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Research Article

Development of UV-spectrophotometric Method and its Validation for Estimating Contents of Prulifloxacin in Simulated Intestinal Fluid

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ABSTRACT

This study was performed with the objective of developing and validating a UV-spectroscopic method for estimating contents of prulifloxacin in simulated intestinal fluid (SIF), i.e., phosphate-buffer media with a pH of 6.8 as per ICH guidelines. The λmax for prulifloxacin in phosphate-buffer media pH 6.8 was found to be 272 nanometers. The calibration curve of the drug followed linearity in-between 1–9 µg/mL concentration range, and the correlation coefficient value was found equal to 0.9995. We tested this proposed method onto the bulk and marketed pharmaceutical formulation (tablets) also to find out the contents of drugs. Using a developed method for estimation of prulifloxacin in simulated intestinal fluid (SIF), the drug was found to be in-between 101.91 and 104.02% in marketed tablets, which shows a good agreement with that of the claimed level. Accuracy of the developed method was established through recovery experimentation, performed for three spiked percent concentrations-75, 100, and 125%. The percentage recovery was found to be in between 97.27 and 101.82%. Low values of percentage RSD supported accuracy as well as the reproducibility of a developed method. The precision of the developed method was established by good in-limit intraday and interday experimental variations and through repeatability tests. Values of % relative standard deviation (RSD) less than two confirmed the precision of a developed method. The ruggedness of the developed method was validated by performing drug estimation by two different performers. This proposed spectroscopic method has proved to be a rapid and successful method for routine analysis of prulifloxacin in simulated intestinal fluid.

Introduction

Prulifloxacin[1,2] (Fig. 1): "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]quinoline-3-carboxylic acid" is a potent orally active 4th generation fluoroquinolone antibiotic. It is a prodrug of ulifloxacin, which has a promising *in-vitro* and *in-vivo* antibacterial effect against a wide variety of gram (-)ve and gram (+)ve microorganisms causing chronic bronchitis, urinary tract infection, lower respiratory tract infections.[3-5] It shows antibacterial activity by inhibiting the enzyme DNA gyrase and thereby preventing DNA replication and synthesis.[6]

To date, several analytical methods for quantification of prulifloxacin in bulk and pharmaceutical preparations have been proposed. Such methods may include UV-spectrophotometric methods for five different media[7] HPLC,[8] RP-HPLC,[9] etc. However, just because of simplicity, economy, specificity, and easy availability, spectrophotometry has always been a method of preference among researchers for the estimation of various drugs. Previously it was assumed that the pH of

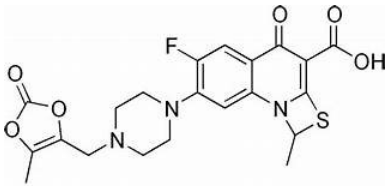

Fig 1: Chemical structure of prulifloxacin

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SIF is 7.5, which is very close to blood plasma. However, subsequent studies revealed that this pH is found in terminal ileum only. In contrast, drug absorption in the intestine is most efficient when a dosage form releases the drug at the proximal small intestine. Therefore, the pH of SIF should be equal to the pH of duodenum and proximal jejunum, which is 6.8.[10] To avoid false-positive results, USP 24/ NF 19[11] has revised the pH of SIF to 6.8, a typical pH of mid-jejunum. The work presented here was done to develop and validate a UV spectroscopic method, as per ICH guidelines, for estimating contents of prulifloxacin in phosphate-buffer-media-pH 6.8 which is simulatory to intestinal fluid. The present piece of work has resulted in a reliable method for the quantitative determination of prulifloxacin in SIF by UV spectrophotometer.

Materials And Methods

We performed this spectroscopic study on 1601 series model UV-Visible Double Beam Spectrophotometer by Shimadzu, Japan. A 1 cm quartz cuvette was used for obtaining absorption spectra and absorbances of reference and test solutions. Crude drug sample of prulifloxacin was procured from Hetero Labs Ltd. and was used as the reference standard. Tablets of prulifloxacin-600 mg- "Percin" by Lupin Ltd. were procured from the local market. Other analytical grade reagents and chemicals were arranged from laboratories and stores of university departments.

Method development and optimization

Prulifloxacin has poor aqueous solubility. During method development, few milliliters of Acetonitrile were used to dissolve the drug in SIF. Accurately weighed 10 mg of prulifloxacin were dissolved in 5 mL of Acetonitrile and diluted with phosphate-buffer-media of pH 6.8 through shaking for 15 minutes, and then the volume was made to 100 mL. This concentrated solution was diluted 10 times to prepare the stock solution (10 µg/mL) of prulifloxacin. The λmax for prulifloxacin was determined by scanning appropriate volumes of 5 µg/mL and 10 µg/mL solutions of prulifloxacin in SIF using UV-spectrophotometer in the scanning ranges of 200–400nm using SIF as blank. Different aliquots were taken from stock solution and diluted with SIF to get concentrations 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/mL, respectively. UV absorbances for these samples were observed at the λmax using SIF as blank. The experiment was performed in triplicate, and averages were calculated, and calibration curves were plotted.

Validation of Developed Method

Validation of a developed method was done through determining linearity and linearity-range, accuracy, preciseness, specificities, ruggedness, and robustness according to ICH guidelines.[12,13]

Linearity and Range

To find out the concentration-range over which the drug solutions of prulifloxacin in SIF show the absorbance as a linear function of concentration, absorbances of 1 to 10 µg/mL solutions of drug in SIF were observed on UV spectrophotometer at λmax. Absorbance for each concentration was observed three times, and averages were calculated. Calibration curves for different ranges of concentrations were plotted. All readings above 1 absorbance were excluded. Regression equations and regression coefficients were determined to study linearity.

Accuracy

Recovery tests were carried out to test the level of accuracy of the proposed analytical method. Under this study equal volumes of standard solutions (10, 12 and 14 µg/ mL respectively) were added to the equal volumes of pre-analyzed samples (4 µg/mL) so as to produce spiking of 75, 100, and 125%. These concentration-spiked samples were reanalyzed for absorbances using the developed method. The recovery study was performed three times for each addition. Percentage recovery and percentage RSD were calculated.

Precision

A precision of the proposed method was ascertained through estimation of intraday and interday variations. For determining intraday preciseness of the developed method –4, 6 and 8 µg/mL solutions of prulifloxacin were analyzed six times in a single day. For determining Interday preciseness 4, 6 and 8 µg/mL of prulifloxacin solutions were analyzed on three different days by developed method. Average concentrations and %RSD were calculated for estimating precision.

Ruggedness

Samples of 8 µg/mL concentrations were analyzed under similar environmental and operational conditions by two different analysts for determining ruggedness.

Repeatability

Repeatability of developed method has been confirmed through analyzing 4 µg/mL of prulifloxacin solution for at least six times.

Estimation of Contents in Prulifloxacin-bulk

10 mg of prulifloxacin were weighed accurately and dissolved in 5 mL of Acetonitrile and then diluted with phosphate-buffer media-pH 6.8 by shaking. Then volume was made up to 100 mL. Then its 1.0 mL was diluted up to 20 mL using SIF. Absorbance of resulting solution (5 µg/mL) was observed at λmax taking SIF as blank. Concentration was calculated from linear regression equation. This procedure was performed six times and standard deviations and % RSD value were calculated.

Table 1: Absorbance values and statistical data of the clibration curve

Concentra^{n} *(µg/ mL)*	*Ab 1*	*Ab 2*	*Ab 3*	*Mean*	*Std. Dev.*	*%RSD*
1	0.119	0.114	0.11	0.114333	0.004509	3.94395
2	0.216	0.223	0.235	0.224667	0.009609	4.277013
3	0.354	0.326	0.334	0.338	0.014422	4.266925
4	0.468	0.452	0.442	0.454	0.013115	2.888739
5	0.575	0.576	0.564	0.571667	0.006658	1.164722
6	0.674	0.672	0.657	0.667667	0.009292	1.391649
7	0.784	0.796	0.765	0.781667	0.015631	1.999723
8	0.881	0.894	0.881	0.885333	0.007506	0.847766
9	0.987	0.976	0.996	0.986333	0.010017	1.015544

Abs stands for absorbance
SD for n = 3 observations.

Estimation of Prulifloxacin in Tablets

20 tablets of prulifloxacin-600 mg were powdered finely and powder-contents equivalent to 10 mg of prulifloxacin were transferred in a 100 mL volumetric flask. This was first dissolved in 5 mL of acetonitrile by shaking for 15 minutes and then sufficient volume of SIF was added and shaken again for 5 minutes. Undissolved excipients were filtered out using whatman filter paper-42 and volume was made to 100 mL using SIF. This solution was then diluted 20 times again using SIF. Absorbance of the resulting solution was noted at λmax taking SIF as blank. The concentration was back calculated from linear regression equation. Experiment was performed in triplicate and the average and %RSD value were calculated.

Results

Method Development and Optimization

The λmax for prulifloxacin in SIF was found to be 272 nm. Data for calibration curves are given in Table 1. Standard deviations for slope, intercept and regression coefficient were calculated using three individual calibration curves. The calibration curve of average absorbance for each concentration was set as optimized standard curve.

Validation of Developed Method

Validation of the developed method was done according to ICH guidelines by performing procedures given under the materials and methods.

Linearity Studies

Equation of linear regression for the developed method came out as y = 0.1101x + 0.0069 for concentrations between 1 to 9 µg/mL. Regression coefficient value was found to be 0.9995 ± 0.000577 for average calibration curve. The results have been shown in Table 2. The linearity curve of prulifloxacin has been presented in Fig. 2.

Table 2: Linearity Parameters

Parameters	*Values*
λmax	272 nm
Linearity range (µg/mL)	1–9 µg /mL
Regression equation	y=0.1101x + 0.0069
Regression coefficient ± SD	0.9995 ± 0.000577
Slope ± SD	0.1101 ± 0.000577
Intercept ± SD	+0.0069 ± 0.004041

SD for n = 3 observations.

Accuracy

Under recovery studies for accuracy testing, % recoveries and their standard deviations from the reanalysis of spiked drug solutions are given in Table 3.

Precision

Through intraday and interday studies for preciseness, average concentrations and % RSD were calculated. % RSD values were found to 1.009, 1.267, 0.93% for intraday studies and 1.271, 1.066, 1.311% for inter-day studies for 4, 6, 8 µg/mL concentrations of prulifloxacin solutions. The results have been shown in Table 4.

Ruggedness

The results obtained by two different analysts adopting the same proposed method for estimation of prulifloxacin in SIF have are shown in Table 5. Calculated % RSD was found to be 1.143%.

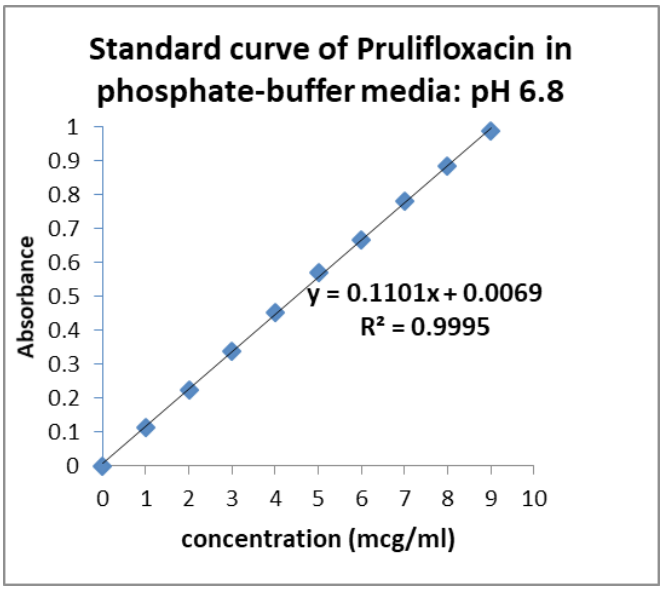

Fig. 2: Standard calibration curve of prulifloxacin in SIF.

Table 3: Recovery studies

Test conc. (µg/mL)	Spiked amount %	(µg/mL)	Final amount (µg/mL)	Recovered amount (µg/mL)	% recovery	% RSD
4	75	7	6.963636	2.963636	98.78788	1.547217
		7	7.027273	3.027273	100.9091	
		7	7.054545	3.054545	101.8182	
4	100	8	7.927273	3.927273	98.18182	1.601163
		8	8.054545	4.054545	101.3636	
		8	7.981818	3.981818	99.54545	
4	125	9	8.863636	4.863636	97.27273	1.583897
		9	9.018182	5.018182	100.3636	
		9	8.963636	4.963636	99.27273	

Concentration level used for ruggedness study was 4 µg/mL. 75, 100, and 125% spiking in concentration was done by adding equal volumes of prulifloxacin solutions of 10, 12, and 14 µg/mL concentrations respectively.

Table 4: Precision studies

S. No.	Concentration (in µg/mL)	Intraday precision (N = 6) Average conc.	% RSD	Interday precision (N = 3) Average conc. found	%RSD
1.	4	3.977273	1.009344	3.981818	1.271179
2.	6	6.030303	1.267288	5.987879	1.066362
3.	8	7.998485	0.930098	7.978788	1.310705

n = 6(6 samples) for intra-day variation and n = 3 (3 samples each) on three different days.

Table 5: Ruggedness studies

Analyst	S. No.	Conc. (µg/mL)	Conc. found	Average conc.	%RSD
1	1.	8	7.872727	7.978788	1.142611
	2.	8	8.081818		
	3.	8	7.981818		
2	1.	8	8.018182	7.957576	
	2.	8	7.845455		
	3.	8	8.009091		

Concentration level used by each analyst for ruggedness study was 8 µg/mL.

Repeatability

Repeatability results are shown in Table 6. %RSD was found to be 0.954%.

Table 6: Repeatability studies

S. No	Conc. (µg/mL)	Conc. found	%RSD
1.	4	3.990909	0.954002
2.	4	4.009091	
3.	4	3.927273	
4.	4	3.954545	
5.	4	4.009091	
6.	4	4.027273	

Concentration level used for repeatability study was 4µg/mL. (n = 6)

Table 7: Estimation of prulifloxacin in bulk

S. No	Concentration (µg/mL)	Conc. found	%RSD
1.	5	4.954545	1.367697
2.	5	5.072727	
3.	5	5.009091	
4.	5	5.072727	
5.	5	5.090909	
6.	5	4.927273	

%RSD stands for percentage relative standard deviation.

Estimation of Prulifloxacin in Bulk

Results are shown in Table 7. The % RSD was found to be 1.3677%.

Estimation of Prulifloxacin in Tablets

The percentage of claimed amounts in tablets was found to be in between 101.91 and 104.02 % and the %RSD for the proposed method has been found as 1.03%. The results have been shown in Tables 8 and 9.

Discussion

Standard calibration curve (drawn between average absorbances obtained from triplicate experiment for each concentration) has shown an excellent linearity in

Table 8: Estimation of prulifloxacin In tablets

S. No.	Amount of Drug (mg/tab) Labeled	Estimated	% of claimed	%RSD
1.	600	618.72	103.12	1.03
2.	600	611.44	101.91	
3.	600	624.12	104.02	

Labeled amount was the amount claimed by the manufacturer and estimated amount was the amount assayed through developed method

Table 9: Summary of validation studies

Parameters (units)	Results
Linearity	
range (µg/mL)	1 - 9 µg /mL
Regression coefficient	0.999 ± 0.000577
Slope	0.110 ± 0.000577
Intercept	+0.006 ± 0.004041
Recovery (%RSD)	
75% spiking	1.555
100% spiking	1.596
125% spiking	1.568
Intra-day precision (%RSD) (n = 6)	
4µg/mL	1.009
6 µg/mL	1.267
8 µg/mL	0.930
Inter-day precision (%RSD) (n = 3)	
4µg/mL	1.271
6 µg/mL	1.066
8 µg/mL	1. 311
Ruggedness (%RSD) (n = 3 for each analyst)	1.143
Repeatability (%RSD)	0.954
Estimation of drug in bulk (%RSD)	1.487
Estimation of drug in tablets (%RSD)	1.030

the concentration range of 1 to 9 µg/mL. Higher value of regression coefficient (0.999 ± 0.000577) confirmed the excellent linearity in the given range (1 to 9 µg/mL) of prulifloxacin in SIF. For recovery studies, the lower values (< 2) of % RSD equal to 1.547, 1.60 and 1.584 for 75, 100 and 125% spiking respectively indicate the accuracy of the developed method. For intraday and interday studies for preciseness, % RSD values were lesser than 2% which confirmed preciseness and reliability of developed method. Again low value of %RSD < 2 ascertained the ruggedness of the developed method. It can be used by different analysts with confidence for a sufficient level of reliability. Repeatability of the proposed method was confirmed by low value of %RSD (< 2).

The percentage RSD less than 2% proved that this method may also be used with confidence for estimating contents of prulifloxacin in the bulk. Using proposed method, contents of prulifloxacin in marketed tablets were found to be in sufficient accord with those of the claimed amounts and the % RSD value, found to be 1.03%, is far < 2, confirms that the proposed method can also be used with confidence for estimating the contents of prulifloxacin in tablet dosage forms. Results showed that excipients did not produce any interference in estimation of drug contents in tablets by proposed method.

In present study, summation of the obtained results inferred that the devised UV-spectroscopic method is an accurate yet simple, economic, and rapid method of estimating contents of prulifloxacin in SIF as well as for quantification of prulifloxacin in bulk and pharmaceutical preparations, especially tablets. The % RSD values for all the parameters were found to be less than 2% for the proposed method. Validation of the proposed method confirms its authenticity as an appropriate method for the routine quantification of prulifloxacin in bulk as well as formulations like tablets. It can also be used as quality control method for the formulations containing prulifloxacin.

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