Int. J. Chem. Sci.: 8(4), 2010, 2309-2324

NEW SPECTROPHOTOMETRIC METHODS FOR THE QUANTITATIVE ANALYSIS OF PRULIFLOXACIN IN PHARMACEUTICAL DOSAGE FORMS

P. RAVI SANKAR[*], Ch. DEVADASU, P. SRINIVASA BABU, G. DEVALA RAO[a], S. GANANATHAMU[b] and S. SOWJANYA[c]

Department of Pharmaceutical Analysis, Vignan Pharmacy College, Vadlamudi, GUNTUR – 522213 (A.P.) INDIA
[a]KVSR Siddhartha College of Pharmaceutical Sciences, VIJAYAWADA (A.P.) INDIA
[b]National Institute of Pharmaceutical Education and Research, HYDERABAD (A.P.) INDIA
[c]Viswa Bharathi College of Pharmaceutical Sciences, Perecherla, GUNTUR (A.P.) INDIA

ABSTRACT

Three simple, accurate, sensitive, precise and economical spectrophotometric methods have been developed for the determination of prulifloxacin in tablet formulation. The developed methods were based on the formation of a blue coloured chromogen due to the reaction of prulifloxacin with Folin-ciocalteu reagent in presence of sodium carbonate (Method-I), formation of a blood-red coloured complex resulting from the reaction of prulifloxacin in acetonitrile with ortho-phenanthroline (Method-II) and formation of blood-red coloured complex resulting from the reaction of prulifloxacin in acetonitrile with 2, 2'-bipyridyl (Method-III). The proposed methods showed the absorption maxima at 650 nm, 510 nm and 520nm and have good linearity in the concentration range of 50-250 µg/mL, 5-25 µg/mL and 4-20 µg/mL for method-I, method-II and method-III, respectively. The results of analysis for the proposed methods were validated statistically by recovery studies.

Key words: Prulifloxacin, Spectrophotometric, Pharmaceutical.

INTRODUCTION

Prulifloxacin[1] (PRF) is a prodrug, and is metabolized in the body to the active compound ulifloxacin . Prulifloxacin appeared as effective as ciprofloxacin, co-amoxiclav or pefloxacin in the treatment of bronchitis exacerbations or lower urinary tract infections. It was tolerated as well as ciprofloxacin. Prulifloxacin has a long half life and may therefore,

[*]Author for correspondence; Ph.: 09000199106; E-mail: banuman35@gmail.com

be taken only once a day. Prulifloxacin has been approved for use in Japan. In the United States, it is undergoing phase III clinical trials for the treatment of traveler's diarrhea. It has been proved that prulifloxacin is more effective than ciprofloxacin in the treatment of adults with complicated urinary tract infections[2]. Prulifloxacin, the lipophilic prodrug of ulifloxacin, is an oral fluoroquinolone antibacterial agent with a broad-spectrum *in vitro* activity against Gram-negative and - positive bacteria, and a long elimination half life, which allows the once-daily administration. In addition, it penetrates extensively into lung tissues. In well designed clinical trials, prulifloxacin (600 mg) is administered once daily for 10 days in patients with AECB[3] (Acute Exacerbation of Chronic Bronchitis) and it showed good clinical and bacteriological efficacy (similar to that of ciprofloxacin or co-amoxiclav). Prulifloxacin, a new thiazetoquinoline derivative with antibiotic properties, was evaluated for cardiac risk[4] both; *in vitro* on the ether-a-go-go-related gene (HERG) K+ channel, and *in vivo* in the conscious dog monitored by telemetry.

Prulifloxacin is available as tablets under brand name Pruquil from Alembic Pharma Private Ltd.

The structure and analytically important functional groups of the drug are shown in Table 1. Therapeutic uses and physical characteristics such as solubility, melting point etc., are given in Table 2.

Table 1: Structure and analytically important functional groups of prulifloxacin

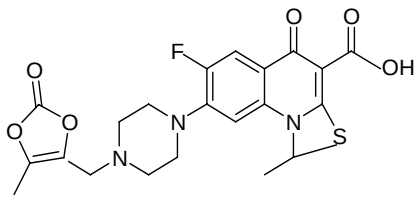
Official name	Chemical name	Structure	Analytically important functional groups
Prulifloxacin	6-Fluoro-1-methyl-7-(4-(5-methyl-2-oxo-1, 3-dioxelen-4-yl) methyl-1-piperazinyl)-4-oxo-4H- (1,3) thiazeto (3,2-a) quinoline-3-carboxylic acid		Ketone functional groups, 3° amino nitrogen, piperazine etc.

Table 2: Therapeutic importance and certain characteristics of Prulifloxacin

Pharmacodynamic/ Therapeutic Category	Characteristics	Therapeutic importance
Broad spectrum antibacterial	Molecular formula: $C_{21}H_{20}FN_3O_6S$ Molecular weight: 461.46 g Solubility: It is soluble in 0.1N NaOH, 0.1N HCl. Freely soluble in acetonitrile and dimethyl formamide. It is also soluble in organic solvents like chloroform and CCl_4. Melting point: 265 -270°C	In the treatment of bronchitis exacerbations or lower urinary tract infections. In the treatment of adults with complicated urinary tract infections

Review of reported and developed methods

Prulifloxacin is not official in any pharmacopoeia. Literature survey revealed that few chromatographic methods have been reported, which include LC-MS[5], HPLC[6], and HPLC with fluorescence detection[7], capillary zone electrophoresis[8] and capillary electrophoresis–chemiluminescence[9] methods for the determination of the active metabolite of prulifloxacin in human plasma and other biological fluids. It has also been reported that there is a sensitive determination of prulifloxacin by its fluorescence enhancement on terbium (III)-sodium dodecylbenzene sulfonate system[10]

There is no analytical report for the estimation of PRF using visible spectrophotometry. This prompted the author to choose PRF for the development of sensitive, precise and accurate visible spectrophotometric methods based on various chemical reactions, involving the analytically important functional groups present in the structure.

EXPERIMENTAL

Instruments used

Systronics double beam UV-Visible spectrophotometer 2201 with 1 cm matched quartz cells was used for all spectral and absorbance measurements. A Systronics digital pH meter was used for all pH measurements.

Preparation of reagents

All the chemicals and reagents FC reagent (Loba), sodium bicarbonate solution (Merck), 1,10-phenanthroline (Qualigens), 2, 2'-bipyridyl (Qualigens), hydrochloric acid (Qualigens) and acetonitrile (Qualigens) used were of analytical grade and solutions were prepared in double distilled water.

Preparation of standard drug solutions

For methods M-I

100 mg of prulifloxacin pure drug was accurately weighed, transferred into a 100 mL volumetric flask containing 20 mL of 0.1N hydrochloric acid and sonicated for about 10 minutes. The volume was made up to the mark with 0.1N hydrochloric acid solution to get the stock solution (1 mg/mL). This solution was further diluted with the same to get the working standard solution.

For methods M-II and M-III

100 mg of prulifloxacin pure drug was accurately weighed, transferred into a 100 mL volumetric flask containing 40 mL of acetonitrile and sonicated for about 10 minutes. The volume was made up to the mark with acetonitrile to get the stock solution (1 mg/mL). This solution was further diluted with same solvent to get the working standard solution.

Spectral characteristics

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}), the spectra were scanned in the wavelength region of 400-800 nm against corresponding reagent blank. The reagent blank absorption spectrum of each method was recorded against solvent employed in each method. The results are graphically presented in the Figs. 1-4. The absorption curves of colored species formed in each method show characteristic absorption maximum, where as the blank in each method has low or no absorption in this region.

Sensitivity of the method

In order to check the validity of Beer's law for these methods, the absorption at appropriate wavelengths of a set of solutions containing different amounts of PRF and specified amounts of reagents (as described in the procedures for each method) were noted against appropriate reagent blanks.

The Beer's law plots of these systems are recorded graphically (Figs. 5-8) Beer's law

limits, molar absoptivity, Sandell's sensitivity and optimum photometric range for prulifloxacin in each method, developed with mentioned reagents, were calculated and the results are given in Table 3. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient values and are recorded in Table 3.

Table 3: Optical characteristics, regression data, precision and accuracy of the proposed methods for prulifloxacin

Parameter	Method-I	Method-II	Method-III
λ_{max} (nm)	650	510	520
Beer's law limits (µg / mL)	50-250	5-25	4-20
Molar absorptivity (L. mole^{-1} cm^{-1})	5.860 x 10^2	2.69 x 10^2	1.430 x 10^4
Detection limits (µg / mL)	0.9720	0.105	0.101
Sandell's sensitivity (µg /cm ^{2}/0.001 absorbance unit)	0.07874	0.9615	0.040
Optimum photometric range (µg / mL)	55-245	4-35	8-24
Regression equation (Y = a+ bc): Slope (b)	0.01280	0.00102	0.03099
Standard deviation of slope (S_b)	1.24 x 10^{-3}	1.003 x 10^{-6}	5.54 x 10^{-5}
Intercept (a)	0.000952	-0.0010	0.00133
Standard deviation of intercept (S_a)	3.773x 10^{-2}	4.83 x 10^{-4}	9.51 x 10^{-4}
Standard error of estimation (S_e)	4.28 x 10^{-3}	8.42 x 10^{-4}	4.28 x 10^{-3}
Correlation coefficient (r)	0.9996	0.9995	0.9999
% Relative standard deviation*	0.4121	0.953	0.8492
% Range of Error (Confidence limits)*			
0.05 level	0.4325	1.0003	0.8913
0.01 level	0.6783	1.568	1.3978
% Error in bulk samples**	0.25	0.44	-0.19

*Average of six determinations.
**Average of three determinations.

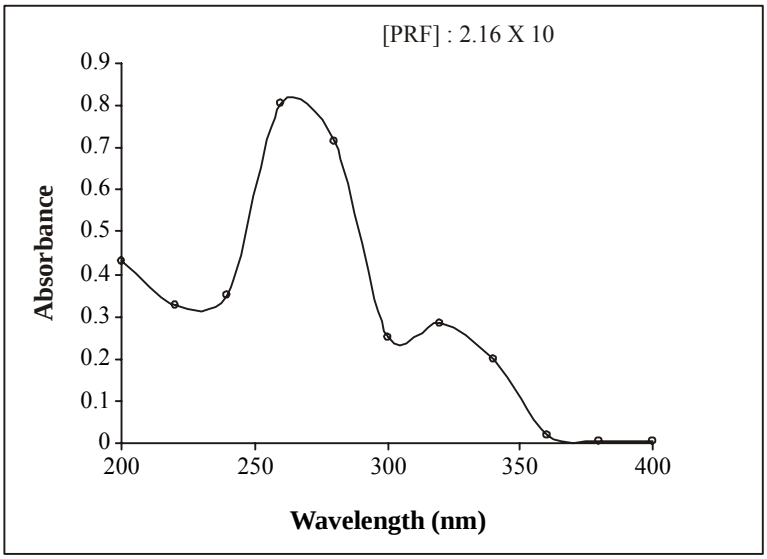


Fig. 1: Absorption spectra of prulifloxacin with 0.1N NaOH (Reference method)

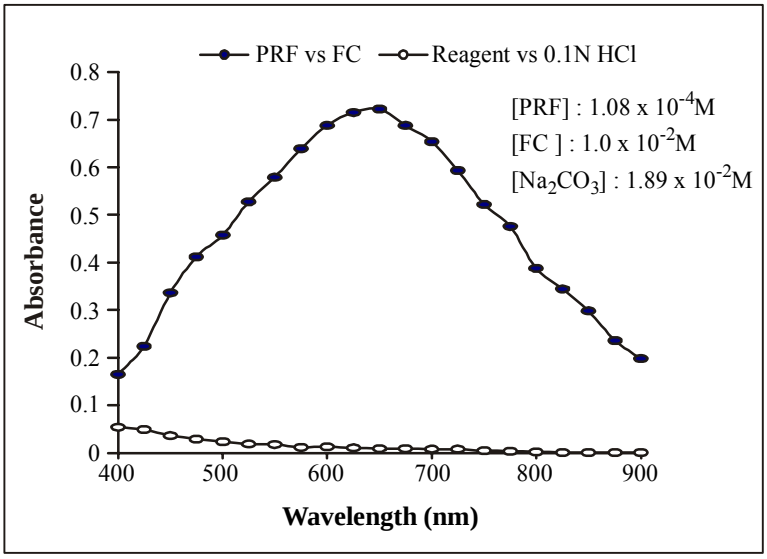


Fig. 2: Absorption spectra of prulifloxacin with FC system and its reagent blank

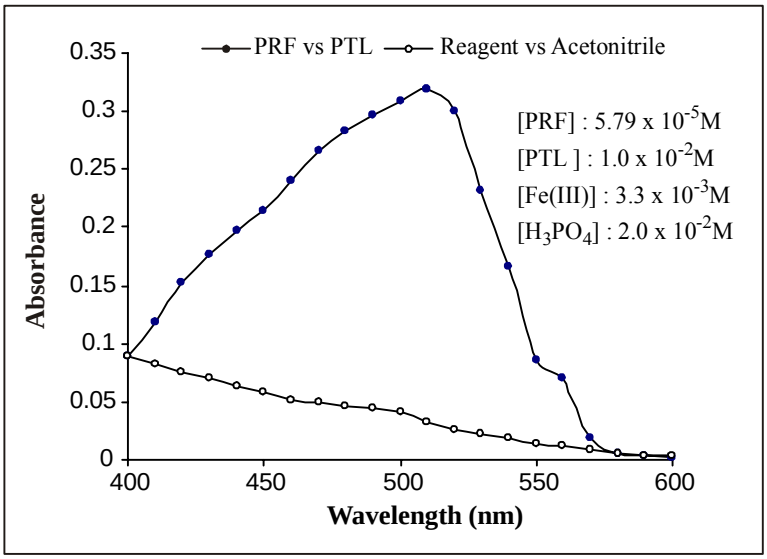


Fig. 3: Absorption spectra of prulifloxacin with ortho-phenanthroline (PTL) system and its reagent blank

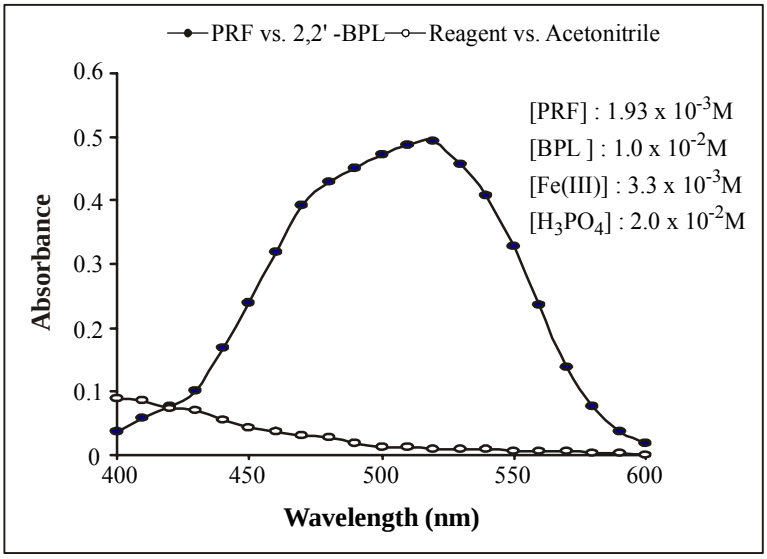


Fig. 4: Absorption spectra of prulifloxacin with 2,2'-bipyridyl system and its reagent blank

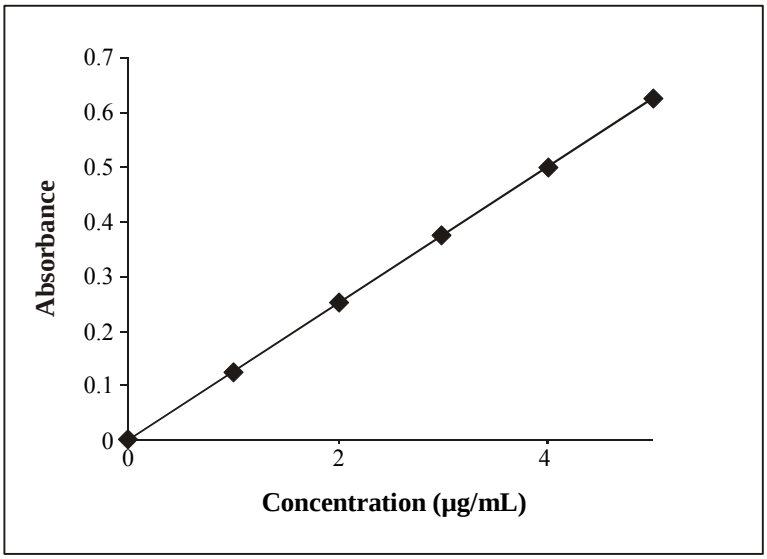


Fig. 5: Beer's law plot of prulifloxacin with 0.1N NaOH system (Reference method)

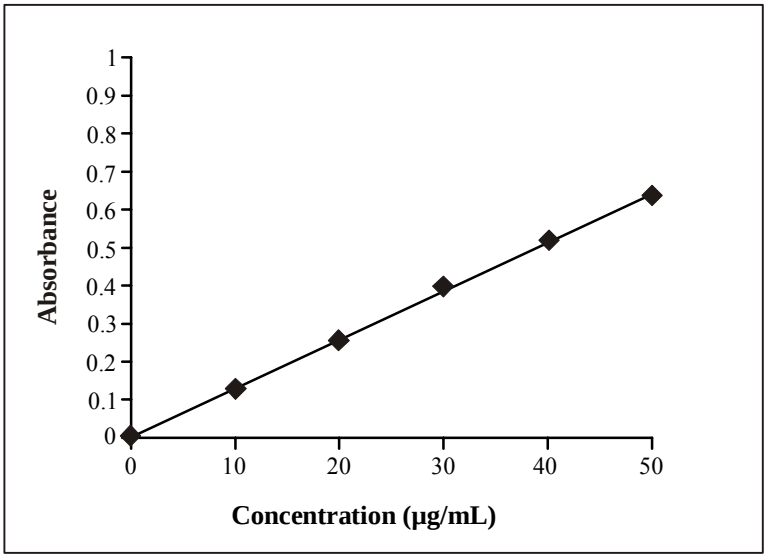


Fig. 6: Beer's law plot of prulifloxacin with FC system

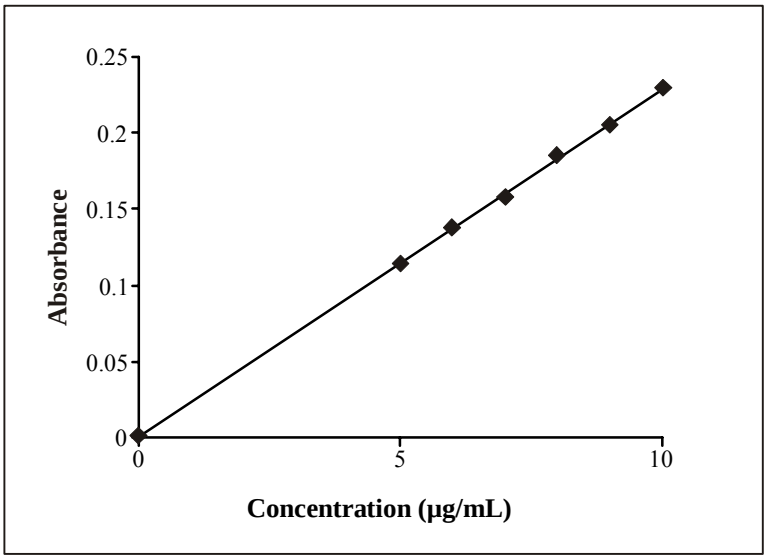


Fig. 7: Beer's law plot of prulifloxacin with ortho-phenanthroline system

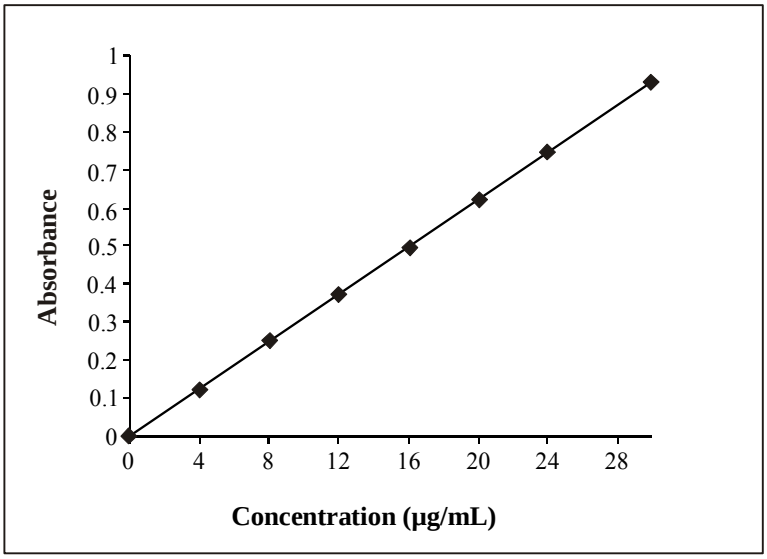


Fig. 8: Beer's law plot of prulifloxacin with 2,2'-bipyridyl system

Recommended procedures

Basing on the above observations, the following procedures have been suggested for the estimation of prulifloxacin in bulk and pharmaceutical preparations.

For bulk drug

Method M-I

Aliquots of standard drug (0.5-2.5 mL, 1000 µg/mL) solution in 0.1N hydrochloric acid were transferred into a series of 10 mL volumetric flasks. To this, 1 mL of Folin-ciocalteu reagent and 2 mL of sodium (5%w/v) solution were added, shacked well and the reaction mixture was allowed to stand for 15 min. The blue colored chromogen; thus formed, was estimated at 650 nm against the reagent blank. The amount of prulifloxacin was computed from its calibration plot.

Methods M-II and M-III

Aliquots of standard drug (0.5-2.5 mL, 100 µg/mL for method-II, 0.4-2.0 mL, 100 µg/mL for method-III) solution in acetonitrile were transferred into a series of 10 mL volumetric flasks. To each flask, 2 mL of $FeCl_3$ and 1 mL of 1, 10-phenanthroline (0.1M) solution or 2,2'-bipyridine (0.1M) solution were added and shaken well and the reaction mixture was heated at 50^{o}C on a boiling water bath for about 15 min. The solution was then cooled and 2 mL of orthophosphoric acid was added in order to prevent further oxidation of $FeCl_3$ and the volume of the resulted solution was made up to the mark with acetonitrile. The blood-red colored complexes; thus formed, were estimated at 510 nm and 520 nm for method-II and Method-III, respectively. The amount of PRF present in the formulation was computed from its calibration plot.

For pharmaceutical formulations

Methods M-I

Twenty tablets of prulifloxacin were weighed and powdered. A quantity of tablet powder equivalent to 50 mg of prulifloxacin was accurately weighed and transferred into a 100 mL volumetric flask containing 50 mL of 0.1N HCl. The solution was sonicated for extracting the drug for about 15 minutes, filtered through a cotton wool and the filtrate was made up to volume with 0.1N HCl. Then it was appropriately diluted with the same solvent and used. Working sample solutions were prepared and the procedure described under bulk samples was followed. The amount of PRF present in the formulation was computed from its calibration plot.

Methods M-I and M-III

Twenty tablets of prulifloxacin were weighed and powdered. A quantity of tablet

powder equivalent to 50 mg of prulifloxacin was accurately weighed and transferred into a 100 mL volumetric flask containing 50 mL of acetonitrile. The solution was sonicated for extracting the drug for about 15 minutes, filtered through a cotton wool and the filtrate was made up to volume with acetonitrile. The solution was further diluted with acetonitrile to get the strength of 100 µg/mL and 1000 µg/mL solution. Working sample solutions were prepared and the procedure described under bulk samples was followed. The amount of prulifloxacin present in the formulation was computed from its calibration plot.

RESULTS AND DISCUSSION

The optical characteristics and the data concerning the proposed method are incorporated in Table 3. The recommended procedure is accurate, sensitive, and precise and it can be adapted to micro determinations of prulifloxacin in bulk and pharmaceutical preparations. It may be used in routine determination of prulifloxacin in pharmaceutical preparations.

Accuracy of the method

The accuracy of the method was determined by taking aliquots containing known quantities of bulk and pharmaceutical preparations of prulifloxacin and estimating them by the proposed and reported methods. To justify the suitability of the proposed method. The recovery studies were also performed.

Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed methods. Three different amounts of pure drug were added to the previously analyzed formulations and the total amount of the drug was once again determined by all the proposed methods after bringing the active ingredient concentration within the Beer's law limits. The results are recorded in Table 4.

Precision

The precision of the method was studied by measuring absorbance of eight samples each containing few µg of PRF in total 10 mL solutions. The percent relative standard deviation and percent range of error (confidence limits P = 0.05 and 0.01 levels) were calculated and presented in Table 3.

Interference studies

The effect of wide range of excipients and other additives, usually present in the

formulations of PRF, in the determination under optimum conditions were investigated separately. The commonly used excipients and additives in the preparation of prulifloxacin tablet formulations such as microcrystalline cellulose, lactose monohydrate, povidone, sodium starch glycolate, colloidal silicon dioxide and magnesium stearate did not interfere with determination of prulifloxacin by the proposed methods; even, if they are present in large amounts than they usually exist.

Table 4: Assay and recovery of prulifloxacin in dosage forms

Method	Sample	Labeled amount (mg)	Proposed method			Found by reference method ± S.D	% Recovery by proposed methods** ± S.D
			Amount found* (mg) ± S.D	T (value)	F (Value)		
I	T_1	600	598.05 ± 0.011	0.572	1.893	595.82 ± 0.014	99.78 ± 0.45
	T_2	600	600.01 ± 0.013	0.457	2.193	601.23 ± 0.016	101.97 ± 0.09
II	T_1	600	598.06 ± 0.009	0.564	1.759	596.05 ± 0.021	99.99 ± 0.41
	T_2	600	600.12 ± 0.009	0.541	1.233	602.5 ± 0.084	99.04 ± 0.12
III	T_1	600	598.92 ± 0.017	1.023	1.651	599.23 ± 0.022	99.95 ± 0.11
	T_2	600	601.52 ± 0.021	0.936	2.825	587.89 ± 0.013	98.99 ± 0.23

T_1 and T_2 are tablets of two different brands.

*Average ± standard deviation of six determinations, the t and F- values refer to comparison of the proposed method with reference method. Theoretical values at 95 % confidence limits t = 2.571 and F = 5.05.

** Average of five determinations.

The chemistry involved in these proposed methods to give various colored chromogens can be explained as -

Method M-I

The colour formation by FC reagent with prulifloxacin may be explained in the following manner based on the analogy with the reports of earlier workers. The mixed acids in FC reagent preparation involve the following chemical species.

$3H_2O.\ P_2O_5.\ 13\ WO_3.\ 5MoO_3.\ 10\ H_2O$ and $3H_2O.\ P_2O_5.\ 14\ WO_3.\ 4MoO_3.\ 10\ H_2O$

PRF probably effects a reduction of 1, 2 or 3 oxygen atoms from the tungstate and/or molybdate; thereby, producing one or more of several possible reduced species, which have characteristic intense blue colour.

Method-II and Method-III

PRF exhibits reducing property due to the presence of functional moieties (one or more) vulnerable to oxidation selectively with oxidizing agents such as Fe (III) under controlled experimental conditions. When treated with known excess of oxidant, PRF undergoes oxidation, giving products of oxidation; inclusive of reduced form of oxidant, Fe (II) from Fe (III), besides unreacted oxidant. It is possible to estimate the drug content colorimetrically, which is equivalent to either the reacted oxidant or reduced form of oxidant formed. The reduced form of Fe (III); (Fe II) has a tendency to give colored complex on treatment with 1,10- phenanathroline[11-13] or 2,2'-bipyridyl.

The first step in these methods is the oxidation of PRF with the oxidant.

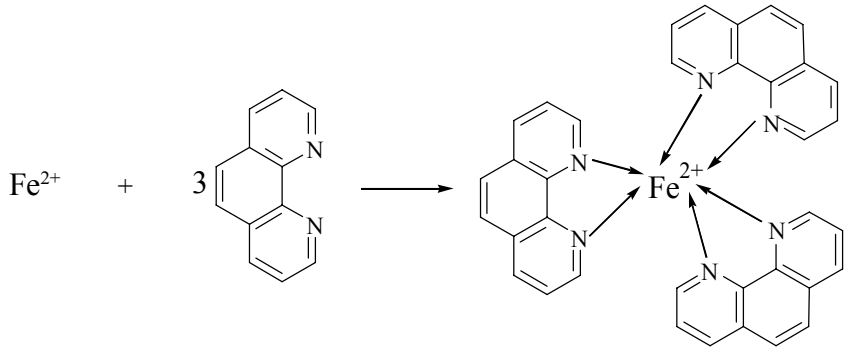
PRF + Fe (III) → Oxidation products + Fe (II) + Fe (III)

(Excess) (Reduced form of oxidant) (Unreacted) …(1)

In this method, as Fe (III) interferes, even to a little extent in the determination of Fe (II), the reactivity of the interfering entity has to be made insignificant by complexing it with o-phosphoric acid.

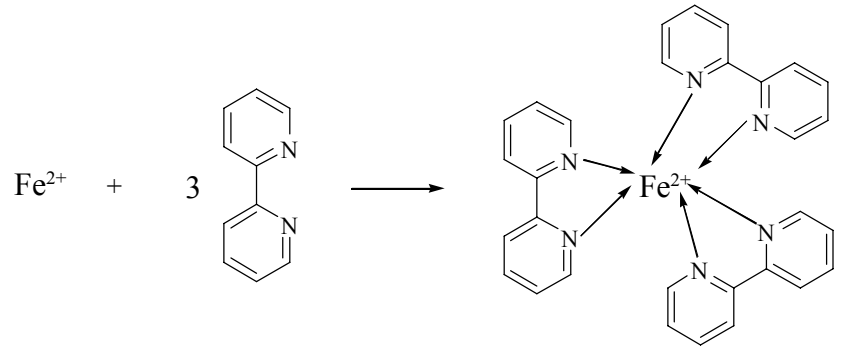
Fe (III) + o-Phosphoric acid → Complex (Not reactive) …(2)

The second step concerns with the estimation of the reduced form of oxidant with appropriate chromogenic agent as described in methods-II and -III. The complex formation for these methods are shown in Scheme 1 and 2, respectively.



Reduced form of Fe^{3+} | 1, 10-Phenanthroline | (Fe^{2+} -Phenanthroline) complex

Scheme 1



Reduced form of Fe^{3+} | 2,2'-Bipyridyl | (Pe^{2+}-2,2'-Bipyridyl) complex

Scheme 2

CONCLUSION

There is no visible spectrophotometric methods reported for the estimation of prulifloxacin in either bulk or pharmaceutical formulations. The authors developed three visible spectrophotometric methods based on the reactivity of different structural units such as piperazine ring and aliphatic tertiary amine in prulifloxacin. Each method uses a specific

reagent and the ε_{max} values of each method are different. The sensitivity order of various proposed methods is –

Method-III > Method-II > Method-I

Statistical analysis of the results shows that all the proposed procedures have good precision and accuracy. Results of analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives present in pharmaceutical formulations. These methods can be adopted for routine quality control of PRF in bulk and pharmaceutical preparations.

ACKNOWLEDGEMENT

The authors are thankful to Alembic Pharma Private Ltd., for providing the gift sample of prulifloxacin. The authors are thankful to the Management, Vignan group of Institutions.

REFERENCES

1. S. C. Sweetman (Ed.), Martindale- The Complete Drug Reference 33 rd Edition, Pharmaceutical Press, London (U.K.),.
2. G. Carmignani and A. F. De Rose, Urol. Int., **74**, 326-331 (2005).
3. Mario Cazzola, Enrica Salvatori, Pulmon. Pharmacol. Therapeut., **Vol.19**, Suppliment, **1**, 30-37 (2006).
4. Francesco Blasi, Stefano Aliberti, Paolo Tarsia, Pierachille Santus, Stefani Centanni and Luigi Allegra. Eur. J. Pharmacol., **477(1)**, 69-72 (2003).
5. Lixia Guo and Meiling Qi. J. Chromatogr. B, **833**, 280-285 (2006).
6. Roberto Maria Pelligrino, Fabio Segoloni, J. Pharm. And Biomed. Ana., **47**, 567-574 (2008).
7. Jun Wen and Zhenyu Zhu, Chromatographia, **66**, 280-285 (2007).
8. Lingli Zhang and Jun Wen, J. Chromatogr. B, **872**, 172-176 (2008).
9. Zhongiu Yang and Xiaoli Wang, Analytica Chimica Acta, **623**, 231-237 (2008).
10. Fengshan Yu and Dazhai Zhou, Anal. Lett., **41**, 3124-3137 (2008).

11. A. Besada, Anal. Lett., **20**, 427 (1987).

12. C. C. Tsen, Anal. Chem., **33**, 849 (1961).

13. M. A. Raggi, A.M. Carvini, Di Pietra and D. Lacche. Pharma, Acta. Hel., **63**, 19, (1988).

Revised : 01.05.2010 *Accepted : 05.05.2010*