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COMPARATIVE STUDY OF PRULIFLOXACIN BY VALIDATED RPHPLC AND MICROBIAL AGAR ASSAY METHOD USING STUDENT'S T TEST

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ABSTRACT

A simple, accurate and precise RPHPLC method was developed for determination of Prulifloxacin in tablet dosage form. The chromatography was performed on a C18 column. Eluents were monitored by UV detection at 279 nm using the mobile phase Acetonitrile: Phosphate buffer pH 7(80:20). The method was statistically validated for linearity, accuracy, precision. The linearity of prulifloxacin was within the concentration range of 16-24 µg/mL. The limits of detection and quantitation were 0.189 µg/ mL and 0.5751 µg/ mL respectively. Microbial agar assay was chosen as a comparison method for the prulifloxacin determination. Assay of prulifloxacin determined in both methods were compared statistically using student's t test which confirmed that the two methods are statistically similar and there is no significant difference.

KEYWORDS: prulifloxacin, RPHPLC, microbial agar assay, student's t test.

INTRODUCTION

Prulifloxacin[1-2] is an older synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It is a prodrug which is metabolized in the body to the active compound ulifloxacin. It has been approved for the treatment of uncomplicated and complicated urinary tract infections, community-acquired respiratory tract infections and gastroenteritis, including infectious diarrhoeas. The chemical name of Prulifloxacin is (*RS*)-6-Fluoro-1-methyl-7-[4-(5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl-1-piperazinyl]-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid, as shown in chemical structure of Prulifloxacin in figure 1.

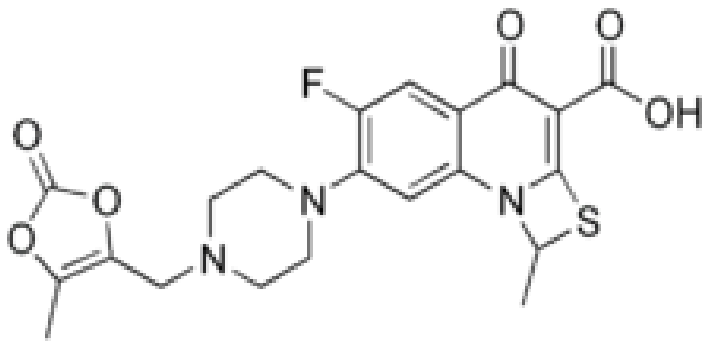

Fig. 1. chemical structure of prulifloxacin.

Literature surveys have revealed a number of methods for the estimation of Prulifloxacin depending on different analytical techniques, LC-MS/MS[3], RPHPLC[4], HPLC[5-6], UV Spectrophotometric methods[7-9].

Present study describes the development and validation of RPHPLC based method for determination of prulifloxacin and comparison of the same with previously validated microbial agar assay method using student's t test.

MATERIAL AND METHOD

Chemicals

All the reagents used for the analysis were of analytical grade. Standard Prulifloxacin was obtained as a gift sample from Micro labs, Bangalore .The Tablet Percin of strength 600 mg were obtained from the market manufactured by Lupin Pharma. Acetonitrile and HPLC grade water were procured from Merck.

Prulifloxacin Reference Solution

Accurately weighed 5 mg of prulifloxacin was transferred into 50 ml of volumetric flask and dissolved in mobile phase to obtain a final concentration of 100 µg/ml. Aliquots of this solution were further diluted with the same to obtain final concentrations.

Preparation of sample solution

Twenty tablets were weighed, crushed and their contents are mixed thoroughly. An accurately weighed portion of powder equivalent to the 10 mg of Prulifloxacin was weighed into a 100 ml volumetric flask containing about 50 ml of mobile phase. It was shaken thoroughly for about 5-10 min. and final volume was made with the same. Further filtered thoroughly with Whatman filter paper no. 41 to remove any insoluble matter. Aliquots of this solution were further diluted with same to obtain final concentration.

Preparation of mobile Phase (1 litre)

800 ml of Acetonitrile was mixed with 200 ml of buffer pH 7 and it was filtered through 0.45µm membrane filter.

Preparation of phosphate buffer pH 7 (1 litre)

0.5 g of anhydrous disodium hydrogen phosphate and 0.301 g of potassium dihydrogen phosphate were dissolved in sufficient water to produce 1000ml.

Chromatographic conditions

The method was conducted using RPHPLC technique. Prulifloxacin was eluted isocratically with a flow rate of 1.0 ml/min using a mobile phase consisting of Acetonitrile and phosphate buffer pH 7 in the ratio of 80:20. The wavelength of the UV–Vis detector was set to 279 nm. The mobile phase was prepared daily, filtered through a 0.45 µm membrane filter. Acclaim ™ 120 C18 5µm 120 A° (4.6 x 250 mm) column was used. The HPLC system was operated at room temperature.

Method Validation[10]

The RPHPLC method was validated as per ICH guidelines Q2 (R1) evaluating for linearity, precision, accuracy and robustness.

Linearity

Prulifloxacin reference solutions were prepared in triplicate at concentrations of 16, 18, 20, 22, 24 µg /ml. Standard plot was constructed and linearity was evaluated statistically by linear regression analysis that was calculated by least-squares regression.

Precision (Analysis of prulifloxacin tablet)

The precision of the method was determined by repeatability (intra-day). Six sample solutions of prulifloxacin tablet dosage form were prepared at 20 µg/ml. The results were expressed as % RSD. The assay was performed and content per tablet were calculated.

Accuracy

The accuracy was determined by the recovery of known amounts of the prulifloxacin reference standard added to the samples. The added levels were 80%, 100% and 120% of the sample concentration (20µg/ml). The results were expressed as the percentage of prulifloxacin reference standard recovered from the sample. All solutions were prepared in triplicate and assayed.

Robustness

Robustness testing was performed to evaluate the susceptibility of measurements under deliberate variations in selected analytical conditions. Factor assayed was the flow rate. Variation was 0.9ml/ min and 1.1 ml/min of the mobile phase flow rate.

Microbial agar assay method

The Microbial agar assay method previously developed and validated was used as a comparison method for the RPHPLC technique. Agar diffusion bioassay for determination of Prulifloxacin was been carried out using the strain *Salmonella typhimurium* ATCC 23564 as a test organism. To the sterile 85mm x 10 mm Petri plates 30 ml of Muller Hinton Agar was poured and allowed to solidify. After solidification of this layer, 0.1 ml of *Salmonella typhimurium* ATCC 23564 culture suspension was poured over the layer and was uniformly spread over the entire area. Using sterile cork borer, wells were bored at the centre of the Petri plates. 200 µl of drug solution was then filled into the well. Inoculated plates were kept in the refrigerator at 2-8 ^{O} C for 10-15 min for the diffusion of the test solution. The plates were then incubated at 37° c for 24 hrs. At the end of incubation period, inhibition zones formed around the well were measured with transparent scale in millimetre. The validation of the method showed good linearity that is R^2=0.997 in range of 20-60 µg/ml, precision was evaluated as RSD were observed RSD was 0, and Mean recovery was found to be 95.37% with RSD of 1.74%.

RESULTS AND DISCUSSION

The goal of this study is to develop rapid RPHPLC methods for the analysis of Prulifloxacin in drug samples and tablet formulations using the most commonly employed column (C18) with UV detection at appropriate wavelength and comparing the assay results using microbial agar method. The representative chromatogram indicating prulifloxacin is shown in figure 2.

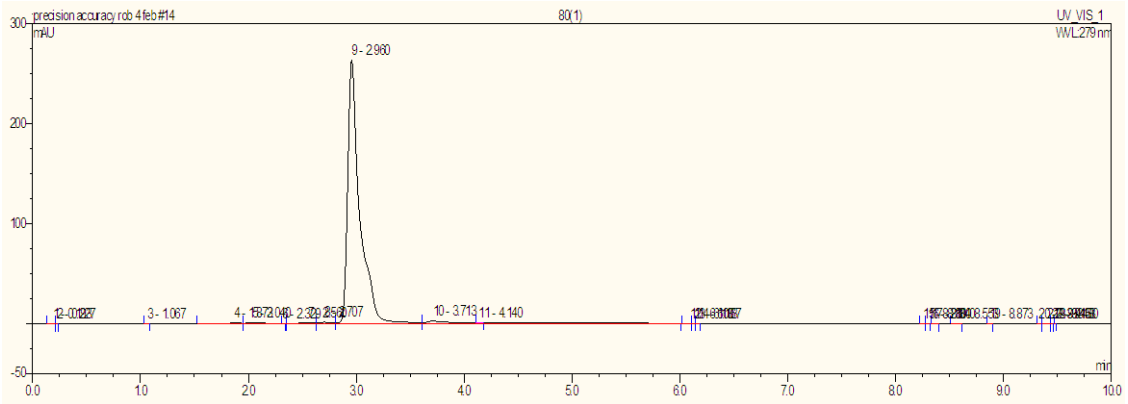

Fig. 2. standard chromatogram of prulifloxacin at 20 µg/ml.

Calibration curve for prulifloxacin was constructed by plotting area versus concentration which showed good linearity in the range of 16-24 µg/ml as indicated in figure 3.

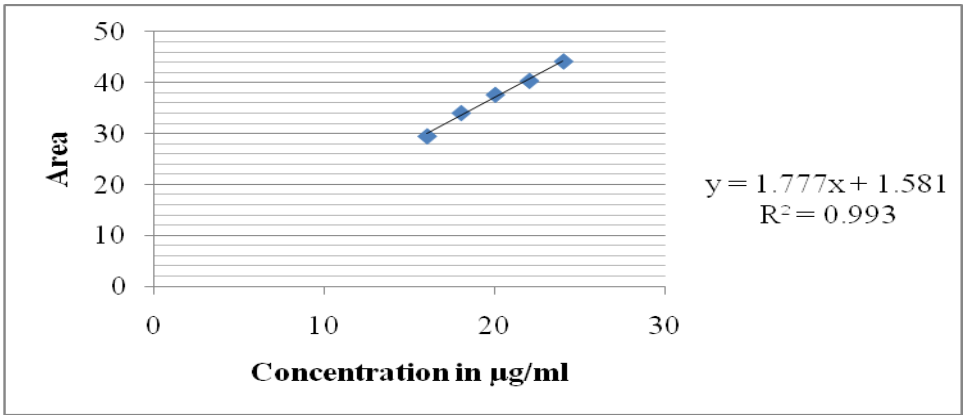

Fig. 3. calibration curve of prulifloxacin

Linearity and Range

A good linear relation was found between prulifloxacin concentration and area. The linearity data and regression analysis data is presented in Table no. 1 and 2 respectively.

Table no. 1: Linearity data

Conc. (µg/ml)	Level (%)	Volume of stock (ml)	Volume of mobile phase (ml)	Total volume	Area*
16	80	1.6	8.4	10 ml	29.50437
18	90	1.8	8.2		34.04403
20	100	2	8		37.61677
22	110	2.2	7.8		40.33313
24	120	2.4	7.6		44.1324

**Average of three determinations*

Table no. 2: Overview of regression analysis data

Parameter	Regression analysis data
Regression coefficient	0.993
Intercept	1.581
Slope	1.777
Regression equation	y = 1.777x + 1.581
Concentration range	16-24 µg/ml
Number of points	5

Precision

Precision was calculated for repeatability using prulifloxacin tablet and assay and content per tablet were evaluated. Precision data is tabulated in Table no. 3.

Table no. 3: Result of precision.

Sr no.	Concentration (µg/ml)	Area	Content	Assay %
1	20 µg/ml	37.953	616.65	102.77
2		37.1245	603.19	100.53
3		37.7374	613.15	102.19
4		38.0082	617.55	102.92
5		37.406	607.77	101.29
6		37.2018	604.45	100.74
		Mean	610.46	101.74
		SD		1.031
		%RSD		1.01%

Accuracy

Accuracy studies were done as percent recovery; it was performed by adding constant amount of the standard drug to the sample at levels of 80%, 100% and 120% of the test concentration. Mean recovery was found to be 100.46%. The results are tabulated in Table no. 4.

Table no. 4 Accuracy Data

Sr no.	Conc. of sample added. (µg/ml)	Level of addition (%)	Conc. of standard added (µg/ml)	Total Conc. (µg/ml)	Area	Conc. found (µg/ml)	% Recovery
1	10	80	8	18	33.9028	18.18	101
2					33.9609	18.22	101.2
3					33.5825	18	100
4		100	10	20	37.2018	20.04	100.2
5					37.5515	20.24	101.2
6					37.5285	20.22	101.1
7		120	12	22	40.8279	22.08	100.36
8					40.1457	21.7	98.63
9					40.8672	22.1	100.45
						Mean	100.46
						SD	0.8242
						%RSD	0.820

Robustness

Robustness is the ability to reproduce the analytical method under different circumstances without the occurrence of unexpected differences in the obtained results. By evaluating the results obtained from the analysis performed under the deliberately varied chromatographic

condition, the LC method developed indicated good performance, demonstrating to be a robust and reliable method in the determination of the drug. Results are shown in table no. 5.

Table no. 5: Results of Robustness

Flow rate	Retention time*	Area*
0.9ml/min	Mean :3.30 SD: 0.01 %RSD: 0.34	Mean:49.7028 SD:0.2841 %RSD:0.5717
1.1ml/min	Mean: 2.711 SD:0.0034 %RSD: 0.127	Mean:33.3878 SD:0.0584 %RSD:0.175

** Average of 3 determinations.*

Analysis of Tablet formulation by Microbiological method

Six sample solutions of prulifloxacin tablet dosage form were prepared at 20 µg/ml. The assay was performed and content per tablet were calculated. Results are tabulated in Table no. 6

Table no 6: Results of formulation analysis by Microbial assay

Sr no.	Concentration (µg/ml)	Zone diameter (mm)	Content per tablet (mg)	Assay %
1	20 µg/ml	30	608.1	101.35
2		30	608.1	101.35
3		30	608.1	101.35
4		30	608.1	101.35
5		30	608.1	101.35
6		30	608.1	101.35
		Mean	608.1	101.35
		SD		0
		%RSD		0

Comparison of RPHPLC and Microbial Assay in Determination of Prulifloxacin in Tablet Dosage Form.

Comparison of assay methods is been tabulated in Table no. 7.

Table no. 7: Comparison of RPHPLC and Microbial assay.

% Assay	
RPHPLC	**Microbial Assay**
102.77	101.35
100.53	101.35
102.19	101.35
102.92	101.35
101.29	101.35
100.74	101.35

Assay results obtained by two methods were statistically evaluated using student's t test, which indicated that assay results are not statistically different ($t_{calculated}$ 0.925 < $t_{theoretical}$ 2.228) which indicated that two methods are statistically same and there is no significant difference between the two. Therefore the methods developed and validated provide similar results for prulifloxacin quantification in tablet dosage form[11].

CONCLUSION

For daily quality control checks of pharmaceutical products it is essential to employ well characterised, fully validated analytical method to obtain reliable results that can be satisfactorily interpreted. The results obtained in this study show that the proposed RPHPLC method for determination of prulifloxacin in tablet dosage form is accurate, specific, robust, having a good linearity and precision characteristics. Moreover there is no statistical significant difference between the RPHPLC and microbial assay (previously developed and validated) for quantification of the drug. There the proposed RPHPLC and microbial assay can both be routinely used for the quantification of the dosage form.

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