IJPT
International Journal of Pharmacy & Technology

ISSN: 0975-766X

Available Online through
www.ijptonline.com

Research Article

A VALIDATED STABILITY INDICATING HPLC METHOD FOR PRULIFLOXACIN

D. R. Chaple*, A. G. Sambhare.
J. L.C. College of Pharmacy, Electronic Zone Building, MIDC Hingna Road, Nagpur-440016
Email:*d.chaple@rediffmail.com*

Received on: 30-01-2010 ***Accepted on: 13-02-2010***

ABSTRACT

A stability indicating reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed for the quantitative determination of prulifloxacin as a bulk drug. The chromatography was performed on a C18 column. Eluents were monitored by UV detection at 273 nm using the mobile phase acetonitrile:water:triethylamine (40:60:0.3%, v/v/v), (pH 3.3). The method was statistically validated for linearity, accuracy, precision and specificity. The linearity of prulifloxacin is within the concentration range of 10–100 µg mL^{-1}. The limits of detection and quantitation were 3.9 and 11.84 µg mL^{-1}, respectively. The method was demonstrated to be precise, accurate and specific with no interference from the peaks of the degradation products (oxidative degradation, photodegradation, acid and base degradation). The results indicated that the proposed method could be used in a stability assay.

KEYWORDS: Spectrophotometry, Prulifloxacin, RP-HPLC.

INTRODUCTION

Prulifloxacin[1,2] 6-Fluoro-1-methyl-7-[4-[(5-methyl-2–oxo-1,3-dioxol-4-yl) methyl]-1-piperazinyl]-4-oxo-1*H*,4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid is an orally active fluroquinolone antibacterial agent (Fig. 1). Prulifloxacin is not official in any Pharmacopoeia. Prulifloxacin belongs to fourth-generation fluroquinolones and has extensive Gram-negative coverage, good Gram-positive coverage and also possesses activity against anaerobes[3]. It is mainly used in the treatment of bronchitis exacerbation and lower urinary tract infection[4]. It shows its antibacterial activity by inhibiting DNA gyrase thus preventing DNA replication and synthesis[5,6].

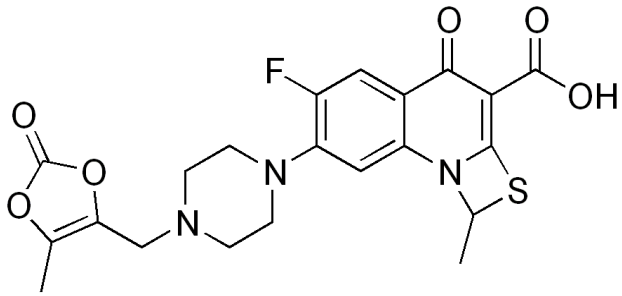


Fig. 1: Structure of prulifloxacin

Various methods have been reported in literature for the analysis of prulifloxacin in biological fluids including spectrofluorimetric[7,8], capillary electrophoresis[9] and HPLC[10-12]. However to our knowledge, no article related to stability-indicating high performance liquid chromatography (HPLC) determination of prulifloxacin in bulk and pharmaceutical dosage forms in presence of its degradation products has reported in literature. According to the International Conference on Harmonization (ICH) guidelines Q1A (R2) entitled 'stability testing of new drug substances and products', stress testing of the drug substance should be carried out

to elucidate the inherent stability characteristics of the active substance[13]. Susceptibility to oxidation is one of the required tests. Also the acid or base hydrolysis and photolytic stabilities studies are required. An ideal stability-indicating method shall quantify the drug per se and also resolve its degradation products.

A simple analytical method is required that can quantitatively estimate prulifloxacin in the presence of its potential degradation products. This paper describes the development and validation of a stability indicating RP-HPLC method for the assay of prulifloxacin as a bulk drug and in its pharmaceutical dosage forms.

EXPERIMENTAL

Materials

Prulifloxacin was obtained as a gift sample from Hetero Drugs Ltd., Hyderabad, India. Prulifloxacin tablets were procured from local pharmacy. All the reagents were of analytical grade. Double distilled water was used throughout the experiment.

LC instrumentation

HPLC model Analytical Technologies Ltd. equipped with ALC pump having variable wavelength UV- Visible (ASPD) detector and auto injector (20 µL).

Chromatography

Chromatography was performed on a Prinoeton SPHER-100, 250 mm x 4.6 mm column using mobile phase containing mixture of acetonitrile:water:triethylamine (40:60:0.3%, v/v/v) and adjusting its pH to 3.3 with phosphoric acid. The mobile phase was filtered through membrane filter (0.45-µm) and vacuum degassed by sonication prior to use. Chromatography was

performed at room temperature under isocratic conditions at a flow rate of 1.0 mL min^{-1}. Detection was done at 273 nm.

Preparation of standard solution

Accurately weighed quantity of Prulifloxacin (10 mg) was dissolved in acetonitrile and volume was made up to 100 mL with acetonitrile (100 µg mL^{-1}).

Preparation of sample solution

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 10 mg of prulifloxacin was shaken with mobile phase for 5-10 min and volume adjusted to 100 mL with mobile phase. The solution was filtered. The filtrate was further diluted with mobile phase to get concentration of prulifloxacin equivalent to about 30 µg mL^{-1}.

Forced degradation of prulifloxacin

Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted using heat, oxidation, light, acid, base and neutral. For thermal degradation, the drug in the solid state kept in oven at 60±2 °C for a period of 7 days. Photodegradation was attempted by exposing accurately weighed quantity of prulifloxacin in the solid state to direct sunlight for a period of 7 days and accurately weighed quantity of prulifloxacin (25 mg) was spread on the petriplates and kept in the UV chamber for 7 days.

Oxidative degradation was induced by accurately weighed quantity of prulifloxacin (25 mg) was added in hydrogen peroxide (25 mL, 3%), the solution was then heated for 2 h at 80°, similarly accurately weighed quantity of prulifloxacin (25 mg) was added in hydrogen peroxide

(25 mL, 6%), the solution was then heated for 2 h at 40^{o} and accurately weighted quantities of prulifloxacin (25 mg) were taken in two separate beaker (100 mL) containing hydrogen peroxide (25 mL, 3%) and hydrogen peroxide(25 mL, 6%). The beakers were covered with petridish and both the reaction mixtures were kept for 7 days in dark. Humidity study was also carried out by subjecting the pure drug to $40^{o}\pm2^{o}$ and 75% relative humidity and $40^{o}\pm2^{o}$ and 96% relative humidity for 7 days.

Acid degradation was attempted by refluxing prulifloxacin with 0.1M HCl (1 mg mL^{-1}) for 4 h. Base degradation was performed by refluxing prulifloxacin with 0.1 M NaOH (1 mg mL^{-1}) for 4 h. To exclude the possible degradative effect of refluxing, the drug was also refluxed with water (1 mg mL^{-1}) for 4 h. After completion of the degradation treatments, the samples were allowed to cool to room temperature, neutralized (when required) and injected into the chromatographic system after appropriate dilution with the mobile phase. The degraded samples were analyzed against a control sample (lacking degradation treatment).

Validation of the assay method

Precision and accuracy

System precision (repeatability) was evaluated by performing five consecutive injections of a 30 µg mL^{-1} prulifloxacin standard solution. Method precision was determined by five repeated assays of the same lot of the tablet formulations. The accuracy of the proposed method was determined by recovery experiments using tablets from the same lot of the commercial and developed formulations. The recovery was assessed at three levels (80, 100 and 120%).

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ concentrations were determined at signal-to-noise ratios of 3:1 and 10:1, respectively.

Linearity

Ten solutions were prepared in the mobile phase containing 10–30 µg mL^{-1} of prulifloxacin. The peak area versus concentration data was treated by least-squares linear regression analysis.

Robustness

The ruggedness was carried out under three different conditions such as: (i) intraday (ii) interday and (iii) different analysts.

RESULTS AND DISCUSSION

Optimization of the chromatographic procedure

The chromatographic conditions were optimized so as to obtain a good separation between the drug and its degradation products. Detection was performed at 273 nm, the λ_{max} of prulifloxacin. The anticipated degradation products were expected to absorb at this wavelength and therefore be detected. Based on the solubility of drug in acetonitrile different compositions of acetonitrile and water at different pH were tried. As a preliminary guide to the selection of the mobile phase, the standard solution of prulifloxacin was injected into the chromatographic system and the elution was studied using mobile phases comprising binary mixtures of acetonitrile and water varying ratios. The results are shown in Table 1. A mobile phase of acetonitrile:water:triethylamine (40:60:0.3% v/v/v, pH 3.3 adjusted with o-phosphoric acid) at a flow rate of 1.0 mL min^{-1} was found to well separate prulifloxacin. The peaks of all the degradation products were well resolved from the drug peak. The percentage of intact prulifloxacin, after forced degradation through various routes, is shown in Table 2 and chromatograms are shown in Fig. 2. Prulifloxacin was quantitatively oxidized when subjected to oxidation in presence of H_2O_2 (3% and 6%) and transition metal with one peak having different

retention time (Rt) than prulifloxacin peak Rt. Similarly, prulifloxacin undergoes degradation under alkali and acid hydrolysis. In alkali hydrolysis two peaks of degradents and in basic condition single peak of degradents were found with different Rt. Prulifloxacin was more susceptible to degradation under acidic conditions than under basic conditions, as evidenced from the percentage of intact drug and difference in hydrolysis times. The very less degradation was observed upon refluxing the drug with water, suggesting that the degradation under acidic and basic conditions was the result of hydrolysis with no influence of heat on the degradation the drug was found to be stable to heat under the conditions of the study, both in the dry state as well as in solution. Exposure to direct sunlight and UV light also shows stability of the drug to the conditions. Samples subjected to humidity study were found to show only one degradation product having different Rt than the pure drug.

Table 1: System suitability report

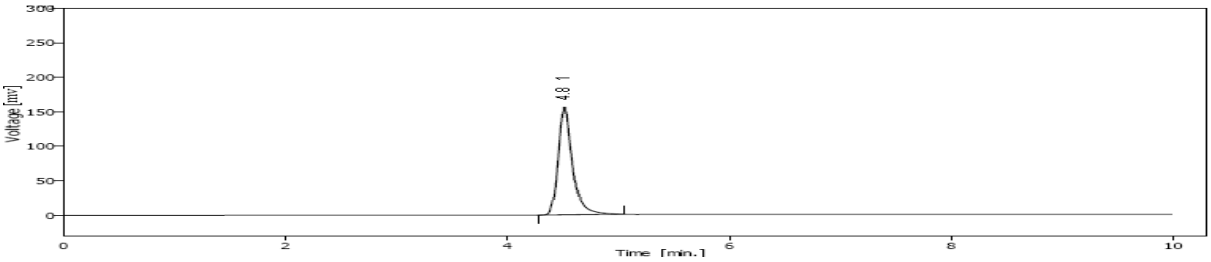
Compound (n = 3)	Rt	N	T
Prulifloxacin	4.88	8148	1.32

n, Number of determination; Rt, retention time; N, number of theoretical plates; T, USP tailing factor.

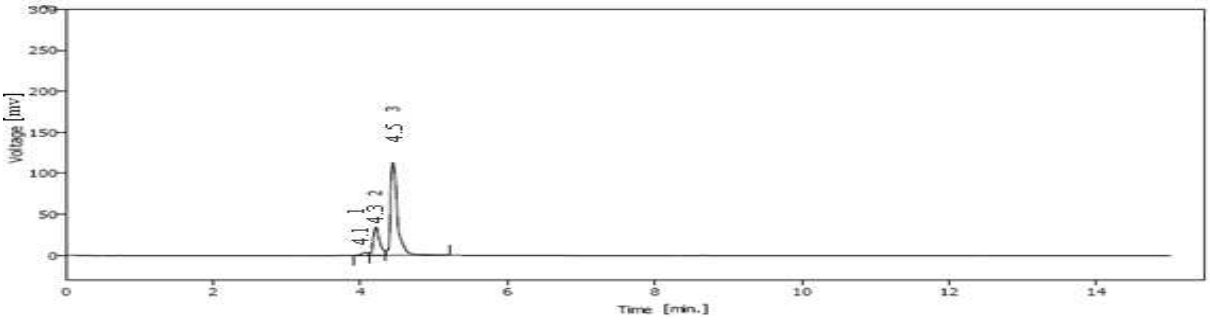
Table 2: Summary of forced degradation results

Stress condition	Time	% Assay of active substance	Mass balance (%assay + %degradation products)
Acid hydrolysis (0.1N HCL)	4 h	76.49	98.4
Base hydrolysis (0.1N NaOH)	4 h	83.96	98.5
Oxidation (3% H_2O_2)	7 days	87.64	98.9
Thermal (60^0 C)	7 days	93.17	98.8
UV (254 nm)	7 days	96.12	98.9

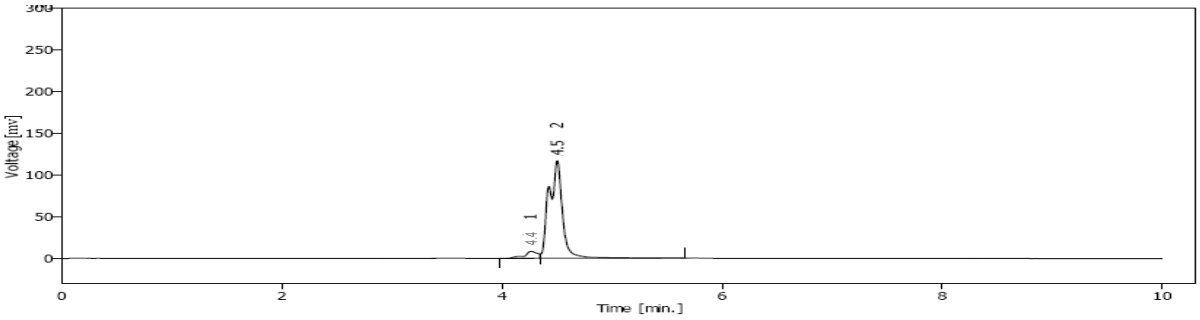
(A) Pure prulifloxacin sample



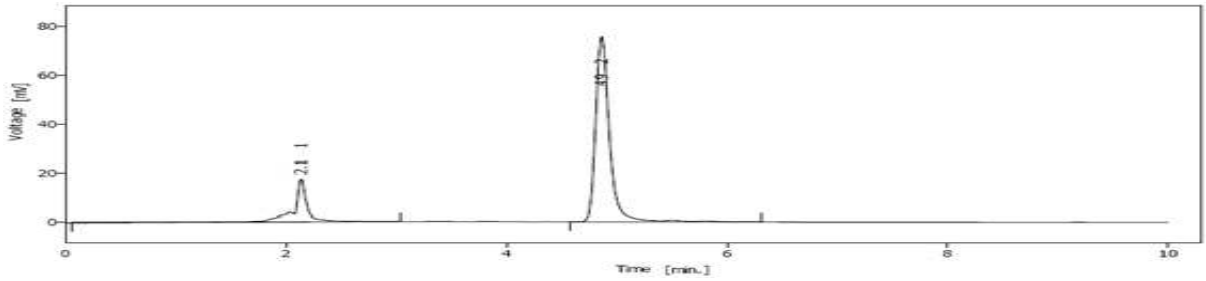
(B) 0.1N HCl



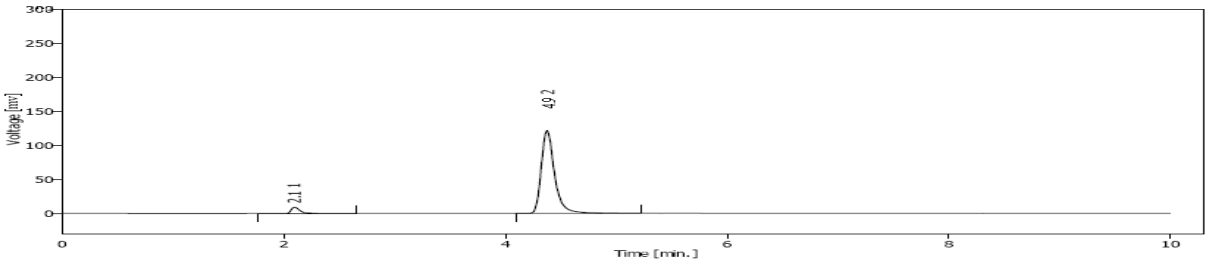
(C) 0.1N NaOH



(D) 3% H_2O_2



(E) UV (254 nm)



(F) 60^0 C

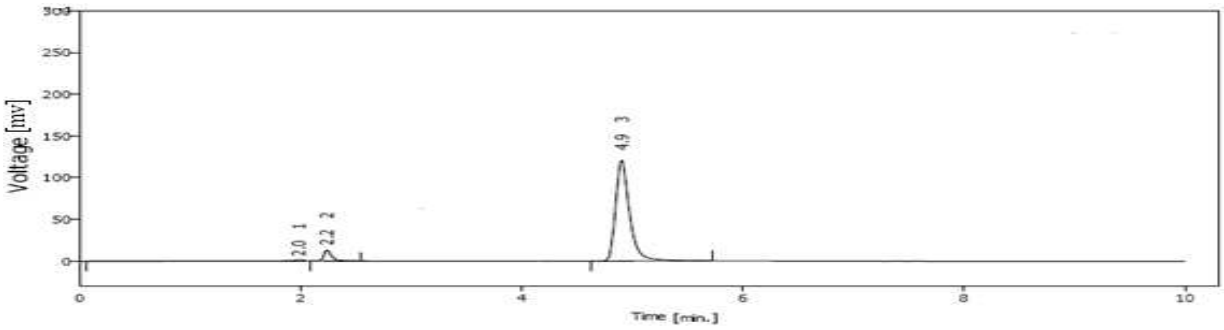


Fig. 2: Typical HPLC chromatograms of prulifloxacin under stressed condition (A) pure prulifloxacin sample (B) 0.1N HCl (C) 0.1N NaOH (D) 3% H_2O_2 (E) UV (254 nm) and (F) 60^0 C.

Validation of assay method

Stability of analyte in solution

Prulifloxacin was found to be stable in solution in the mobile phase, when the standard solution was analyzed at 0, 6, 24 and 48 h post preparation. No peaks corresponding to the degradation products were observed and there was no significant change in the drug peak area.

Precision and accuracy

System precision (repeatability) was evaluated by performing six consecutive injections of the 30 µg mL^{-1} standard solution, giving a low R.S.D. value of 0.16% and no change in retention time of the drug. The Prulifloxacin contents were found in the tablet formulations using the

proposed method. The low R.S.D. values indicate that the proposed method is precise. Recovery data obtained from the study of the tablet formulations ranged from 98–101% with low R.S.D. values (0.7%) (Table 3).

Table 3: Recovery results of prulifloxacin sample

Added (µg) (n = 3)	Recovered (µg)	% Recovery	% R.S.D.
8	8.01	100.13	0.7
10	10.14	101.40	0.8
12	12.03	100.25	0.7

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ concentrations were found to be 3.9 and 11.84 µg mL^{-1}, respectively.

Linearity

The plot of the drug peak area versus concentration was linear over the concentration range of 10–100 µg mL^{-1}. The regression parameters showing slope of 4679.4 and intercept at 5542.15 line equation calculated by the least-squares method was y = 3841.x - 136.5 with a coefficient of correlation of 0.9999. The intercept value was found to be -3778.73.

Specificity

Placebo formulation samples yielded clean chromatograms with no interference from the tablet excipients. The ability of the method to separate the drug from its degradation products and the non-interference from the tablet excipients indicates the specificity of the method.

Robustness: There was no significant change in retention time, peak shape and assay results upon introduction of intentional variations in parameters such as pH of mobile phase, (from 3.2 to 3.4).

CONCLUSION

The RP-HPLC assay method developed for prulifloxacin is rapid, precise, accurate, specific and stability indicating. The method may be used for assessing the stability of prulifloxacin as a bulk drug. The method may be extended to study the degradation kinetics of prulifloxacin and also for the estimation of prulifloxacin.

ACKNOWLEDGEMENTS

The authors would like to thank management of Hetero drugs Ltd., Hyderabad, India for the donation of drug used in this investigation.

REFERENCES

1. The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals, 14th edn., Merck & co. Inc, white house station, New Jercy, 2004, pp7908.
2. Martindale: The complete drug reference, 36th edn. Pharmaceutical Press, London, 2007, pp286.
3. G. Giannarini, C. Tascini, C. Selli, Future Microbiology, 2009, Vol 4, pp13-24.
4. Hooper, D. Q., in: Mandell, G. L., Bennett J. E., Dolin R. M., Douglas and Bennett's principles and practice of infectious diseases, Philadelphia, Churchil Livingstone, 2000, pp. 656-662.
5. Hooper, D. C., In: Wolfson, J. S., Quinolone Antimicrobial Agents, American society for microbiology, Washington, 1993, pp 53-57.
6. D. C.Hooper, Drugs, 1999, Vol 58, pp6.
7. Fengshan Yu, Lin Li , Fang Chen, Anal. Chimi. Acta, 2008, Vol 610, pp257-262.

8. Fengshan Yu, Yongwei Gao, Man Cui, Fang Chen , Yanbin Ding, Analytical Letters, 2009, Vol 41, pp2933-2943.

9. L. Zhang, J. Wen, Y. Pan, Z. Li, G. Fan, Y. Wu, J. Chrom. B, 2008, Vol 287, pp172-177.

10. L. Guo, M. Qi, X. Jin, P. Wang , H. Zhao, J. Chrom. B., 2006, Vol 832, pp280-285.

11. R. M. Pellegrino, F. Segoloni, C. Cagini, J. Pharm. Biom. Anal.,2008, Vol 47, pp567-574.

12. J. Wen, Chromatographica, 2009 Vol 69, pp605-609.

13. ICH, Stability Testing of New Drug Substances and Products (Q1AR2), International Conference on Harmonization, IFPMA, Geneva, 2003.

*Corresponding Author:

D. R. Chaple*,
J. L.C. College of Pharmacy,
Electronic Zone Building,
MIDC Hingna Road,
Nagpur-440016
Email:*d.chaple@rediffmail.com*