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A VALIDATED RP-HPLC METHOD FOR THE ASSAY OF PRULIFLOXACIN IN MARKETED DRUG PRODUCT USING LEVOFLOXACIN AS AN INTERNAL STANDARD

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ABSTRACT

A simple, accurate, precise, specific isocratic reversed phase-high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative estimation of prulifloxacin in pharmaceutical formulations. RP-HPLC method was developed by using Welchrom C-18 Column (4.6 x 250 mm, 5 µm), Shimadzu LC-20AT prominence liquid chromatograph. The mobile phase used is phosphate buffer (pH-3.2): acetonitrile (60 : 40 v/v) with a flow rate of 1 mL/min. The responses are measured at 285 nm using Shimadzu SPD-20A prominence UV-Vis detector. Levofloxacin is used as internal standard. The retention times of prulifloxacin and levofloxacin are found to be 7.093 and 2.780 min respectively. The method posses linearity in the range of 2-12 µg/mL. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity, precision and specificity according to ICH guidelines. The proposed method provides an accurate and precise quality control tool for routine analysis of prulifloxacin in tablet dosage forms.

Key words: Prulifloxacin, Internal standard, RP-HPLC, UV-Vis detector.

INTRODUCTION

Prulifloxacin[1] is a lipophilic prodrug of ulifloxacin is an oral fluoroquinolons with broad spectrum activity against various Gram-positive and Gram-negative microorganisms[2-4]. Literature, survey revealed that very few methods have been reported for the analysis of prulifloxacin which include LC-MS[5], HPLC with fluorescence detection[6-7], capilary zone

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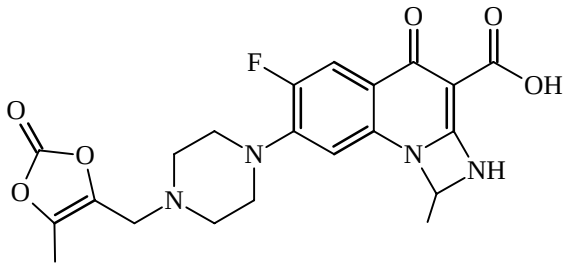
electrophoresis[8], capillary electrophoresis chemiluminescence[9] and few spectrophotometric methods[10-11]. The present study illustrates development and validation of simple, sensitive, precise and accurate RP-HPLC method for the determination of prulifloxacin in pharmaceutical tablet dosage forms.

Chemical name and structure of prulifloxacin



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

Quantitative HPLC was performed on a high pressure gradient high performance liquid chromatograph (Shimadzu LC-20AT prominence liquid chromatograph) with two LC-20AT VP pumps, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A prominence UV-Vis detector and WELCHROM C-18 Column (4.6 x 250 mm, 5 µm). The HPLC system was equipped with "Spincotech" software.

Standards and chemicals used

Prulifloxacin was provided by Hetero Drugs Limited, Hyderabad, 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 6.056 g of potassium dihydrogen orthophosphate in 445 mL of HPLC grade water. To this 55 mL of 0.1 M

phosphoric acid was added and pH was adjusted to 3.2 with triethylamime. The above prepared buffer and acetonitrile were mixed in the proportion of 60 : 40 v/v and was filtered through 0.22 µm nylon membrane filter and degassed by sonication.

Preparation of internal standard solution

About 100 mg of pure Laevofloxacin was accurately weighed and dissolved in 100 mL of mobile phase to get 1 mg/mL stock solution. From this solution 10 µg/mL of levofloxacin solution was prepared by serial dilution with mobile phase.

Preparation of calibration standards

About 100 mg of pure Prulifloxacin was accurately weighed and dissolved in 100 mL of mobile phase to get 1 mg/mL stock solution. Working standard solution of prulifloxacin was prepared with mobile phase. To a series of 10 mL volumetric flasks, standard solutions of prulifloxacin in the concentration range of 2, 4, 6, 8, 10 and 12 µg/mL were transferred and 10 µg of standard solution of levofloxacin was added to each flask. The final volume was made with the mobile phase.

Table 1: Chromatographic conditions for Prulifloxacin

The following chromatographic conditions were established for assay of prulifloxacin tablets.

Parameter	Chromatographic conditions
Column	Welchrom C_{18} Column (4.6 x 250 mm, 5 µm)
Mobile phase	Buffer: ACN (60 : 40 v/v)
Flow rate (mL/Min)	1
Detection	By UV at 285 nm.
Run time (min)	15
Column back pressure (Kg/cm^2)	119-125
Column temperature (^oC)	Ambient
Volume of injection loop (µL)	20
Retention time (min)	2.780 and 7.093 for Levofloxaxin and Prulifloxacin respectively

System suitability

The HPLC system was stabilized for forty 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 calibration standard solution was injected to check the system suitability parameters like symmetric factor, number of theoretical plates and resolution.

Recommended procedure

Construction of calibration curve

Three replicates of each calibration standard solutions were (2, 4, 6, 8, 10 and 12 µg/mL) were injected in to the chromatogram, the retention times and average peak areas were recorded. Calibration graph was plotted by taking concentration of prulifloxacin on X-axis and ratio of peak areas of standard prulifloxacin to internal standard on Y-axis.

Assay of prulifloxacin

The content of twenty tablets was transferred into a mortar and ground to a fine powder. From this, tablet powder which is 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. 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 elutes were monitored with UV detector at 285 nm. The amount of drug present in sample was computed from the calibration graph.

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

Prulifloxacin sample solution spiked with levofloxacin was analyzed in triplicate and the resolution of the two compounds found to be greater than 10.0 indicates the specificity of the method. 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 prulifloxacin.

Precision

Six replicate injections of sample solution were given in to the HPLC system. Amount of prulifloxacin in sample for each injection is computed form its calibration curve. Low percent relative standard deviation and percent range of errors (0.005 and 0.001 confidence limits) were calculated and it reveals that the proposed method is precise.

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. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient values.

Accuracy

Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed method. 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.

Recovery studies

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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.

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

Parameter	Method
Retention time (t) minutes	2.780 and 7.093 for laevofloxacin and prulifloxacin respectively
Plates per meter (N)	311531
Peak asymmetry	1.026
Resolution	25.390
Linearity range (µg/mL)	2-12 µg/mL
Detection limits (µg/mL)	0.2613
Regression equation (Y = a + bc)	y = 0.109 x + 0.007
Slope(b)	0.109
Standard deviation of slope (S_b)	0.00143
Intercept (a)	0.007
Standard deviation of intercept (Sa)	0.0087
Standard error of estimation (Se)	0.231
Correlation coefficient (r)	0.999
Relative standard deviation (%)*	0.2008
Percentage range of errors* (Confidence limits)	
0.005 level	0.6020
0.001 level	0.7900
% error in bulk samples**	0.036

*Average of six determinations
** Average of three determinations

RESULTS AND DISCUSSION

The goal of this study is to develop rapid HPLC methods for the analysis of Prulifloxacin in bulk drug samples and tablet formulations using the most commonly employed column (C18) with UV detection at appropriate wavelength. The representative chromatograms indicating the PRF are shown in Fig. 1 to 6. The linearity of the method lies between 2-12 µg/mL (Fig. 7). System suitability, precision and accuracy of the proposed method for Prulifloxacin were represented in Table 2. Assay and recovery of PRF in dosage forms were presented in Table 3.

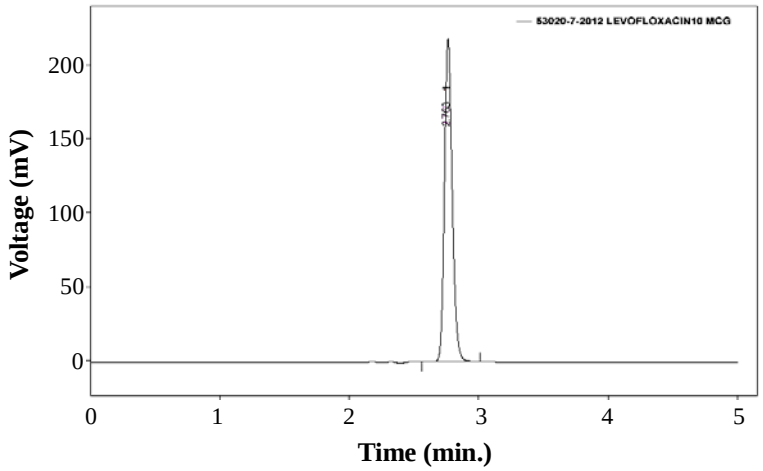

Fig. 1: Standard chromatogram of Levofloxacin (10 µg/mL)

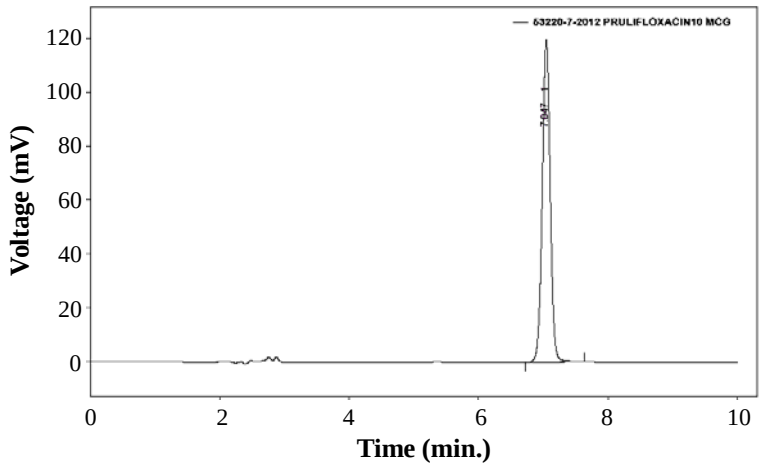

Fig. 2: Standard chromatogram of Prulifloxacin (10 µg/mL)

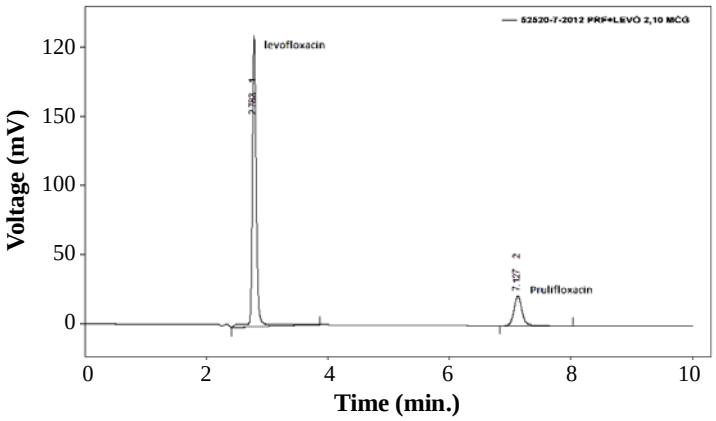

Fig. 3: Standard chromatogram of Prulifloxacin and Levofloxacin (2, 10 µg/mL)

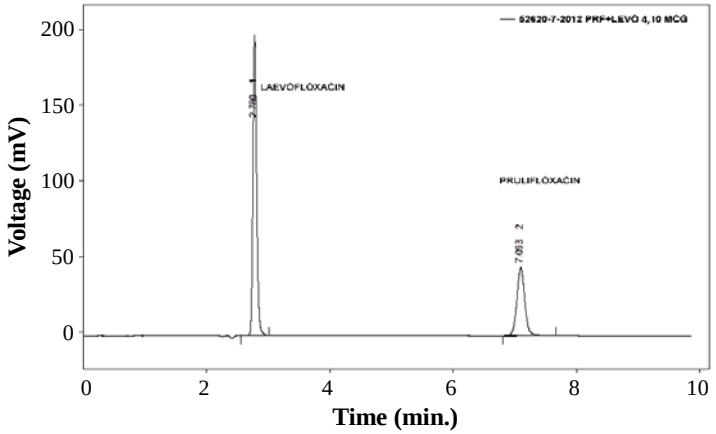

Fig. 4: Standard chromatogram of Prulifloxacin and Lovofloxacin (4 µg/mL)

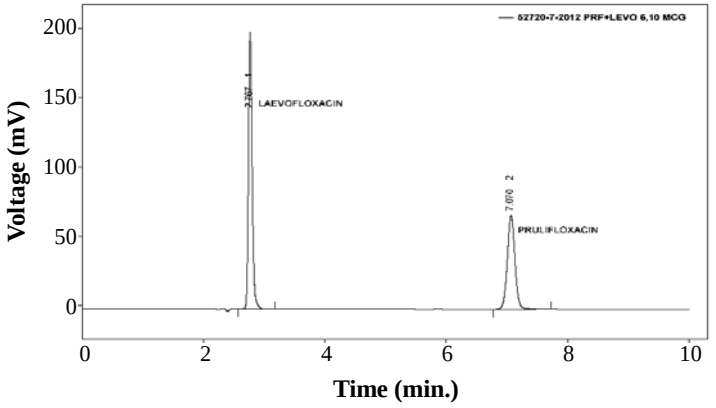

Fig. 5: Standard chromatogram of Prulifloxacin and Levofloxacin (6 µg/mL)

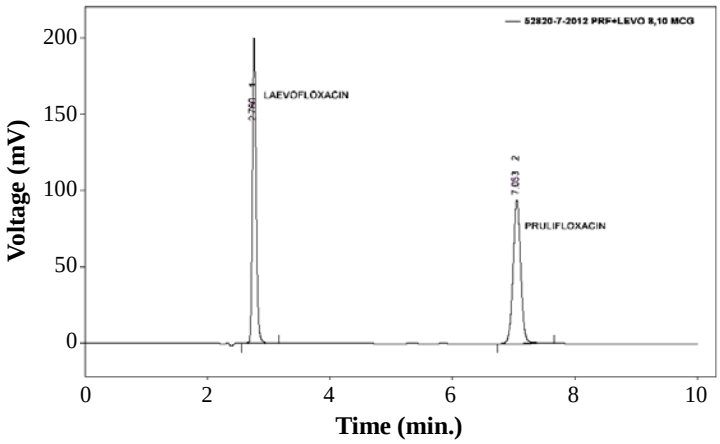

Fig. 6: Standard chromatogram of Prulifloxacin and Levofloxacin (8 µg/mL)

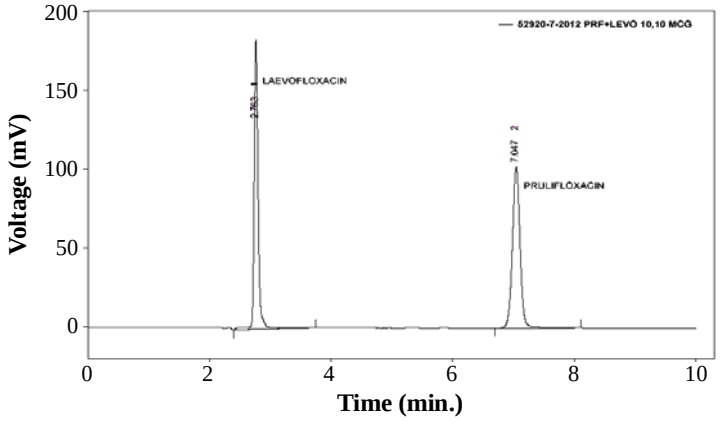

Fig. 7: Standard chromatogram of Prulifloxacin and Levofloxacin (10 µg/mL)

Table 3: Assay and recovery of Prulifloxacin in pharmaceutical dosage forms

Pharma-ceutical 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	0.831	1.831	596.72 ± 0.014	100.20 ± 0.52
T_2	600	599.92 ± 0.08	1.421	2.621	595.81 ± 0.013	99.78 ± 0.44

*Average ± standard deviation of six determinations: The t and F-values refer to comparison of 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 brand names of PRF. T_1 is pruflox (Cipla) and T_2 is Prudila (cadila)

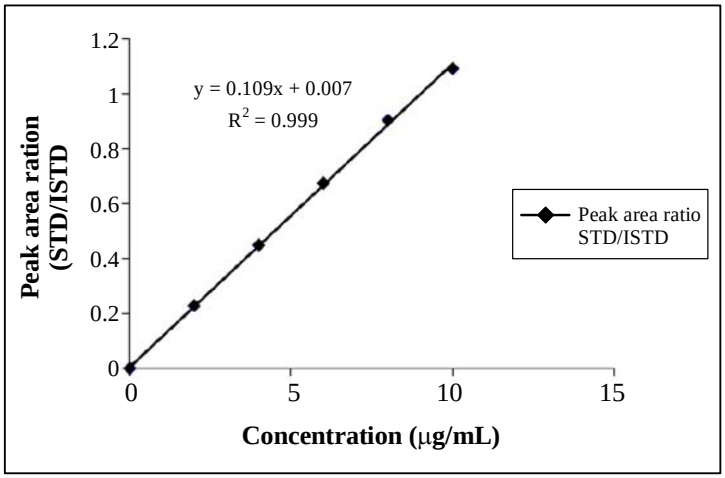

Fig. 8: Calibration plot of Prulifloxacin

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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