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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF RELATED SUBSTANCES IN BULK FORM OF PRULIFLOXACIN AND SUBSEQUENT DEGRADATION STUDIES

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ABSTRACT

A stability-indicating reversed-phase HPLC method has been developed, optimized and validated for quantitative analysis of degradation products and process impurities of prulifloxacin in the bulk drug. Separation of the drug from possible impurities was achieved by reverse-phase YMC-Pack ODS AQ, 150 x 4.6mm, 5µm particle size column with the mobile phase consisted of A and B, 0.04 M ammonium acetate buffer adjusted to a pH of 3.5 ± 0.05 with acetic acid as solvent A and degassed mixture of 0.04 M ammonium acetate buffer and acetonitrile in the ratio of 80:20 as solvent B, at an flow rate of 0.8 mL/min with UV detection wavelength of 277 nm with 10 µL sample volumes enabled separation of the drug from its degradation products. The retention time of prulifloxacin was found to be 19.04 minutes. The developed method was validated for linearity, accuracy, robustness and system suitability as per guidelines recommended by ICH. Prulifloxacin was subjected to stress conditions such as hydrolysis (acid and base), oxidation (photolysis, and thermal), the stressed samples were analyzed by use of this method. Maximum degradation was observed in acid and base hydrolysis and oxidation. The drug was also susceptible to degradation under photolytic and thermal conditions. The degradation products of PFN were well resolved from main peak thus proving the stability indicating nature of the method. The method developed was suitable for stability indicating nature, in process and quality control analysis which is simple, robust, linear and precise.

***KEYWORDS**: Prulifloxacin (PFN), Reverse Phase – HPLC, Validation, Degradation.*

INTRODUCTION

Antimicrobial activity, efficacy and relative safety of fluoroquinolones have been attractive in the last few years for the treatment of community acquired and nosocomial infections [1]. Prulifloxacin (PFN) is a newer fluoroquinolone broad spectrum antibiotic used for the treatment of complicated and uncomplicated urinary tract infections, whose chemical name is 6-fluoro-1-methyl-7-[4-[5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]-1-piperazinyl]-4-oxo-1H,4H-[1,3]thiazeto[3,2-a]quinolone-3-carboxylic acid Figure 1. It is a lipophilic prodrug of ulifloxacin [2].

According to review of literature, determination of prulifloxacin have been reported in the past such as spectrophotometric method [3], RP-HPLC method [4, 5] and stability indicative assay method [6], degradation studies [7]. Although RP-HPLC method development, validation and degradation for prulifloxacin has been developed but no specific RP-HPLC had been developed for the estimation of relative substances in prulifloxacin and its degradation studies together in bulk form.

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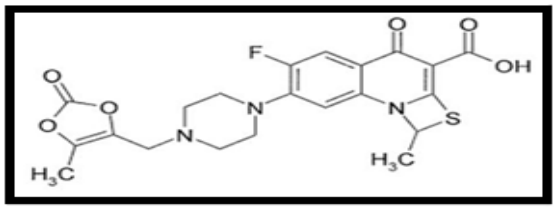

Fig. 1: Chemical Structure of prulifloxacin

The main objective of this work was to develop a new analytical reverse phase HPLC method for PFN. The developed method was validated as per ICH regulatory guidelines and subsequently the degradation of PFN was studied using the same method.

MATERIALS AND METHODS

Instrumentation:

Quantitative HPLC was performed using a high performance liquid chromatography (Shimadzu LC-20AT prominence liquid chromatography), auto injector with loop volume of 10 µL, and YMC-Pack ODS AQ 150 x 4.6mm, 5µm Column. The HPLC system was equipped with "Empower 3" software.

Chemicals:

Prulifloxacin was kindly provided by Hetero Labs Ltd., Hyderabad, India. Triethyl amine and methanol of HPLC grade, hydrochloric acid, hydrogen peroxide and sodium hydroxide of GR

grade, ethanol of AR grade are from Merck Pharmaceuticals Ltd., Mumbai, India. Milli Q purification system was used to get milli Q water.

Characterization of Prulifloxacin:

Selection of wavelength: UV spectra of prulifloxacin solution was scanned at a range of 190-320nm using double beam UV-VIS spectrophotometer Shimadzu UV-1800 using UV probe software to optimize the wave length.

Preparation of mobile phase:

A 0.04M buffer Ammonium Acetate was prepared by dissolving about 3.08g of solid Ammonium Acetate in 1000 mL of millipore water and pH was adjusted to 4.0 ± 0.05 with acetic acid. The prepared solution was filtered through 0.45µm membrane filter and degassed by sonication and thus taken as Solvent A.

The above prepared buffer and acetonitrile were mixed in the proportion of 80 : 20 v/v and was filtered through 0.22 µm nylon membrane filter and degassed by sonication and thus taken as Solvent B.

Sample Preparation: A mixture of standard solution was prepared by weighing prulifloxacin and its impurities and dissolving it in diluent containing acetonitrile and methanol in the ratio of 50:50 (v/v).

Chromatographic Conditions for Prulifloxacin:

Chromatographic separation was achieved on an YMC-Pack ODS AQ reversed phase column (4.6mm × 150) packed with 5µm diameter particles. RP-HPLC separation for PFN was observed at 277 nm and column temperature at 30^{O}C and sample temperature at 5^{O}C were maintained. The flow rate and injection volume were 0.8 mL/min and l0µl, respectively. Gradient program was set as Time per percentage (%) of solution B [T/%]: 0.01/10, 5/10, 45/80, 55/80, 60/10, 65/10. All chromatographic operations were carried out over a run period of 65 minutes.

System suitability:

The HPLC system was stabilized for 60 minutes by following the chromatographic conditions as described above to get a stable base line. Injections containing of single calibration standard solution was given to check the system suitability parameters like symmetric factor, number of theoretical plates and resolution.

Method Validation: The developed method was validated in terms of accuracy, precision, linearity and robustness according to ICH guidelines.

Forced degradation: Prulifloxacin was subjected to stressed conditions such as hydrolysis (acid, base), Oxidation, thermal and photolytic, the stressed samples were analyzed.

RESULTS AND DISCUSSION

HPLC method development and optimization:

The main objective of this study was to develop more specific RP-HPLC method for PFN to achieve good separation between prulifloxacin and all its related impurities. The wavelength detection was selected at 277nm as all the relative impurities and prulifloxacin shown maximum absorbance at this wavelength. Resolution, peak symmetry was satisfactory by using the optimized chromatographic conditions.

System Suitability:

The peak shape of prulifloxacin was found to be symmetric and well separated by its relative components as shown in Figure 2. The theoretical plate count was found to be more than 2000, and retention time was found to be 19.04 for PFN. The system suitability parameters were evaluated from standard chromatograms shown in Figure 2 and results in Table 1.

Validation Results:

Precision: Precision was performed and % RSD of six replicate injections was determined and observed to be within the acceptable limits. Results are tabulated in Table 2.

Linearity: Linearity was performed by using varying concentrations ranging from 0.03 level to 0.15 level of prulifloxacin and its impurities. Linearity graphs were constructed for prulifloxacin and for its impurities by plotting the concentration of PFN versus absorbance. Correlation coefficient was evaluated. The correlation coefficient of all linearity plots was equal to 0.999 as shown in linearity plot Figure 3. The regression equations, slope and intercept were calculated and results were shown in Table 3. Linearity plots for all the related impurities were obtained and are found to be linear.

Accuracy: Accuracy of the proposed method was performed and the obtained recovery values indicate the trueness of the method to estimate PFN related impurities. The acceptance criteria are 80-120%. The obtained percent recovery values of PFN and its impurities in Table 4 are in the range of 96.3-114.5% which declares that the method as accurate.

Robustness: Robustness study was performed by making variations in chromatographic conditions such as pH and temperature keeping other parameters constant to prove the reliability of the method for PFN. The results of pH variation and temperature are shown in Table 5 and Table 6 respectively.

Forced degradation studies:

The degradation studies of Prulifloxacin and its impurities indicated that the compound was stable when exposed to thermal, humidity, water hydrolysis and photolytic conditions. Significant degradation was observed upon exposure to acid, base hydrolysis and oxidation conditions. The standard and its impurities were screened from 200 nm to 400 nm and apart from above no other significant impurities were found. It is observed that the proposed method was capable to separate all the process and degradation impurities. Therefore the related compounds of Prulifloxacin by RP-HPLC was proved to be a stability indicative method. The results for solid and liquid state forms of PFN and its impurities shown in Table 7 and Table 8.

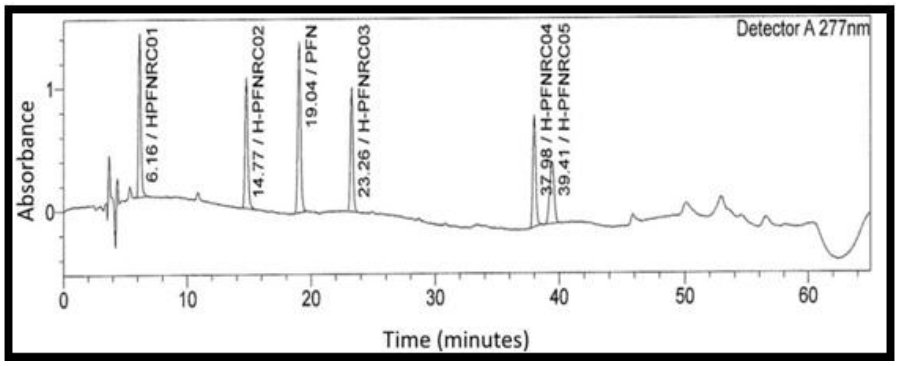

Fig. 2: System suitability chromatogram of PFN and its impurities

Table No. 1: System suitability results

Peak#	RT	Area	Height	Area%	RRT	Resolution	Name
1	6.16	17861	1335	17.94	0.32	....	HPFNRC01
2	14.77	16650	1065	16.72	0.78	22.79	HPFNRC02
3	19.04	22544	1381	22.64	1.00	10.22	PFN
4	23.26	14973	1010	15.04	1.22	10.23	HPFNRC03
5	37.98	14470	904	14.53	1.99	35.87	HPFNRC04
6	39.41	13083	503	13.14	2.07	2.55	HPFNRC05
Total		99581	6198	100.1			

Table No. 2: Precision results

S. No.	Area counts	Area counts	Area counts	Area counts	Area counts	Area counts
	PFN	HPFNRC01	HPFNRC02	HPFNRC03	HPFNRC04	HPFNRC05
1	22544	17861	16650	14973	14470	13083
2	22540	17641	16529	15178	14554	13171
3	22619	17656	16689	15019	14346	13350
4	22610	17392	16546	15134	14596	12921
5	22931	17570	16682	15039	14534	13024
6	22747	17439	16740	15239	14536	13047
Average	22665	17593	16639	15097	14506	13099
St. dev	150	169	84	103	88	147
% RSD	0.66	0.96	0.51	0.68	0.61	1.12

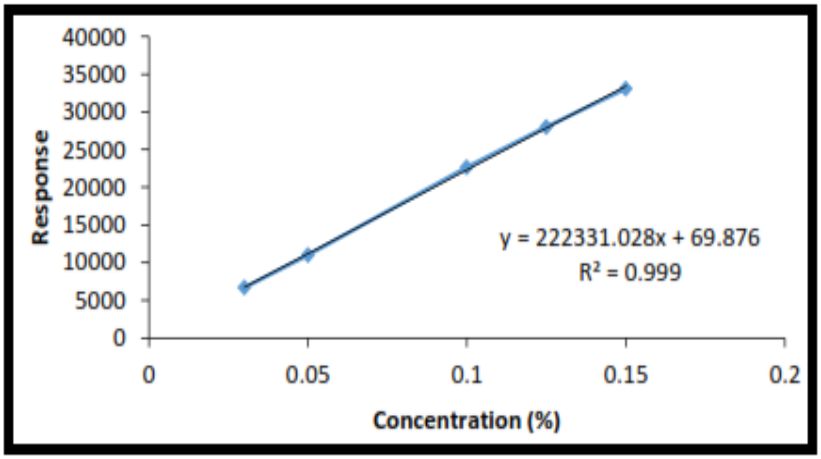

Fig. 3: Linearity plot of PFN

Table No. 3: Linearity of PFN Concentration v/s Response

Concentration in % (X axis)		Area
1	0.0300	6690
2	0.0500	11023
3	0.1000	22665
4	0.1200	28030
5	0.1500	33102
Correlation coefficient		**0.9997**
Intercept		**70**
% Y Intercept		**0.21**

Table No. 4: Accuracy results of PFN

% Recovery	Accuracy at		
	0.05% Level	0.10% Level	0.15% Level
H-PFNRC01	108.5	107.8	114.5
H-PFNRC02	99.4	100.0	103.0
H-PFNRC03	99.5	97.9	101.5
H-PFNRC04	97.2	96.3	110.5
H-PFNRC05	103.1	100.8	105.4

Table No. 5: Results of robustness study by variation in pH (by +/- 0.1 unit of initial pH)
[MSUI: Maximum Single Unknown Impurity TI: Total Impurity]

Name of the Impurity	Initial pH 3.5 Results (%)	pH 3.4 Results (%)	Variation	pH 3.6 Results (%)	Variation
H-PFNRC01	14.25	12.62	1.63	13.14	1.11
H-PFNRC02	0.33	0.32	0.01	0.32	0.01
H-PFNRC03	0.27	0.26	0.01	0.26	0.01
H-PFNRC04	0.22	0.25	0.03	0.24	0.02
H-PFNRC05	0.25	0.26	0.01	0.25	0.00
MSUI	0.13	0.11	0.02	0.15	0.02
TI	15.70	14.01	1.69	14.59	1.11

From the above Table 5 it can be observed that there was no significant change except in H-PFNRC01. Results were comparable proving that proposed method for PFN was robust.

Table No. 6: Results of robustness study by variation in temperature (by +/- 2^{o}C of initial temperature)
[*MSUI: Maximum Single Unknown Impurity TI: Total Impurity*]

Name of the Impurity	Initial 40 °C Results (%)	38 °C Results (%)	Variation	42 °C Results (%)	Variation
H-PFNRC01	14.25	13.68	0.57	14.43	0.18
H-PFNRC02	0.33	0.32	0.01	0.32	0.01
H-PFNRC03	0.27	0.25	0.02	0.25	0.02
H-PFNRC04	0.22	0.23	0.01	0.23	0.01
H-PFNRC05	0.25	0.26	0.01	0.26	0.01
MSUI	0.13	0.48	0.35	0.35	0.22
TI	15.70	15.61	0.09	16.48	0.78

From the above Table 6 it can be observed that there was no significant change. Results were comparable proving that proposed method for PFN was robust.

Table No. 7: Degradation conditions of PFN and its impurities (solid state)
[MSUI: Maximum Single Unknown Impurity TI: Total Impurity]

Name of the Sample	Mother sample (As such)	Thermal sample (at 150 °C)	UV light exposure sample
% of H-PFNRC01	0.10	0.07	0.06
% of H-PFNRC02	0.01	0.01	0.01
% of H-PFNRC03	ND	ND	ND
% of H-PFNRC04	0.15	0.15	0.14
% of H-PFNRC05	0.02	0.02	0.01
% of MSUI	0.01	0.01	0.95
% of TI	0.28	0.28	1.20

Table No. 8: Degradation conditions of PFN and its impurities (liquid state)

Name of the Sample	Mother sample (As such)	Water hydrolysis	Acid hydrolysis	Oxidation	Base hydrolysis	Humidity Exposure solution
% of H-PFNRC01	0.10	0.47	20.75	0.66	90.53	0.08
% of H-PFNRC02	0.01	ND	ND	0.01	ND	0.01
% of H-PFNRC03	ND	ND	0.01	ND	ND	ND
% of H-PFNRC04	0.15	0.14	ND	0.14	ND	0.15
% of H-PFNRC05	0.02	0.01	0.21	0.01	ND	0.01
% of MSUI	0.01	0.03	34.10	0.10	2.69	0.01
% of TI	0.28	0.66	59.24	0.98	97.42	0.27

The drug underwent mild degradation in all the stressed condition and the degraded peak was found to be well separated from the main peak thus proving the method as the stability – indicating.

CONCLUSION

A RP-HPLC method was developed for the estimation of relative substances in prulifloxacin. The developed method was subjected to validation parameters as per ICH guidelines. By assessing forced degradation studies stability indicating nature was established confirming that the prulifloxacin was free of interferences. Simple, accurate, precise, reliable RP-HPLC method was optimized, developed and validated as per ICH guidelines for the estimation of relative substances in bulk form of prulifloxacin and subsequent degradation studies were performed.

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