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Research Article

**RP-HPLC PDA METHOD FOR ESTIMATION OF
PRULIFLOXACIN IN BULK AND PHARMACEUTICAL
DOSAGE FORM****Rameshpetchi Rajendran¹, Devikasubramaniyan^{2*}, Ramya Sri Sura³, E. Veeranna⁴,
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Khammam, Telangana, 507318³University of Technology, Osmania University, Hyderabad, Telangana, 500007**Abstract:**

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Prulifloxacin in its pure form as well as in tablet dosage form. Chromatography was carried out on Symmetry C18 (4.6×150mm, 5μ) column using a mixture of Methanol and water (85:15 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 242nm. The retention time of the Prulifloxacin was 3.0 ±0.02min. The method produce linear responses in the concentration range of 15-75μg/ml of Prulifloxacin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Prulifloxacin, RP-HPLC, validation.**Corresponding author:****Devikasubramaniyan,**
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INTRODUCTION:

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages [1].

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 μm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance [6].

Types of HPLC Techniques [2-7]**Based on Modes of Chromatography**

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted [4].

Based on Principle of Separation [8-12]

Liquid/solid chromatography (Adsorption): LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and

classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.³

Liquid/Liquid chromatography (Partition Chromatography): LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with β , β' -oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later [1,2]

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions [3,16,17].

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers.^{3,15}

Chiral chromatography: In this type of chromatography optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity.³

Based on elution technique

Isocratic separation: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

Gradient separation: In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength [3].

Based on the scale of operation [13-15]

Analytical HPLC: Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: μg quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC [4].

Based on type of analysis

Qualitative analysis: Which is used to identify the compound, detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample [3].

INSTRUMENTATION OF HPLC

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the

detector and finally a means of presenting and processing the results.

Mobile phase (solvent) reservoirs and solvent degassing

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase.

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity [8,20].

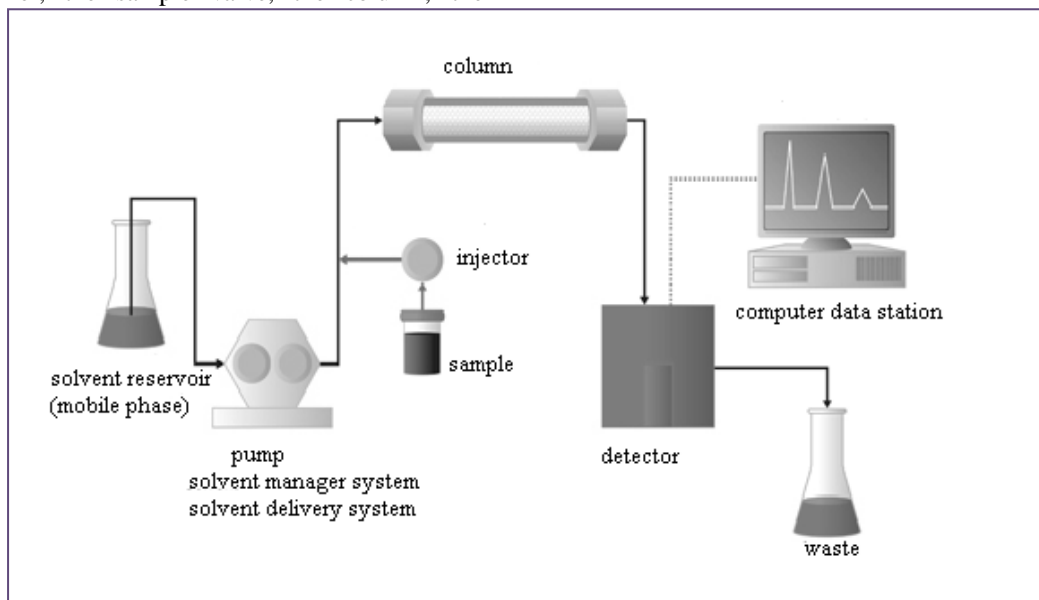


Fig.1: Components of HPLC instrument block diagram. ²²

Mobile phase preparation

Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤ 1 μm pore size filters and be degassed before use.

Degassing of solvents

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction

into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

1. By warming the solvents
2. By stirring vigorously with a magnetic stirrer
3. By subjecting to vacuum filtration
4. By ultra sonication (using ultrasonicator)

Prulifloxacin (RS)-6-Fluoro-1-methyl-7-[4-(5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl-1-piperazinyl]-4-oxo-4H-[1,3]thiazeto[3,2-a]quinoline-

3-carboxylic acid. Prulifloxacin has been investigated for the treatment of Urinary Tract Infection.

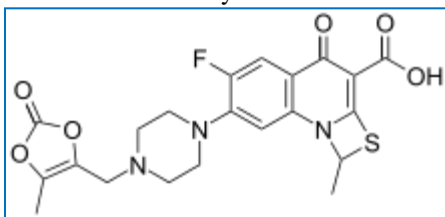


Fig. 2: chemical structure of Prulifloxacin

MATERIALS AND METHODS:

Accurately measured 950ml (95%) of HPLC Methanol and 50ml of HPLC Water (5%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Instrumentation and Chromatographic conditions

The analysis was performed by using Symmetry C18 column, 4.6 \times 250mm internal diameter with 5 micron particle size column and UV detector set at 242nm nm, in conjunction with a mobile phase of Methanol:water in the ratio of 85:15v/v (pH 5 adjusted with OPA) at a flow rate of 1 ml/min. The retention time of Prulifloxacin was found to be 3.050 minute. The 10 μ l of sample solution was injected into the system

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Prulifloxacin working standard into a 10ml of clean dry volumetric

flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.45ml of the above Prulifloxacin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Water in proportion 85:15 v/v respectively.

Optimization of Column:

The method was performed with column like Symmetry C18 (4.6 \times 250mm, 5 μ m) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow

(Optimized chromatogram):

Column	:	Symmetry C18
(4.6 \times 250mm) 5 μ		
Column temperature	:	Ambient
Wavelength	:	242nm
Mobile phase ratio	:	
Methanol:water(85:15% v/v)		
Flow rate	:	1ml/min
Injection volume	:	10 μ l
Run time	:	5min

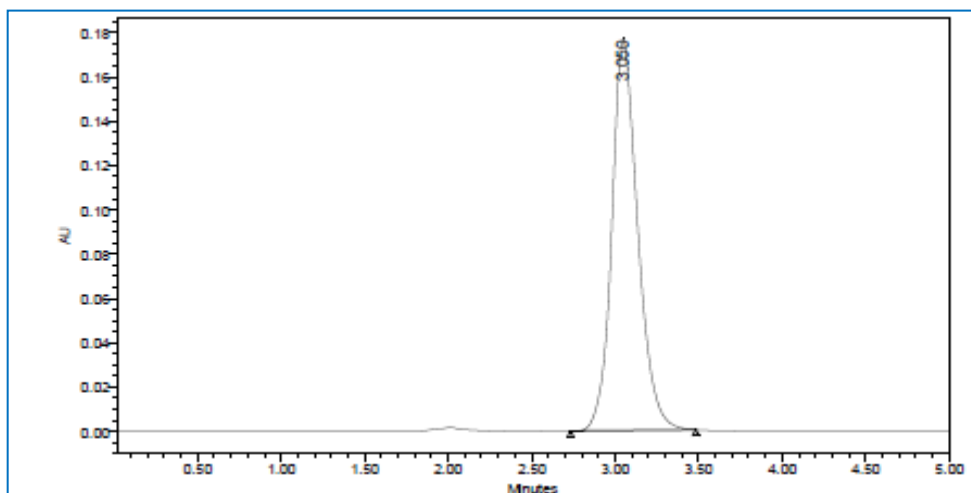


Fig. 3 Typical chromatogram of mixture of Standard solution.

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 950ml (95%) of HPLC Methanol and 50ml of HPLC Water (5%) were mixed and degassed in a digital ultrasonicator for 10

minutes and then filtered through 0.45 μ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Linearity: The linearity of was obtained in the concentration ranges from 15-75 µg/ml

Table 1: Linearity data of Prulifloxacin

Concentration Level (%)	Concentration µg/ml
60	15
80	30
100	45
120	60
140	75

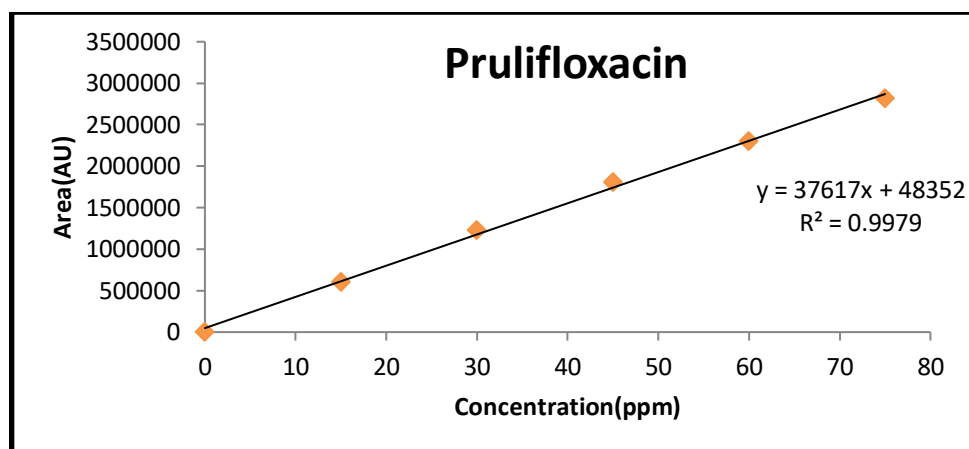


Fig.4: calibration graph of Prulifloxacin

LINEARITY PLOT

Linearity of detector response of assay method was found by injecting seven standard solutions with concentration ranging from 15-75 µg/mL for Balofloxacin. The graph was plotted for concentration versus peak area. The results were shown in Table-1 and fig 4.

Precision

Repeatability

The precision of test method was determined by preparing six test preparations using the product blend and by mixing the active ingredient with excipients as per manufacturing formula. And the relative standard deviation of assay results was calculated. The results were shown in Table 2

Table 2: Results of repeatability for Prulifloxacin

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Prulifloxacin	3.050	1913224	179836	8328	1.1
2	Prulifloxacin	3.057	1915644	178276	7828	1.2
3	Prulifloxacin	3.058	1913674	176819	9872	1.1
4	Prulifloxacin	3.058	1912535	179371	8376	1.1
5	Prulifloxacin	3.046	1916435	179383	7493	1.2
Mean			1914302			
Std.dev			1660.528			
%RSD			0.086743			

Accuracy

Prulifloxacin tablets content were taken at various concentrations ranging from 50 % to 150 % (50 %, 75 %, 100 %, 125 %, and 150 %) to accurately quantify and to validate the accuracy. The assay was performed in triplicate. The results were shown in Table-3

Table 3: The accuracy results for Prulifloxacin

% Concentration (at specification Level)	Peak area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1918358	67.5	67.48	99.9	99.9%
100%	3774514	135	134.8	99.8	
150%	5630870	202.5	202.39	99.9	

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 LOD value for Prulifloxacin 12.4 µg/ml.

Quantitation limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The LOQ values for Prulifloxacin 37.5 µg/ml

ROBUSTNESS

The robustness was performed for the flow rate 1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Prulifloxacin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase $\pm 5\%$. The standard samples of Prulifloxacin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. Table 4

Table 4: Results for Robustness of Prulifloxacin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1ml/min	1913251	3.050	8937	1.2
Less Flow rate of 0.7mL/min	179049	3.405	8033	1.1
More Flow rate of 0.9mL/min	173553	2.770	9173	1.2
Less organic phase (about 5 % decrease in organic phase)	170033	3.736	7971	1.1
More organic phase (about 5 % Increase in organic phase)	178837	2.692	8822	1.1

SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 242nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry C18 (4.6 x 150mm, 5µm) because it was giving good peak. 35° C temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: water was fixed due to good symmetrical peak. So this mobile phase was used for the

proposed study. Methanol: water(85:15%v/v) was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Prulifloxacin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Prulifloxacin was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol:

water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

Table 5. Summary data for Prulifloxacin

Parameters	Prulifloxacin
Retention Time (min.)	3.050
Linearity ($\mu\text{g/ml}$)	15-75
Correlation Coefficient (r^2)	0.999
Slope	37617
Y - intercept	48352
LOD ($\mu\text{g/ml}$)	12.4
LOQ ($\mu\text{g/ml}$)	37.5
Repeatability (% RSD) n=6	0.086743
Intraday Precision (% RSD) n=6	0.148577
Interday Precision (% RSD) n=6	1.386878
Accuracy (%)	99.9%

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