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

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ABSTRACT

A simple, accurate and precise RP-HPLC method has been developed for the quantitative estimation of prulifloxacin in pharmaceutical formulations. Prulifloxacin is a prodrug, and is metabolized in the body to the active compound ulifloxacin. Prulifloxacin appeared as effective as ciprofloxacin, co-amoxiclav or pefloxacin in the treatment of bronchitis exacerbations or lower urinary tract infections. A RP-HPLC method was developed by using a Phenomenex Luna C_{18} column (250 mm length, 4.6 mm internal diameter and 5µm particle size) and a 60 : 40 v/v mixture of 10 mM phosphate buffer (pH adjusted to 3.0 after addition of 2.5 mL of triethyl amine) and acetonitrile was used as mobile phase. The analyte was monitored with UV detector at 275 nm. Typical retention time for prulifloxacin was found to be 6.8 min. The method was statistically validated for its linearity, accuracy and precision. Due to its simplicity and accuracy, the method can be used for routine quality control of prulifloxacin in pharmaceutical formulations.

Key words: Prulifloxacin, C18 column, RP-HPLC method, Validation.

INTRODUCTION

Prulifloxacin[1,] is a prodrug and it is metabolized in the body to the active compound ulifloxacin. Prulifloxacin appeared as effective as ciprofloxacin, co-amoxiclav or pefloxacin in the treatment of bronchitis exacerbations or lower urinary tract infections. It was tolerated as well as ciprofloxacin. Prulifloxacin has a long half-life and may therefore be taken only once a day. Prulifloxacin has been approved for use in Japan. In the United States, it is undergoing phase III clinical trials for the treatment of traveler's diarrhea. It has been proven that prulifloxacin is more effective than ciprofloxacin in the treatment of adults with

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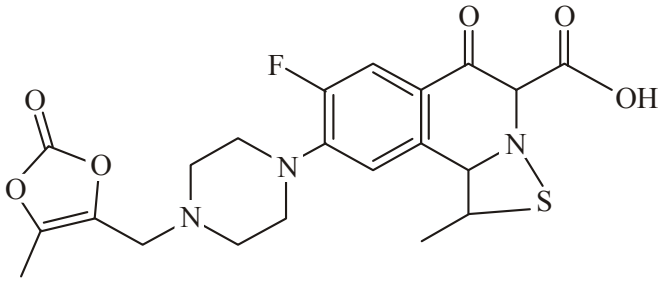
complicated urinary tract infections[2]. Prulifloxacin, the lipophilic prodrug of ulifloxacin, is an oral fluoroquinolone antibacterial agent with a broad-spectrum *in vitro* activity against gram-negative and gram-positive bacteria, and a long elimination half-life, which allows the once-daily administration. In addition, it penetrates extensively into lung tissues. In well-designed clinical trials, prulifloxacin 600 mg administered once daily for 10 days in patients with AECB[3] (Acute Exacerbation of Chronic Bronchitis) showed good clinical and bacteriological efficacy (similar to that of ciprofloxacin or co-amoxiclav). Prulifloxacin, a new thiazeto-quinoline derivative with antibiotic properties, was evaluated for cardiac risk[4] both *in vitro* on the ether-a-go-go-related gene (HERG) K+ channel, and *in vivo* in the conscious dog monitored by telemetry.

PRF is not official in any pharmacopoeia. Literature survey revealed that few chromatographic methods have been reported, which include LC-MS[5], HPLC[6], HPLC with fluorescence detection[7], capillary zone electrophoresis[8] and capillary electrophoresis–chemiluminescence[9] methods for the determination of the active metabolite of prulifloxacin in human plasma and other biological fluids. It has been also reported that there is a sensitive determination of prulifloxacin by its fluorescence enhancement on terbium (III)-sodium dodecylbenzene sulfonate system[10].

There is no analytical report for the estimation of PRF using visible spectrophotometry and also no RP-HPLC method was reported for this drug to estimate in pharmaceutical dosage forms (tablets). This prompted the author to choose PRF for the development of sensitive, precise and accurate visible spectrophotometric methods based on various chemical reactions, involving the analytically important functional groups present in the structure and one sensitive and precise HPLC method for determination of PRF in bulk samples and pharmaceutical formulations.



6-Fluoro-1-methyl-7-(4-(5-methyl-2-oxo-1, 3-dioxelen-4-yl) methyl-1-piperazinyl)-4-oxo-4H-(1, 3) thiazeto (3, 2-a) quinoline-3-carboxylic acid.

EXPERIMENTAL

Materials and methods

Instrumentation

Quantitative HPLC was performed on a high pressure gradient high performance liquid chromatograph (Shimadzu HPLC, Class VP series) with two LC-10AT VP pumps, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength UV detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu) and Phenomenex C_{18} column (250 mm length, 4.6 mm internal diameter and particle size 5 µm). The HPLC system was equipped with "Spincotech" software.

Standards and chemicals used

Prulifloxacin was provided by Sun Pharma Limited, Mumbai, India. All the chemicals were analytical grade: potassium dihydrogen orthophosphate and phosphoric acid from S.D Fine-Chem Ltd., Mumbai, India, while acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Limited (Mumbai, India).

Commercial tablets of prulifloxacin were purchased from local market. Unidrox, a product by Cipla Limited (Mumbai, India) and Pruquil from ranabaxy contained 600 mg tablets

Preparation of mobile phase

A 10 mM phosphate buffer was prepared by dissolving 1.3609 g of potassium dihydrogen orthophosphate in 1000 mL of water. To this 1.5 mL of triethyl amine was added and pH was adjusted to 3.00 with orthophosphoric acid. Above prepared buffer and acetonitrile were mixed in the proportion of 60 : 40 v/v. The mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and degassed by sonication.

Preparation of standard drug solutions

About 100 mg of pure PRF was accurately weighed and dissolved in 50 mL of mobile phase in 100 mL volumetric flask to get 1 mg/mL stock solution. A series of standard solutions in the concentration range of 2, 4, 6, 8, 10 and 12 µg/mL were prepared followed by a suitable dilution of stock solution with the mobile phase.

Sample preparation

The content of twenty tablets was transferred into a mortar and ground to a fine

powder. From this tablet powder, equivalent to 100 mg of PRF was taken and the drug was extracted in 100 mL of mobile phase. The resulting solution was filtered through 0.22 μm nylon membrane filter and degassed by sonication. This solution was further suitably diluted for chromatography.

Chromatographic conditions

The HPLC system consisting of Phenomenex Luna C18 column (250 mm length, 4.6 mm internal diameter and 5 μm particle size) was stabilized with the mobile phase at a flow rate of 1.0 mL/min. The test solutions were injected into the system by filling a 20 μL fixed volume loop manual injector. The chromatographic run time of 10 min. was maintained for the elution of the drug from the column. The eluates were monitored with UV detector at 275 nm.

Table 1: Chromatographic conditions for PRF

Parameter	Method
Stationary phase	Phenomenex C18 column (length: 250 mm, Internal diameter: 4.6 mm, Particle size: 5 μm)
Mobile phase	Buffer: ACN (60 : 400)
Flow rate (mL/min)	1.0
Column back pressure (kg/cm^2)	118-125
Run time (min)	10
Column temperature (^{o}C)	Ambient
Volume of injection loop (μL)	20
Detection Wavelength (nm)	By UV at 275nm
Retention time (min)	6.83

Recommended procedures

For bulk samples

The HPLC system was stabilized for thirty min. by following the chromatographic conditions as described in Table 1 to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Six

replicates of each standard solutions were 2, 4, 6, 8, 10 and 12 µg/mL. The retention time and average peak areas were recorded. Calibration graph was plotted by taking concentration of PRF on X-axis and peak areas on Y-axis. The amount of drug present in sample was computed from the calibration graph.

For pharmaceutical formulations

The content of twenty tablets was transferred into a mortar and ground to a fine powder. From this tablet powder, equivalent to 50 mg of PRF was taken and the drug was extracted in 100 mL of mobile phase. The resulting solution was filtered through 0.22 µm nylon membrane filter and degassed by sonication. This solution was further suitably diluted for chromatography. Working sample solutions were prepared and the procedure described under bulk samples was followed.

RESULTS AND DISCUSSION

The goal of this study is to develop rapid HPLC methods for the analysis of PRF in bulk drug samples and tablet formulations using the most commonly employed column (C_{18}) with UV detection at appropriate wavelength. The representative chromatograms indicating the PRF are shown in Fig. 1 to 6.

Parameter fixation

In developing these methods, a systemic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

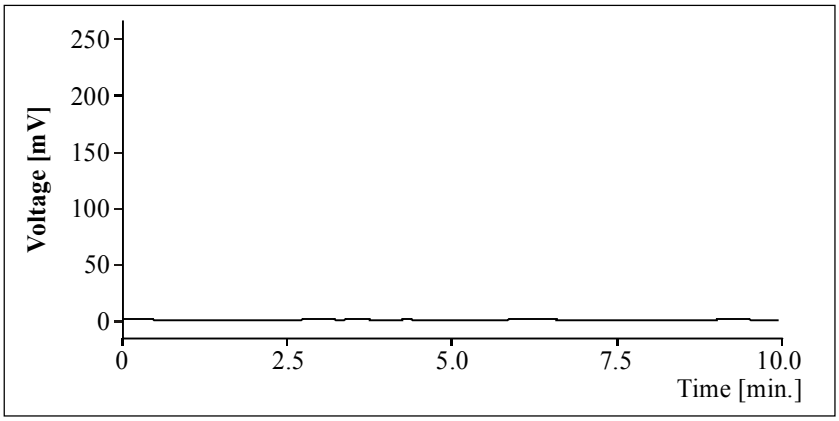


Fig. 1: Blank chromatogram of PRF

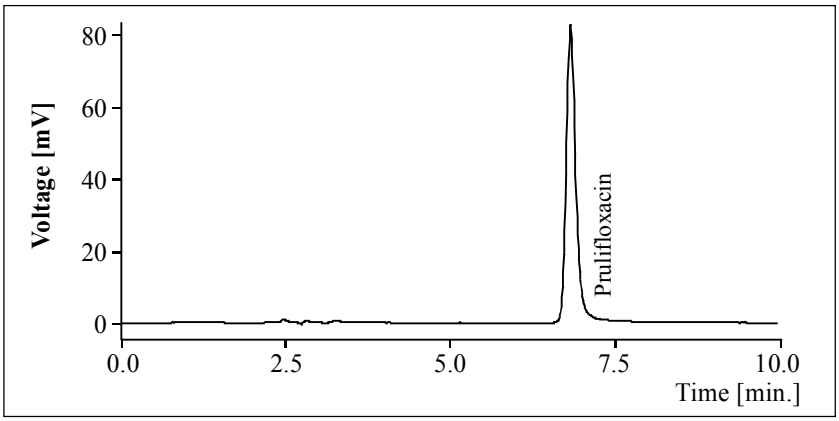


Fig. 2: Standard chromatogram of PRF (2 µg/mL)

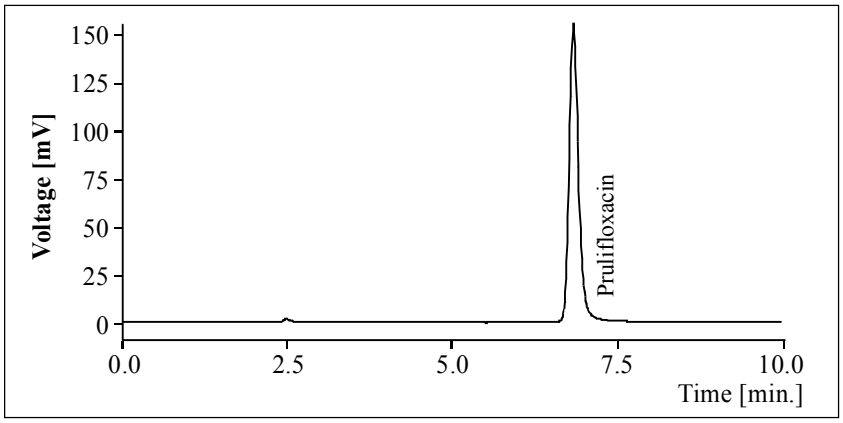


Fig. 3: Standard chromatogram of PRF (4 µg/mL)

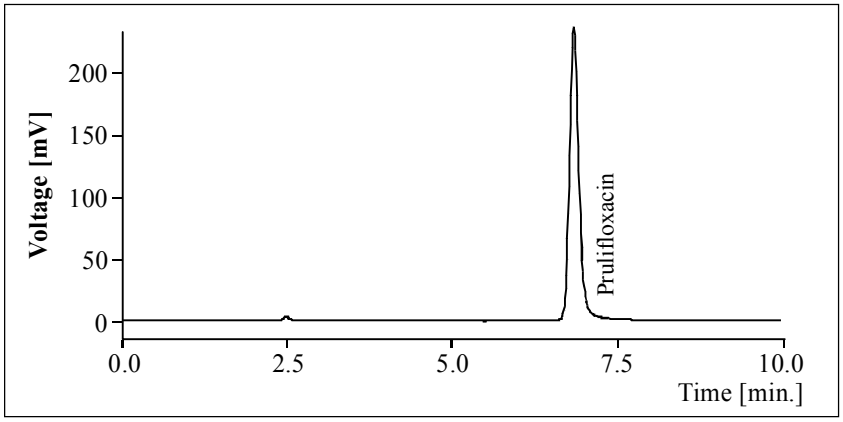


Fig. 4: Standard chromatogram of PRF (6 µg/mL)

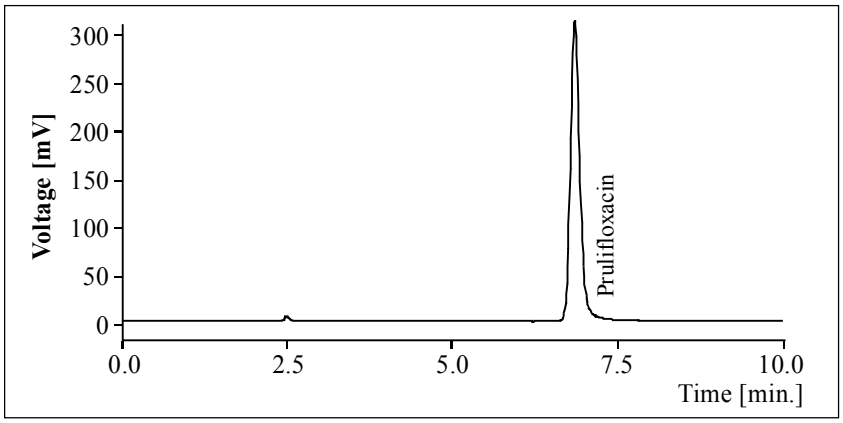


Fig. 5: Standard chromatogram of PRF (8 µg/mL)

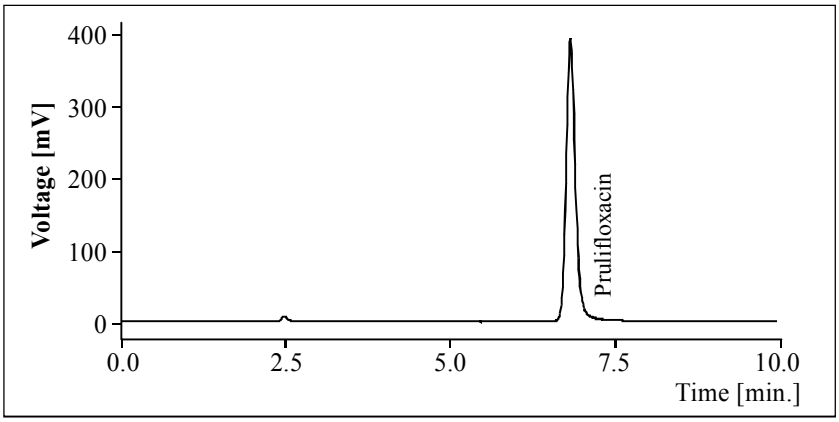


Fig. 6: Standard chromatogram of PRF (10 µg/mL)

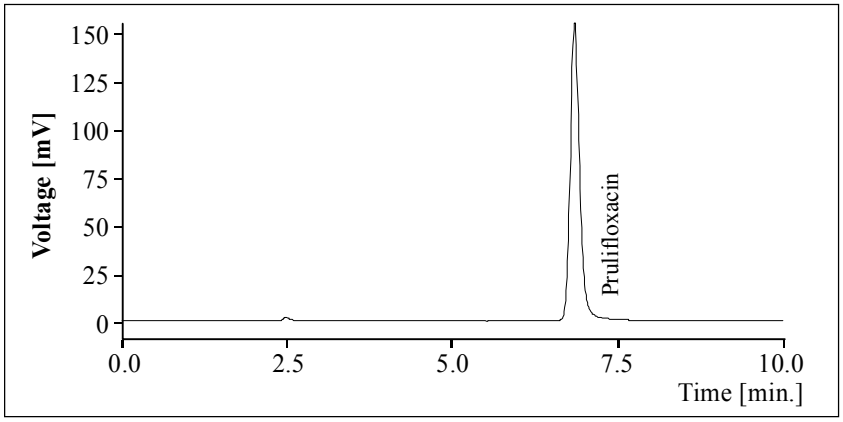


Fig. 7: Sample chromatogram of PRF

Stationary phase characteristics

PRF is having solubility in aqueous solvents buffered to low pH. Based on the solubility characteristics, the reverse phase mode of HPLC was selected for chromatography. Among the different RP-HPLC stationary phases tried, C_{18} was found to be optimum.

Mobile phase characteristics

In order to get narrow peaks and base line separation of the components, the authors have carried out number of experiments by varying different components like percentage of methanol in the mobile phase, pH of the aqueous phase and flow rate by changing one at a time and keeping all other parameters constant.

Method validation

An integral part of analytical method development is validation. Once the method has been devised, it is necessary to evaluate under the conditions expected for real samples before being used for a specific purpose. The following parameters were evaluated.

Specificity

The effect of wide range of excipients and other additives usually present in the formulations of PRF in the determinations under optimum conditions was investigated. The common excipients such as lactose anhydrous, microcrystalline cellulose, crosscaramellose sodium and magnesium stearate have been added to the sample solution and injected. They do not disturb the elution or quantification of PRF. In fact, many have no absorption at this UV maximum.

Precision

The precision of the method was ascertained from the peak area of PRF obtained by determination of six replicates of fixed amount of PRF. The percent relative standard deviation and percent range of errors (0.05 and 0.01 confidence limits) were calculated and were presented in the Table 2.

Linearity

The linearity graphs for the proposed assay methods were obtained over the concentration range of 2 – 12 µg/mL PRF containing fixed quantity of internal standard. The linearity graph was given in Fig. 8. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient values and the results were presented in Table 2.

Table 2: System suitability, precision and accuracy of the proposed method for PRF

Parameter	Method
Retention time (t) (min)	6.83
Theoretical plates (n)	7503
Plates per meter (N)	30012
Peak asymmetry	1.364
Resolution gactor	-
Linearity range (µg/mL)	2-12
Detection limits (µg/mL)	0.01446
Regression equation (Y = a + bc)	
Slope (b)	126.0357
Standard deviation of slope (S_b)	7.659×10^{-2}
Intercept (a)	0.0714
Standard deviation of intercept (S_a)	0.5523
Standard error of estimation (S_e)	0.8106
Correlation coefficient (r)	0.9995
Relative standard deviation (%)*	
Retention time	0.0854
Peak area/peak area ratio	0.0996
Percentage range of errors* (Confidence limits)	
0.05 level	0.6023
0.01 level	0.7915
% Error in bulk samples**	0.037

*Average of six determinations

**Average of three determinations

Accuracy

To determine the accuracy of the proposed method, different amounts of bulk samples of PRF in between the upper and lower linearity limits were taken and analyzed by the proposed method. The results (percent error) are recorded in Table 2.

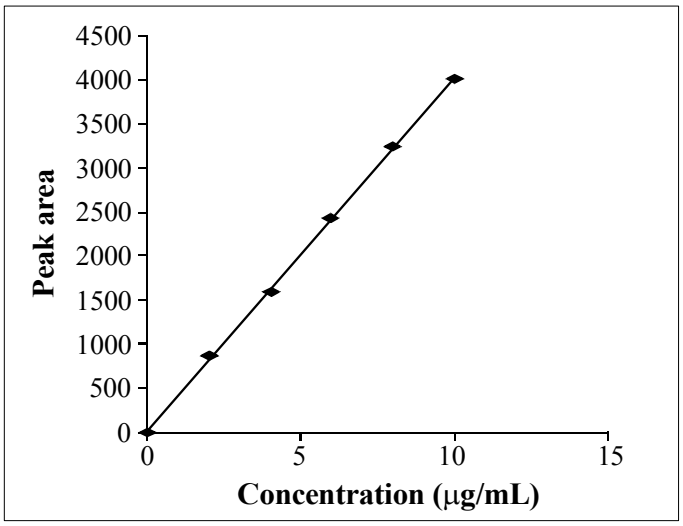


Fig. 8: Calibration plot of PRF

Recovery studies

Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed methods. Known amounts of pure drug was then added to each of the previously analyzed formulation and the total amount of drug was once again determined by the proposed methods after bringing the active ingredient concentration within the linearity limits. The results were recorded in Table 3.

Robustness

Robustness of the proposed methods was evaluated by making small changes in flow rate, buffer concentration, pH of the buffer solution, organic modifier concentration and temperature. The results were found to be not affected by these small alterations.

System suitability

To ascertain the system suitability for the proposed method, a number of parameters such as relative retention, theoretical plates, resolution, peak asymmetry, % RSD for

retention times, peak areas of PRF have been calculated with the observed reading and the results are recorded in Table 2.

Analysis of formulations

Commercial formulations (capsules) containing PRF were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for the formulations were compared statistically with t- and F- tests and found not to differ significantly. The results were summarized in Table 3.

Table 3: Assay and recovery of PRF in dosage forms

Pharmaceutical formulation	Labelled amount (mg)	Proposed method			Found by reference method ± S.D	% Recovery by proposed methods** ± S.D
		Amount found* (mg) ± S.D	t (value)	F (Value)		
T_1	600	599.89 ± 0.014	0.820	1.832	596.78 ± 0.015	100.21± 0.52
T_2	600	599.92 ± 0.09	1.426	2.623	595.82 ± 0.014	99.78 ± 0.45

*Average ± standard deviation of six determinants; the t and F-values refer to comparison of the proposed method.
Theoretical values at 95 % confidence limits t = 2.571 and F = 5.05.
**Average of six determinations.

T_1 and T_2 are the brand names of PRF. T_1 are Unidrox from Cipla and $T_{2\ is}$ Pruquil from Ranbaxy.

CONCLUSION

Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. Results of analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations. These methods can be adopted for routine quality control of prulifloxacin in bulk and pharmaceutical preparations.

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