DOI - 10.22270/jmpas.v8i3.839
Journal of Medical Pharmaceutical And Allied Sciences
RESEARCH ARTICLE

www.jmpas.com
ISSN 2320-7418

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

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Keywords
Ulifloxacin, RP-HPLC, Stress testing. Antimicrobial, Non-polar

Received
15/04/2019
Reviewed
29/04/2019
Revised/ Accepted
04/05/2019

ABSTRACT

The current research study, a successful practice was done for **"Validated HPLC method development for the estimation of Prulifloxacin in marketed formulation".** Analytical method validation of Prulifloxacin tablets was developed via material and method plan which is based on logical experimental procedure through literature survey associated with statistical tools. Major instrument which was used is UV spectrophotometer (make shimadzu) and HPLC system was Shimadzu LC-2010CHT with Chromalion software. The isocratic mobile phase consisted of mixture of phosphate buffer preparation and acetonitrile in the ratio of 150:850 ml v/v and with PH 7.4 by adding triethylamine. Chromatographic parameters was follow is Column Xterra C18 125Å (250mm x 4.6mm x 0.5µm), Flow rate 1 ml/min, Injection volume 20µl, Column oven temperature 35ºC, Sampler temperature 2-8ºC and Run time 10 minute. Assay of prulifloxacin tablets (Alpruli 600 mg) in three set occur average %age is 100.63, all value between 98-102%. Proposed method was found to be linear in the range of 20-100 µg/ml prulifloxacin with the correlation coefficient near to one respectively. The validation and the reliability of proposed method was assessed by recovery study. The recovery of added standards (80%, 100% 120%) 99.7 %, 100.7 % and 100.4 % for prulifloxacin respectively. found prulifloxacin is stable in low temperature between 2-8ºC upto 48 Hours.

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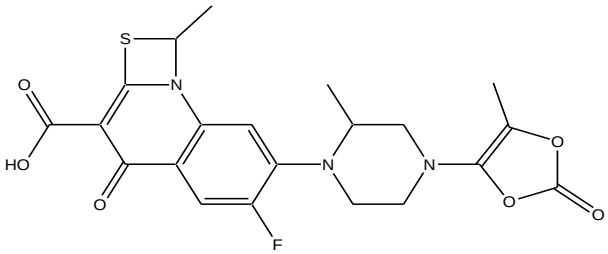
INTRODUCTION

The RP-HPLC method developed for quantitative and qualitative analysis of prulifloxacin tablets was rapid, simple, accurate, precise and specific. Here conclude that this method is one of best method available in market because it is economically proved. Recovery study on tablet formulation gave accurate prediction of good relation between label claim and practical value. Method is specific due to it shows no interference in analyte peak of any unknown or known compounds. Method was validated as per USP acceptance criteria and ICH guidelines. Hence present validated method can be confidently utilize as an alternative method to reporting of result in routine practice or release of product report in quality control compliance (1).

Prulifloxacin is a bactericidal antimicrobial agent, it inhibit bacterial DNA gyrase enzyme and lead to prevent DNA transcription, replication, repair. Prulifloxacin have broad spectrum antimicrobial activity it also inhibit topoisomerase IV which tend to lead bactericidal effect. Maximum daily dose 600 mg in tablet form day 1 to maximum 10 days. For treatment of lower urinary tract infection and acute exacerbation of chronic bronchitis, the duration of treatment according to the severity of the disease and on patient response and should be continued for at least 48-72 hour after mild cure /recovery of symptoms (2).

Molecular Structure and IUPAC name of Prulifloxacin



6-Fluoro-1-methyl-7-[4-(5-methyl-2-oxo-1, 3-dioxolen-4yl) methyl-1-piperazinyl]-4-oxo-4*H*-[1, 3] thiazide [3, 2-*a*] quinoline-3-carboxylic acid.

MATERIAL AND METHOD

Major instrument which was used is UV spectrophotometer (make shimadzu) and HPLC system was Shimadzu LC-2010CHT with Chromalion software. Shown maximum absorbance at 282 nm. The isocratic mobile phase consisted of mixture of phosphate buffer preparation and acetonitrile in the ratio of 150:850 ml v/v and with PH 7.4 by adding triethylamine. Chromatographic parameters was follow is Column Xterra C18 125Å (250mm x 4.6mm x 0.5µm), Flow rate 1 ml/min, Injection

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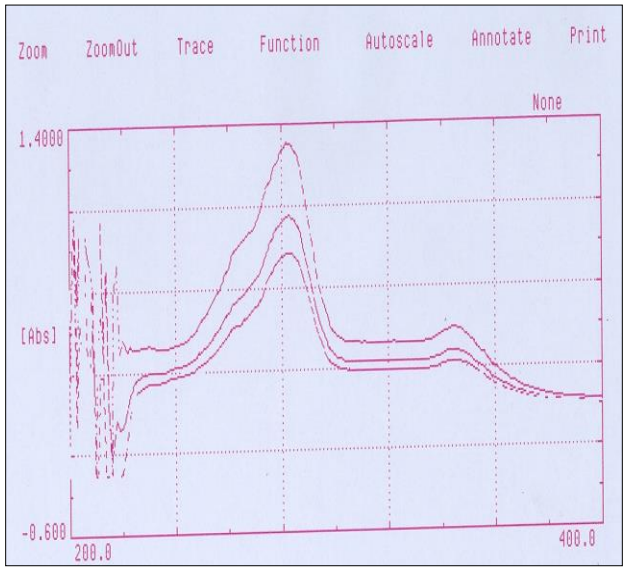
volume 20µl, Column oven temperature 35ºC, Sampler temperature 2-8ºC and Run time 10 minute (3).

DETERMINATION OF ʎmax

Determination of λ_{max} was done by scanning of solution which contain Prulifloxacin in various solvent system such as maximum absorbance at 282 nm which is reference for chromatographic development (4).

1. Dimethyl formamide: Methanol (40: 60 % V/V)
2. Dichloromethane: Acetonitrile (20: 80 % V/V)
3. Acetonitrile: Methanol (50: 50 % V/V)



MOBILE PHASE PREPARATION

Weighed accurately and transferred 1.36 gram of Potassium dihydrogen phosphate in 1000 ml of water, adjusted pH 7.4 with triethylamine filter through 0.45 µ nylon membrane filter under vacuum. Then prepared a degassed mixture of above buffer preparation and acetonitrile in the ratio of 150:850 ml v/v and mixed well, sonicated for 10 minutes, allowed to cool at room temperature. Mobile phase was developed for further validation study (5).

VALIDATION OF DEVELOPED HPLC METHOD

LINEARITY:

Linearity plot was on five different concentration of Prulifloxacin. A stock solution of 1000 µg/ml was prepared in mobile phase and successively diluted from stock to get concentration from 20-100 µg/ml of Prulifloxacin. Each concentration was injected triplicate in chromatographic system. Area was recorded against concentration then plotted by using linear regression slope, intercept and co-efficient of co-relation with in 98% confidence was established (6).

Acceptance criteria: Co-relation co-efficient ($R^{2)} \geq 0.999$

Concentration (µg/ml)	Area response	Statistical Analysis
20	435.453	Slope = 32.899
40	858.952	Intercept = 22.53
60	1298.378	
80	1767.472	

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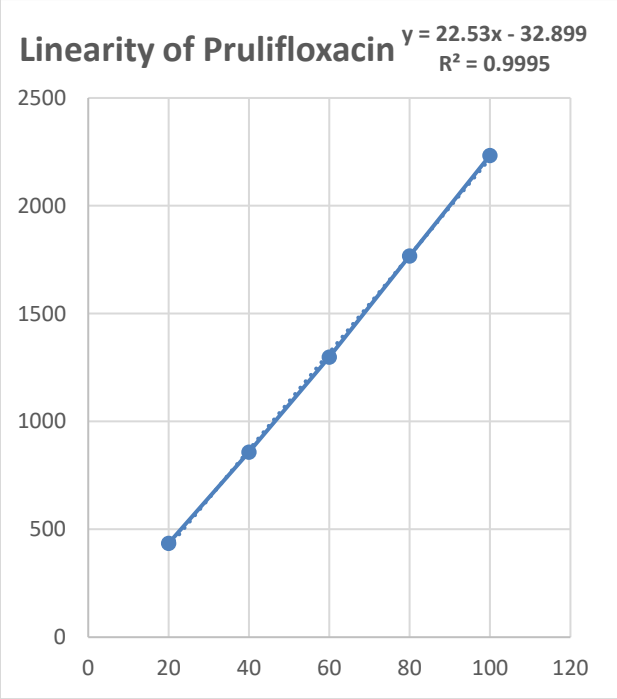
100	2234.164	Correlation coefficient = 0.999



X axis = Concentration in µg/m

Y axis = (AUC)/ Area response.

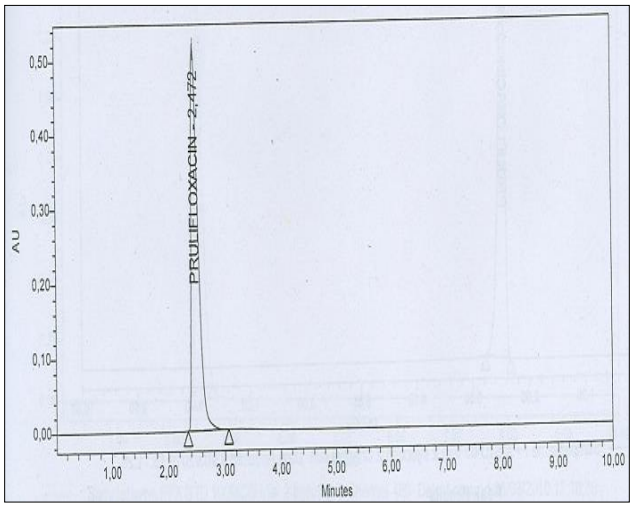
ACCURACY AS RECOVERY:

True value obtained after test result which is closeness to achieve Accuracy. Perform recovery study in means of percentage by applying analytical procedure of the standard sample from the range of 80%, 100% and 120% level of the test concentration. Here calculated the drug content in marketed formulation (Tablets) by addition of known quantity of standard this method is called as standard addition method (7).

Recovery study for Prulifloxacin

Initial Amount (mg) [A]	Addition of known quantity (µg/ml) [B]	Final concentration (µg/ml)	% Recovery	Average Recovery n=3
100	800	80	98.17	99.4
			101.32	
			98.82	
100	1000	100	100.54	100.7
			102.39	
			99.07	
100	1200	120	100.22	100.4
			101.17	
			99.71	

HPLC chromatogram of linearity



Preparation of Standard Solution:

Weighed accurately 100 mg of Prulifloxacin reference standard into 100 ml volumetric flask, add 50 ml of mobile phase sonicated to dissolve, made volume with mobile phase and mixed well. Diluted accurately 1.0 ml of Prulifloxacin Stock Solution into 10 ml volumetric flask, made volume with mobile phase and mixed well. This solution was

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80% sample solution. The resulting concentration of test sample 100 µg/ml of Prulifloxacin injected in triplicate (8).

Recovery is calculated as

Concentration obtained

$$= \frac{\text{Area of test} \times \text{dilution of std.} \times \text{potency}}{\text{Area of std.} \times \text{dilution of test}}$$

Acceptance criteria: Range 99.0% to 110.0%

Preparation of sample Stock Solution:

Marketed formulation 20 tablets of Prulifloxacin were taken and calculate average weight then made a fine powder with the help of mortar pestle. Recorded average weight 1027 mg. weighed accurately and transferred powder equivalent to 100 mg ≈ (About 172 mg) of Prulifloxacin were taken and into 100 ml volumetric flask added 50 ml of mobile phase sonicated for 30 minutes with intermittent shaking , allowed to attain room temperature made volume upto the mark with mobile phase and mixed (9). Centrifuge solution at 3500 rpm for 10 minutes made volume upto the mark with mobile phase and mixed. The resulting concentration of test sample 1 mg/ml of Prulifloxacin were used in the further study (10).

PREPARATION OF SAMPLE SOLUTION

Preparation of 80% sample solution

Diluted accurately 0.8 ml of Prulifloxacin Stock Solution into 10 ml volumetric flask, made volume with mobile phase and mixed well. This solution was 80% sample solution. The resulting concentration of test sample 80 µg/ml of Prulifloxacin injected in triplicate (11).

Preparation of 100% sample solution

Diluted accurately 1.0 ml of Prulifloxacin Stock Solution into 10 ml volumetric flask, made volume with mobile phase and mixed well. This solution was 80% sample solution. The resulting concentration of test sample 100 µg/ml of Prulifloxacin injected in triplicate (12).

Preparation of 120% sample solution

Diluted accurately 1.2 ml of Prulifloxacin Stock Solution into 10 ml volumetric flask, made volume with mobile phase and mixed well. This solution was 80% sample solution. The resulting concentration of test sample 120 µg/ml of Prulifloxacin injected in triplicate (13).

Precision:

A) Intra Day Precision

Repeatability was performed by injecting the sample in triplicate. Stock solution of 1000 µg/ml Prulifloxacin was prepared and diluted to get concentration of 10µg/ml to 100 µg/ml. Each diluted solution was

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injected triplicate in chromatographic system. The area of analyte peak of all three injections was recorded and calculated mean RSD (14).

Acceptance criteria: % RSD Not more than 2.0 %

Intra Day Precision Study by HPLC

Conc. of Prulifloxacin µg/ml	Area response	Statistical analysis
40	848.452	Mean= 847.984
40	853.476	% R.S.D= 0.68
40	842.024	
60	1375.4476	Mean = 1388.247
60	1389.563	% R.S.D = 0.88
60	1399.731	
80	1787.476	Mean = 1781.131
80	1765.465	% R.S.D = 0.77
80	1790.452	

B) Inter Day Precision

This was done on different day using previous day prepared three different concentration levels 10µg/ml to 100 µg/ml. Each diluted solution was injected in triplicate. Each diluted solution was injected triplicate in chromatographic system. The area of analyte peak of all three injections was recorded and calculated mean RSD (15).

Acceptance criteria: % RSD not more than 2.0%

Inter Day Precision Study by HPLC

Conc. of Prulifloxacin (µg/ml)	Area response	Statistical analysis
40	868.568	Mean= 866.671
40	872.286	% R.S.D= 0.78
40	859.158	
60	1387.487	Mean =
60	1379.057	1386.310
60	1392.386	% R.S.D = 0.49
80	1757.89	Mean =
80	1765.255	1767.635
80	1779.761	% R.S.D = 0.63

RANGE: The range was established from the accuracy, linearity and precision studies.

SPECIFICITY:

Specificity is the term extent that analyte may be exist without interference from other related compound in a mixture. Specificity able to differentiate all possible impurities by applying forced stress testing. When in any chromatogram analyte peak not affected from other known or unknown impurity it indicates that chromatographic parameters good as per specificity point of view (16).

Acceptance Criteria:

a) There should not be any interference from blank, excipient and reagent peaks with main peak.

b) The peak purity index for the main peaks and degradation product peaks in standard preparation and sample

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S.No.	Chromatographic conditions	Level	Prulifloxacin retention time
A	**pH of buffer**		**Retention Time**
	7.2	-2	2.552
	7.4	0	2.572
	7.6	+2	2.547
Mean 2.557			**%RSD** 0.52
B	**Flow rate(ml/min)**		**Retention Time**
	0.8	-2	2.507
	1	0	2.545
	1.2	+2	2.592
Mean 2.548			**%RSD** 1.67
C	**% of Acetonitrile**		**Retention Time**
	890	-2	2.565
	900	0	2.547
	910	+2	2.499
Mean 2.537			**% RSD** 1.34

preparation should be equal to or more than 0.990.

Specificity Testing (Acid stress)

Concentration µg/ml	Time (hours)	Retention time	RT of degraded product
100	0	2.510	----
100	8	2.511	----
100	24	2.512	----

Specificity Testing (Base stress)

Concentration µg/ml	Time (hours)	Retention time	RT of degraded product
100	0	2.510	----
100	8	2.511	----
100	24	2.510	----

Specificity testing (Peroxide stress)

Concentration µg/ml	Time (hours)	Retention time	RT of degraded product
100	0	2.510	----
100	8	2.511	----
100	24	2.512	----

ROBUSTNESS:

Robustness is the degree of deliberate changes on chromatographic parameters which is show reproducible results. A working solution of 100 µg/ml (100 PPM) for Prulifloxacin was taken and the following method parameters were changed independently of each other.

- Mobile phase ratio (±2%)
- Flow rate (±0.2 ml/min)

Injected three times each with changes in parameters (17).

Acceptance criteria: - % RSD Not more than 2.0%

ANALYSIS OF MARKETED FORMULATION OF PRULIFLOXACIN BY HPLC:

Marketed formulation 20 tablets of Prulifloxacin were taken and calculate average weight then made a fine powder with the help of mortar pestle. Recorded average weight 1027 mg. weighed

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accurately and transferred powder equivalent to 100 mg ≈ (About 172 mg) of Prulifloxacin were taken and into 100 ml volumetric flask added 50 ml of mobile phase sonicated for 30 minutes with intermittent shaking , allowed to attain room temperature made volume upto the mark with mobile phase and mixed. Centrifuge solution at 3500 rpm for 10 minutes (18). Diluted 1.0 ml of above preparation to 10 ml with mobile phase and mixed well. Filter through 0.45 µm PVDF filter by discarding first 5 ml of the filtrate. The resulting concentration of test sample 100 µg/ml of Prulifloxacin were used in the further study (19).

Assay of Prulifloxacin by HPLC

S. No.	Theoretical content (mg/ml)	Amount of drug recovered (mg)	% Recovery	Mean % recovery
1	**600**	**601.673**	100.28	**100.63**
2	**600**	**609.941**	101.66	
3	**600**	**599.742**	99.96	

The LOD was calculated as 0.1504 µg/ml of Prulifloxacin.
The LOQ was calculated as 0.4353µg/ml of Prulifloxacin.

SOLUTION STABILITY

In present study shows stability of drug in solution form in different condition and measured it in area response form. Here taken same solution which was injected as… Standard solution and assay sample. Solution concentration was 100 µg/ml (20).

1. After 24 Hours in 2-8°C Temperature
2. After 48 Hours in 2-8°C Temperature
3. After 24 Hours in Room Temperature
4. After 48 Hours in Room Temperature

After 24 Hours in 2-8°C Temperature			
Standard solution		**Tablet sample solution**	
Initial Area response	**After Area response**	**Initial Area response**	**After Area response**
Set 1 - 2254.157	2255.573	Set 1 - 2305.562	2301.487
Set 2 - 2267.386	2266.027	Set 2 - 2300.462	2308.478
Set 3 - 2243.573	2251.472	Set 3 - 2299.341	2298.487

After 48 Hours in 2-8°C Temperature			
Standard solution		**Tablet sample solution**	
Initial Area response	**After Area response**	**Initial Area response**	**After Area response**
Set 1 - 2254.157	2234.484	Set 1 - 2305.562	2299.676
Set 2 - 2267.386	2247.438	Set 2 - 2300.462	2297.238
Set 3 - 2243.573	2228.398	Set 3 - 2299.341	2291.376

After 24 Hours in Room Temperature			
Standard solution		**Tablet sample solution**	
Initial Area response	**After Area response**	**Initial Area response**	**After Area response**
Set 1 - 2254.157	2198.237	Set 1 - 2305.562	2257.347

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Set 2 - 2267.386	2201.357	Set 2 - 2300.462	2221.720
Set 3 - 2243.573	2189.326	Set 3 - 2299.341	2230.106

After 48 Hours in Room Temperature			
Standard solution		**Tablet sample solution**	
Initial Area response	**After Area response**	**Initial Area response**	**After Area response**
Set 1 - 2254.157	2101.478	Set 1 - 2305.562	2198.471
Set 2 - 2267.386	2105.094	Set 2 - 2300.462	2179.295
Set 3 - 2243.573	2100.462	Set 3 - 2299.341	2135.572

CONCLUSION

The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value and %RSD less than 2), Precise and it can be used for intended purpose on tablet dosage form. The Simplicity, Rapidly and Reproducibility of the developed method qualify the objective of research. Results of analysis of the prulifloxacin tablet formulations are arranged in result and discussion section. The portion of prulifloxacin found on terms of quantity was between 98-102% and also within USP acceptance criteria.

The RP-HPLC method developed for quantitative and qualitative analysis of prulifloxacin tablets was rapid, simple, accurate, precise and specific. Here conclude that this method is one of best method available in market because it is economically proved. Recovery study on tablet formulation gave accurate prediction of good relation between label claim and practical value. Method is specific due to it shows no interference in analyte peak of any unknown or known compounds. Method was validated as per USP acceptance criteria and ICH guidelines. Hence present validated method can be confidently utilize as an alternative method to reporting of result in routine practice or release of product report in quality control compliance.

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