Int J Pharm Bio Sci 2015 July; 6(3): (P) 318 - 324

Research Article **Analytical chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF PRULIFLOXACIN IN FORMULATIONS

DEEPAK POKHARKAR,[1] RUPESH PINGALE,[2] SUVARNA PHADATARE[3] AND LAWANYA LATA PANDEY[4]

[1,2]NCRD'S Institute of Pharmacy Nerul, Navi Mumbai 400706, India
[3, 4] NCRD'S Institute of Pharmacy Nerul, Navi Mumbai 400706, India

ABSTRACT

A new Simple and Rapid high-performance thin-layer chromatographic method was developed and validated for quantitative determination of Prulifloxacin. Chromatographic separation studies were carried out on prulifloxacin. TLC procedure was optimized with varying ratios of n-propanol, methanol and ammonia. The mobile phase n-propane: methanol: ammonia with ratio (5.1:0.9:0.9 v/v/v) gave good resolution as well as a well defined peak at Rf value of 0.18..The linear regression analysis data for the calibration plots showed a good linear relationship with r 2 = 0.9972 in the concentration range 20–160ng/spot. The method was validated for precision, recovery, repeatability, and robustness as per the International Conference on Harmonization guidelines. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selected for the analysis of Prulifloxacin. The method is successfully employed in the estimation of equilibrium solubility, quantification of prulifloxacin as a bulk drug, in commercially available preparation.

KEY WORDS: HPTLC, Prulifloxacin, Method Validation, Calibration curve.

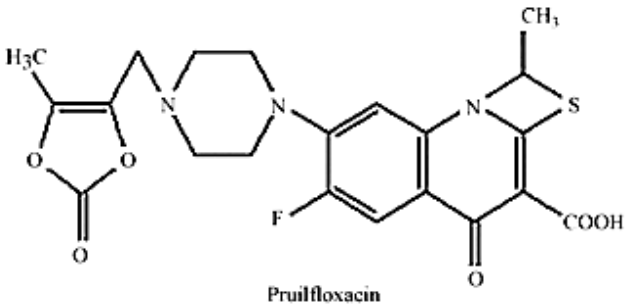
DEEPAK POKHARKAR
NCRD'S Institute of Pharmacy Nerul, Navi Mumbai 400706, India

*Corresponding author

1. INTRODUCTION

Prulifloxacin (Fig.1) is the lipophilic prodrug of Ulifloxacin, a new thiazeto-quinolone antibacterial agent with broad spectrum activity against various Gram- positive and Gram negative bacteria, acts directly on bacterial DNA gyrase inhibiting cell reproduction that leads to cell death.Prulifloxacin has a chemical structure that allows its absorption from the gastrointestinal tract and can therefore administered orally. Its half-life is quite long and the molecule remains in the bloodstream for about 11 hours. This characteristic allows a 600 mg tablet to be administered only once a day, for a very convenient dosing. The active metabolite of Prulifloxacin (Ulifloxacin) is mostly cleared, in an unchanged form, through the urinary tract, thus allowing the drug to be consistently active until its clearance. Literature survey revealed that only a few methods on validation have been reported for quantitative estimation of Prulifloxacin in biological samples and tablet dosage forms. In the present paper, we describe a simple, accurate, precise and sensitive HPTLC method for determination of Prulifloxacin in tablet dosage forms [1,2]

Figure 1
Chemical structure of prulifloxacin



Pruilfloxacin

Various methods have been reported for the determination of pruliflioxacin in pharmaceutical preparations including spectrophotometric methods [3–7] spectrofluorimetry method [8], gas-liquid chromatography (GC) [9,10] FT-Raman spectroscopy, planar chromatography [12,] and high performance liquid chromatography (HPLC) [13-17.] Most of the methods reported are highly sophisticated, costly, and time consuming and require special sample preparation. The HPLC technique is excellent with respect to selectivity and sensitivity, but it cannot be used for routine analysis because of their specialty requirement and cost In view of this, high-performance thin layer chromatography- (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters. Furthermore, in case of HPTLC, there are no restrictions on the choice of solvents and mobile phases; drug and lipophilic excipients can be dissolved in a suitable solvent that would evaporate during spotting on TLC plate, leaving behind analyte as a thin band. Therefore, for such methods, extraction procedure is not required always and could be developed for analyzing drug without any interference from excipients [18–21]. The present paper describes the development and validation of HPTLC method for routine estimation of prulifloxacin from bulk and pharmaceutical dosage forms such as tablets

2. EXPERIMENTAL

2.1. Apparatus

The HPTLC system (Camag, Muttenz, Switzerland) consists of Limomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (10 × 10) cm, a derivatization chamber, and a plate heater. Precoated silica gel 60 F_{254} TLC plates (10 × 10) cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. TLC plates were prewashed twice with 10ml of methanol and activated at 80 ◦ C for 5 min prior to sample application. Densitometric analysis was carried out using a TLC scanner III with win CATS software.

2.2. Reagents and Materials

Prulifloxacin pure powder was obtained as sample from Cipla Ltd (India). All other chemicals and solvents were of analytical reagent grade and used as received without further purification**.**

2.3. HPTLC Method and Chromatographic Conditions

2.3.1. Sample Application

The standard samples of prulifloxacin were spotted on Merck TLC plates precoated with silica gel 60 F254 (20cm ×10 cm with 200 µm layer thicknesses) from E. Merck, Germany. The samples were applied onto the plates as a band with 8 mm width using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Camag Linomat 5 applicator (Camag, Switzerland).

2.3.2. Mobile Phase

The mobile phases comprising of n-propanol, hexane, cyclohexane, acetone, methanol, ammonia, toluene, and ethyl acetate were tried in different combinations and proportions. Out of this n-propanol, methanol and ammonia combination gave best separations, based on this criteria HPTLC method for analysis was developed.

2.3.3. Preparation of Prulifloxacin Standard Stock Solution

10 mg of prulifloxacin was weighed and dissolved in 10 mL of acetonitrile to obtain 1000 µg/mL. This was used as a standard. This was diluted to get concentration in the range of 20-160 ng/spot. The solvent system n-propanol: methanol: ammonia (5.1:0.9:0.9 v/v/v) was used as the mobile phase. Chromatogram was developed in a twin trough glass chamber, using 20 minutes chamber saturation time. The length of chromatogram run was 80 mm. The developed plate was air-dried. Scanning was performed in the fluorescence mode at 278 nm. The slit dimension was kept at 6 x 0.45 mm at scanning speed of 100 nm/s. After completion of scanning, peak areas of marker compound were noted. Peak areas were plotted against corresponding concentrations to obtain the regression equation.

2.3.4 Sample preparation for tablet analysis

To determine the content of prulifloxacin in conventional tablets (label claim: 600 mg prulifloxcin per tablet) twenty tablets were weighed, their mean weight was determined and were finely powdered and powder equivalent to 1 tablets of prulifloxacin was weighed. Then equivalent weight of the drug was transferred into a 10 ml volumetric flask containing 8 ml acetonitirile stir it properly in order to dissolve the drug and filter it with watman filter paper and make it the volume up to 10 ml and this was sonicated for 30 minutes. Further dilution was made to obtain different concentration. The sample solution was filtered through Whatman qualitative filter paper.

3. RESULTS AND DISCUSSION

Method Validation

Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) for specificity, sensitivity, accuracy, precision, repeatability, and robustness [22]

3.1 Linearity

Prulifloxacin showed a good correlation coefficient when peak area of the resolved spot was plotted against concentration in the range of 20-160ng/spot. The equation of the regression line was $y = 5.808 x + 343.032$, $r^2 = 0.9972$ (Fig. No. 2), Table no 1.

Table 1
Data for Calibration Curve

Sr. No.	Conc. (ng)	Area
1	60	670.54
2	80	812.72
3	100	943.11
4	120	1054.78
5	140	1152.88
6	160	1257.17

Table 2
Method validation data

Parameters	Results
Method precision (CV%, n =6)	0.702
Limit of Detection (ng)	9.146
Limit of quantitication (ng)	27.71
Specificity	Specific
Linearity (correlation coefficient)	0.99972
Range (ng per spot)	20-160
Robustness	Robust

Table 3
Intra-day and inter-day precision of the method (n = 6)

Amount (ng/ spot)	Intra- day precision			Inter- day Precision		
	Mean area	SD	% RSD	Mean area	SD	% RSD
60	678.1	2.961	0.436	662.8	5.603	0.846
100	950.18	6.581	0.693	956.3	5.577	0.583
160	1236	5.495	0.445	1247.8	2.739	0.220

Table 4
Recovery studies (n = 6)

Amount of drug spiked (ng)	Amount of drug found (ng)	Recovery (%)	Average Recovery (%)
60	59.60	99.33	99.72
100	98.21	98.21	
160	162.6	101.62	

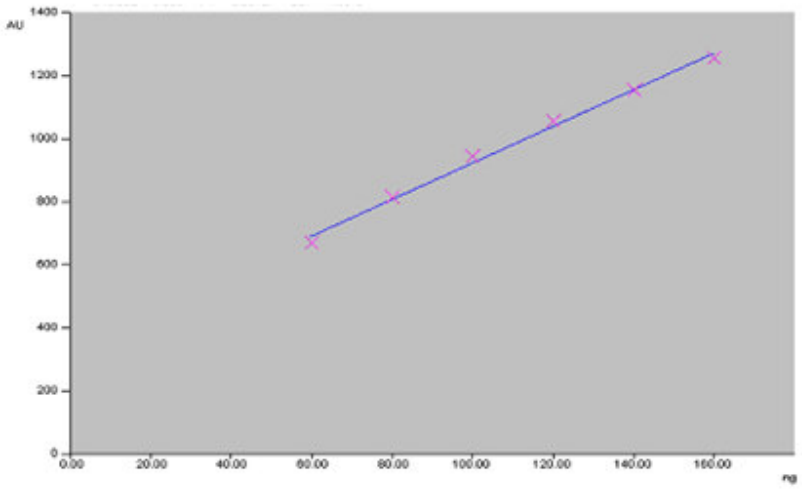
Table 5
Robustness of the method

Amount (ng/ spot)	Change in Mobile Phase (%RSD)	
	n-propenol:methanol:ammonia acetate (5.1:0.9:0.9) + 5%	n-propenol:methanol:ammonia acetate (5.1:0.9:0.9) – 5%
100	0.95	1.08
160	0.98	1.11
	Change in Wavelength (%RSD)	
	278 (+5%)	278 (-5%)
100	1.56	1.89
160	1.76	1.05

Table 6
Assay results

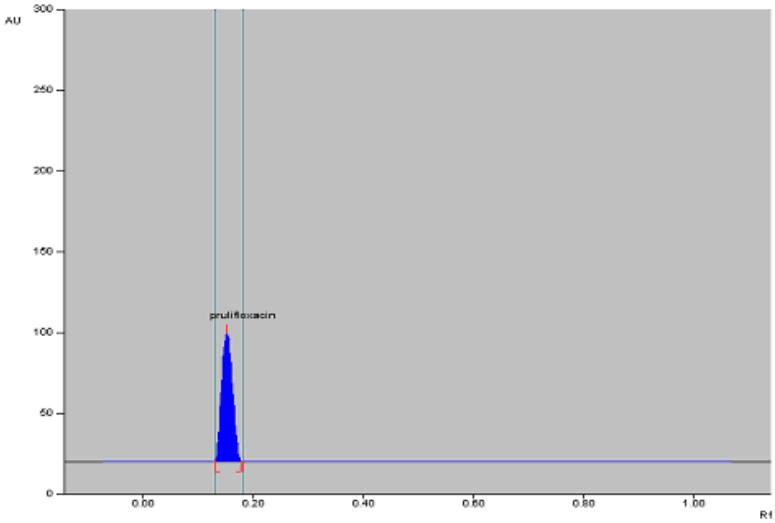
Label claim in mg	Amount found in mg	% Recovery
600	596.48	99.41

Figure 2
Calibration plot obtained by chromatography of Prulifloxacin



Y = 5.808 x + 343.032 *r2 = 0.9972*

Figure 3
Chromatogram of Prulifloxacin



3.2 Precision

The proposed method was found to be precise as indicated by percent RSD (Relative Standard Deviation) not more than 1.5 (Table No.3)

3.3 Accuracy and Recovery

The proposed method when used for quantification of drug after spiking with standard average recovery obtained was 99.72 %. (Table No. 4) The accuracy of the proposed method was determined by recovery

experiments using same solution. The recovery was assessed at three levels (80, 100 and 120%).

3.4 Limit of Detection and Limit of Quantification

The limit of detection was found to be 9 ng/spot while the limit of quantification was found to be 27.71 ng/spot. (Table No.2)

3.5 Robustness

The different values of % RSD obtained after introducing small changes in mobile phase composition and wavelength indicated the robustness of the method. (Table No. 5)

3.6 Specificity

The specificity of the method was ascertained by analyzing Prulifloxacin. The ability of the method to separate the drug from tablet excipients indicates the specificity of the method. There was no interference or co elution from ecxcipients at the Rf value (0.18) of drug.

3.7 Application of Proposed method to solid dosage form

The assay of commercial Prulifloxacin tablets showed that the method shown in the table no - 6 was accurate and reliable with mean drug content of 99.41 % of the labeled claim. No interference peaks were found in the chromatogram and indicating that the determination of the drug content was free from interference by excipients.

4. CONCLUSION

A new HPTLC method has been developed for the identification and quantification of prulifloxacin. Low cost, faster speed, and satisfactory precision and accuracy are the main features of this method. Method was successfully validated as per ICH guidelines and statistical analysis proves that method is sensitive, specific, and repeatable. It can be conveniently employed for routine quality control analysis of prulifloxacin as bulk drug in marketed tablets, without any interference from excipients.

ACKNOWLEDGMENT

The authors wish to thanks Cipla Ltd, Mumbai for providing gift sample of Prulifloxacin.

REFERENCES

1. General Chapter 1225, Validation of Compendial Methods, USP/NF 30/25, Rockville, The United States Pharmacopeial Convention Inc, USA, 2007.
2. www.medicinescomplete.com/mc/merck/current/07908.htn
3. F. Jaffery, S. N. Ahmad, and B. L. Jailkhani, “A spectrophotometric method for simultaneous estimation of phenytoin and carbamazepine,” *Journal of Pharmacological Methods*, vol. 9, no. 1, pp. 33–39, 1983.
4. L. E. Riad, K. K. H. Chan, W. E. Wagner Jr., and R. J. Sawchuk, “Simultaneous first- and zero- order absorption of carbamazepine tablets in humans,” *Journal of Pharmaceutical Sciences*, vol. 75, no. 9, pp. 897–900, 1986.
5. M. S. Cámara, C. Mastandrea, and H. C. Goicoechea, “Chemometrics-assisted simple UV- spectroscopic determination of carbamazepine in human serum and comparison with reference methods,” *Journal of Biochemical and Biophysical Methods*, vol. 64, no. 3, pp. 153–166, 2005.
6. Z. Rezaei, B. Hemmateenejad, S. Khabnadideh, and M. Gorgin, “Simultaneous spectrophotometric determination of carbamazepine and phenytoin in serum by PLS regression and comparison with HPLC,” *Talanta*, vol. 65, no. 1, pp. 21–28, 2005.
7. A. J. Fellenberg and A. C. Pollard, “A rapid spectrophotometric procedure for the simultaneous micro determination of carbamazepine and 5,5 diphenyl hydantoin

in blood," *Clinica Chimica Acta*, vol. 69, no. 3, pp. 429–431, 1976.

8. C. Huang, Q. He, and H. Chen, "Flow injection photochemical spectrofluorimetry for the determination of carbamazepine in pharmaceutical preparations," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 30, no. 1, pp. 59–65, 2002.
9. A. Frigerio, K. M. Baker, and G. Belvedere, "Gas chromatographie degradation of several drugs and their metabolites," *Analytical Chemistry*, vol. 45, no. 11, pp. 1846–1851, 1973.
10. K. Chen and H. K. Bashi, "Comparative analysis of antiepileptic drugs by gas chromatography using capillary or packed columns and by fluorescence polarization immunoassay," *Journal of Analytical Toxicology*, vol. 15, no. 2, pp. 82–85, 1991.
11. M. E. Auer, U. J. Griesser, and J. Sawatzki, "Qualitative and quantitative study of polymorphic forms in drug formulations by near infrared FT-Raman spectroscopy," *Journal of Molecular Structure*, vol. 661-662, no. 1–3, pp. 307–317, 2003.
12. S. Mennickent, R. Fierro, M. Vega, M. de Diego, and C. G. Godoy, "Instrumental planar chromatographic method for determination of carbamazepine in human serum," *Journal of Separation Science*, vol. 32, no. 9, pp. 1454–1458, 2009.
13. T. D. Cyr, F. Matsui, R. W. Sears, N. M. Curran, and E. G. Lovering, "Liquid chromatographic methods for assay of carbamazepine, 10,11-dihydrocarbamazepine, and related compounds in carbamazepine drug substance and tablets," *Journal of the Association of Official Analytical Chemists*, vol. 70, no. 5, pp. 836–840, 1987.
14. M. E. Abdel-Hamid, "Comparative LC-MS and HPLC analyses of selected antiepileptics and beta-blocking drugs," *Farmaco*, vol. 55, no. 2, pp. 136–145, 2000.
15. M. K.M. Babu, "Simultaneous separation and quantitation of four antiepileptic drugs—a study with potential for use in patient drug level monitoring," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 34, no. 2, pp. 315–324, 2004.
16. M. W. Lam, C. J. Young, R. A. Brain et al., "Aquatic persistence of eight pharmaceuticals in a microcosm study," *Environmental Toxicology and Chemistry*, vol. 23, no. 6, pp. 1431–1440, 2004.
17. C. González-Barreiro, M. Lores, M. C. Casais, and R. Cela, "Simultaneous determination of neutral and acidic pharmaceuticals in wastewater by high-performance liquid chromatography- post-column photochemically induced fluorimetry," *Journal of Chromatography A*, vol. 993, no. 1-2, pp. 29–37, 2003.
18. E. K. Oh, E. Ban, J. S. Woo, and C.-K. Kim, "Analysis of carbamazepine and its active metabolite, carbamazepine-10, 11-epoxide, in human plasma using high-performance liquid chromatography," *Analytical and Bioanalytical Chemistry*, vol. 386, no. 6, pp. 1931–1936, 2006.
19. R. B. Patel, M. B. Shankar, M. R. Patel, and K. K. Bhatt, "Simultaneous estimation of acetylsalicylic acid and clopidogrel bisulfate in pure powder and tablet formulations by high- performance column liquid chromatography and high-performance thin-layer chromatography," *Journal of AOAC International*, vol. 91, no. 4, pp. 750–755, 2008.
20. R. B. Patel, A. B. Patel, M. R. Patel, M. B. Shankar, and K. K. Bhatt, "Estimation of alprazolam and sertraline in pure powder and tablet formulations by high-performance liquid chromatography and high-performance thin-layer chromatography," *Analytical Letters*, vol. 42, no. 11, pp. 1588–1602, 2009.
21. M. R. Patel, R. B. Patel, J. R. Parikh, and B. G. Patel, "HPTLC method for estimation of tazarotene in topical gel formulations and *in vitro* study," *Analytical Methods*, vol. 2, no. 3, pp. 275–281, 2010.
22. "Validation of analytical procedures: text and methodology,Q2(R1)ICH Harmonized Tripartite Guideline Q2(R1),November 2005.