacin as a bulk drug and in its pharmaceutical dosage forms. Chemical structure of prulifloxacin is shown in figure 1.

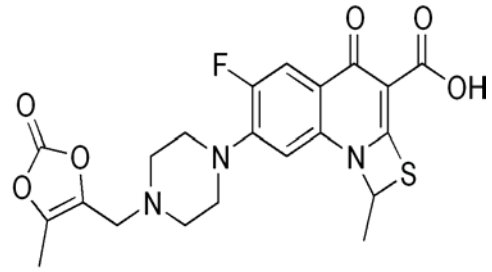


Figure 1: Chemical structure of Prulifloxacin

MATERIALS AND METHODS

Materials

Prulifloxacin pure drug was obtained as a gift sample from Hetero Drugs Ltd., Hyderabad, A.P, India and tablets were procured from local pharmacy. Acetonitrile used as a HPLC grade (filter through 0.2µ filters), Potassium dihydrogen phosphate and 0.1M ortho phosphoric acid were used as AR grades and purchased from RANKEM, RFCL limited, New Delhi, India.

HPLC instrumentation and conditions

Instrumentation

A waters HPLC system consisting of a Waters 2695 separation module, an inbuilt auto sampler, a column oven and Waters 2996 photodiode array detector was (PDA) employed for throughout the analysis. Chromatography was performed on an Inertsil ODS C18 (250mm × 4.6mm x 5 µm) column. A digisum DI 707 digital pH meter used for pH adjustment and a bandline sonerex sonicator was used for sonication. The data were acquired using the EM Power-2 software and other details of the instrumentation are given in table 1.

Optimized chromatographic conditions

Chromatography was performed on a Inertsil ODS C18 column (250mm × 4.6mm x 5 µm) column using mobile phase containing mixture of phosphate buffer: Acetonitrile 30:70%v/v and adjusting its pH to 3.5 with ortho phosphoric acid. The mobile phase was filtered through membrane filter (0.45 µm) and vacuum degassed by sonication prior to use. The pump pressure and run

time was maintained at 1500-2000 psi and 10 min respectively. Chromatography was performed at ambient temperature (30°C) under isocratic conditions at a flow rate is 1.0 ml/min and detection was done at 278 nm. Optimized Chromatographic conditions and other details are shown in table 1.

Table 1: Instrumentation and Optimized chromatographic conditions for proposed method

Instrumentation	Optimized chromatographic conditions
HPLC	Waters Alliance: 2695 separation module
Detector	Waters: 2996 PDA
Column	Intersil ODS C 18
Column temperature	30°C
Flow rate	1.0 ml/min
Injection volume	10µL
Wavelength	278 nm
Run time	10 minutes
Diluent	Mobile phase
pH	3.5
Mobile phase composition	Phosphate buffer: ACN 30:70% v/v

Preparation of solutions

Preparation of phosphate buffer solution

Dissolve 6.8g of potassium dihydrogen phosphate in 1000 ml of Milli-Q water. Filter through 0.45µm nylon membrane filter and degassed. Adjust the pH to 3.2 by 0.1M ortho phosphoric acid.

Preparation of stock solution

100 mg of Prulifloxacin pure drug was dissolved in 100ml of mobile phase to get a concentration of 1000 µg/ml.

Preparation of working standard solution

10 ml of stock solution was taken in 100 ml volumetric flask and diluted up to the mark with mobile phase to get a concentration of 100µg/ml.

Preparation of sample solution

20 tablets of Prulifloxacin were powdered and an amount of the powder equivalent to 100 mg of the drug was accurately weighed and transferred to the 100 ml volumetric flask, made up to the volume with mobile phase. The solution was placed in an ultrasonicator for 20min and filtered through a 25 mm, 0.45 µm nylon syringe filter. 10ml of this solution was taken and diluted to 100 ml by using a mobile phase to get a final concentration of 100 µg/ml. five replicate sample solutions were prepared in similar manner.

RESULTS AND DISCUSSION

Validation study of prulifloxacin

The Method validation was performed as per ICH guidelines for the determination of prulifloxacin in bulk and in the pharmaceutical formulations. The method was validated with respect to parameters including specificity, accuracy, precision, linearity, robustness, system suitability, limit of detection and limit of quantification.

Assay of Prulifloxacin tablets

The developed method was applied to the assay of PFX tablets. The content was calculated as an average of six determinations and experimental results were given in table 2. The results were very close to the labeled value of commercial tablets. The representative standard and sample chromatograms of Prulifloxacin were shown in Figure 2 and 3 receptively.

Table 2: Assay results of Prulifloxacin formulations

Formulations	Labeled Amount (mg)	Amount Found (mg)	% Assay ±RSD*
PERCIN TABLETS	600	597	98.83±0.35
ALPRULI TABLETS	600	596	98.58±0.33

*Average of 6 determinations

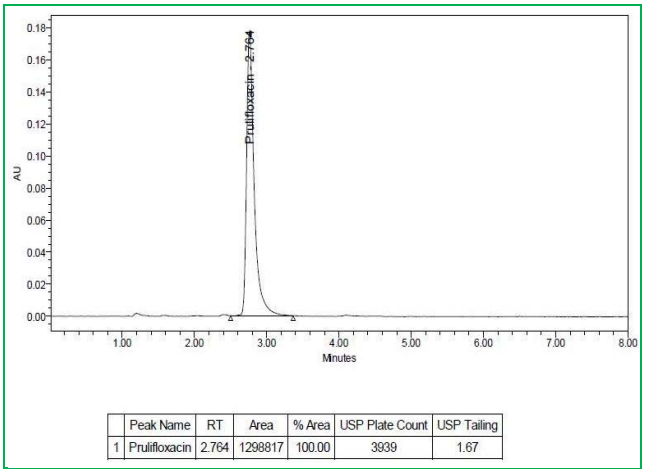


Figure 1: RP-HPLC Chromatogram of Prulifloxacin

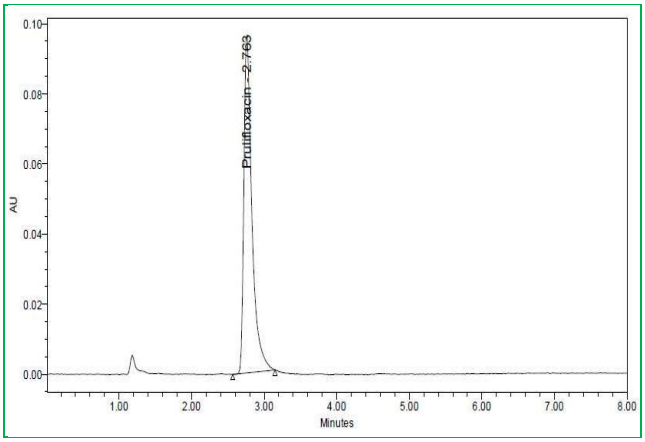


Figure 2: RP-HPLC chromatogram of Prulifloxacin formulation (Tablets)

Specificity

The specificity of the Reverse phase-HPLC method was established by injecting mobile phase and placebo solution in triplicate and recording the chromatograms. While the comparison of chromatograms there was no interference from the placebo with the sample peak. Therefore, it was concluded that the method is specific.

Accuracy (Recovery studies)

The accuracy was determined by calculating the recovery of prulifloxacin at 50%, 70%, 100%, 120% and 150% was added to a pre-quantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of PFX at each level was not less than 95% and not more than 105%. The percentage recovery of Prulifloxacin was found to be in the range of 99.83% to 100.21%. The results are shown in the table 3.

Table 3: Recovery data for the proposed RP-HPLC method

Concentration	Amount added (µg/ml)	Area obtained	Average area	Mean % Recovery ± S.D* (n=3)	% RSD #
50%	60	375737	375110	99.83843 ± 0.780	0.781955
	60	377679			
	60	371914			
100%	120	750099	753023.70	100.2115± 0.34	0.339995
	120	754112			
	120	754860			
150%	180	1131455	1122514	99.58859±0.708	0.711281
	180	1119992			
	180	1116095			

*S.D is standard deviation; # %RSD is percentage relative standard deviation

Table 5: Linearity and Statistical Analysis data for Prulifloxacin

Linearity level	Concentration (PPM)	Area	Average area **	Statistical Analysis		
				Slope	Y-Intercept	Correlation coefficient (r^2)
50%	60	385621	752891.2	6448	2129	0.999
70%	84	549210				
100%	120	754390				
120%	144	914221				
150%	180	1161014				

** Average of 6 determinations

Table 6: Robustness results of Prulifloxacin

Parameters			Peak areas	Retention time	Tailing factor
Optimized		Used			
Flow rate (±0.2)	**1 ml/min**	0.8	937010	4.577	1.79
		1	1298817	2.764	1.67
		1.2	658618	2.990	1.39
Temperature (±5^{0}c)	**30°C**	25	814535	3.565	1.57
		30	1298817	2.764	1.67
		35	813680	3.558	1.59
Mobile phase composition (± 3%)	**30:70**	37:63	788945	3.255	1.69
		30:70	1298817	2.764	1.67
		33:73	791155	3.233	1.69
pH (±0.5)	**3.5**	3.0	784739	3.560	1.59
		3.5	1298817	2.764	1.67
		4.0	817781	3.536	1.58

Precision

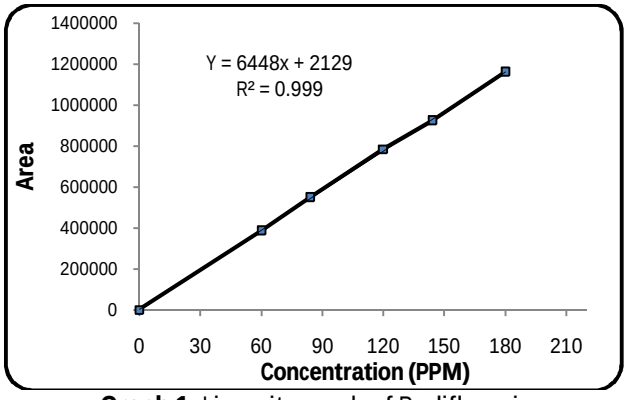
Precision should be investigated using homogeneous, authentic samples. Precision of analytical method was expressed as standard deviation and percentage relative standard deviation of series of replicate measurements. Precision of PFX estimation by proposed method was ascertained by replicate analysis of homogeneous samples of prulifloxacin standard solutions in the intraday under the similar conditions. The results were shown in table 4.

Table 4: System Precision results of the proposed RP-HPLC method

Injections	Retention Time (Rt)	Peak Area
1	2.764	746891
2	2.763	750590
3	2.763	758964
4	2.764	758663
5	2.767	758874
6	2.770	757346
MEAN	2.76517	755221
SD	0.00279	5187.53
%RSD	0.10079	0.68689

Linearity

Linearity of the proposed method was established by using series of standard solutions of Prulifloxacin at concentration levels from 50 to 150%, like 50%, 70%, 100%, 120% and 150%. This linearity studies are repeated for three times with different stock solutions. The curve obtained by concentration on x axis and peak area on y-axis against showed linearity in the concentration range of 60-180 µg/ml of PFX and linearity graph is shown in Graph 1. The regression equation was found to be y = 6448x + 2129 with correlation coefficient is r^2 = 0.999. The statistical analysis of data is shown in table 5.



Graph 1: Linearity graph of Prulifloxacin

Robustness

The robustness was evaluated by the analysis of PFX under different experimental conditions such as slight changes in chromatographic conditions like change of flow rate (± 0.2 ml/min), temperature (± 5^0C), mobile phase composition (± 3%) and pH of buffer in mobile phase (± 0.5%). It was observed that there were no marked changes in the chromatograms and the parameters are within the limit, which indicates that the method has robustness and suitability for routine use. The complete results are shown in Table 6 and the method is having good system suitability.

System suitability

The system suitability test was carried out on freshly prepared PFX standard solution (100%) was used for the evaluation of the system suitability parameters such as area, retention time, USP peak tailing, the number of theoretical plates, LOD and LOQ. Five replicate injections for a system suitability test were injected into the chromatographic system and system suitability of the system results are given in table 7.

Limit of Detection (LOD)

The limit of detection has established the minimum concentration at which the analyte can be reliably detected. LOD is determined by the signal to noise ratio and signal to noise ratio 3:1 is generally considered acceptable for estimating the detection limit and it was found to be 0.84µg/ml.

Table 7: System suitability results for proposed RP-HPLC method

Injections	Area	Retention Time	USP Plate count	USP tailing
1	757346	2.764	3503	1.73
2	750590	2.763	3274	1.74
3	758964	2.763	3479	1.70
4	758663	2.764	3541	1.69
5	758874	2.767	3423	1.70
MEAN	756887.4	2.7642		
SD	3580.474	0.001643		
%RSD	0.473052	0.059445		

Limit of Quantitation (LOQ)

The limit of quantitation was established the minimum concentration at which the analyte can be reliably quantified. LOQ is determined by the signal to noise ratio and a typical signal to noise ratio is 10:1 is acceptable for estimating the quantitation limit and it was found to be 1.8µg/ml. Finally the proposed method is having good system suitability and system suitability parameters as shown in table 8.

Table 8: System Suitability Parameters of proposed RP-HPLC method

Parameters	Values
Wavelength (λmax)	278 nm
Regression equation	Y=6448x+2129
Correlation coefficient(r^2)	0.999
Retention time (min)	2.764
Theoretical plates	3939
Tailing factor	1.67
Limit of detection (µg/mL)	0.84
Limit of quantitation	1.8
Capacity factor (k)	1.2112

CONCLUSION

A Novel RP-HPLC method for quantitative determination of PFX in bulk and in pharmaceutical dosage forms is established. This method is simple, reliable, linear, accurate, sensitive and reproducible as well as economical for the effective quantitative analysis of PFX in bulk and formulations. The method was completely validated showing satisfactory data for all the method validation parameters tested and method is free from interference of the other active ingredients and additives used in the formulation. Therefore the method is suitable for use for the routine analysis of Prulifloxacin in API or in pharmaceutical dosage forms.

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REFERENCES

1. Merck & co. Inc., The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals, 14th Edn., white house station, New Jersey, 2006, 1359.
2. Sean C. Sweetman., Martindale: The complete drug reference, 37th Edn. Pharmaceutical Press, London, 2011, 348.
3. Gianluca Giannarini, Carlo Tascini and Cesare Selli, Prulifloxacin: clinical studies of a broad spectrum quinolone agent, Future Microbiology, 4 (1), 2009, 13-24.
4. Hooper DQ, in: Mandell, GL, Bennett JE, Dolin RM, Douglas and Bennett's Principles and practice of infectious diseases, 5th Edn, Philadelphia, Churchil Livingstone, 2000, 656-662.
5. Hooper DC, Wolfson JS, Mechanisms of quinolone action and bacterial killing, in: Quinolone antimicrobial agents, 2nd Edn. Washington, D.C. American Society for Microbiology, 1993, 53-57.
6. Hooper DC, Mode of action of fluoroquinlones. Drugs, 58 (supply 2), 1999, 6-10.

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