Research Article

Development of A Rapid and Sensitive Spectrofluorimetric Method for the Estimation of Ofloxacin in Bulk and Formulations

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ABSTRACT

In the present work a rapid, sensitive and economic spectrofluorimetric method has been developed for determination of ofloxacin at nanogram concentration in bulk and its formulation. The relative fluorescence intensity of ofloxacin at buffer pH 1.2 was measured at excitation wavelength (λ_{exc}) of 295nm and emission wavelength (λ_{em}) of 485nm. Linearity range was found to be 200-1400ng/ml and the regression equation obtained is, relative fluorescent intensity = 1369 x concentration (in ng/ml) – 166.1 with regression coefficient (r^2) = 0.99. The method was tested and validated for various parameters according to ICH guidelines and USP. The detection and quantification limits were found to be 30.25 and 91.20 ng/ml, respectively. From the results it was observed that the procedure is accurate, precise and reproducible with low relative standard deviation <2%. The % recovery achieved is also efficient. This analytical method can be used for spectrofluorimetric determination of various fluoroquinolones including ofloxacin in different dosage forms. This method can be further extended for bioanalytical estimation of ofloxacin in blood samples.

Keywords: Ofloxacin, Spectrofluorimetric Method, Validation.

INTRODUCTION

Quinolones have emerged as one of the most important classes of antibiotics of the past decade. Ofloxacin {9-fluoro-2, 3-dihydro-3-methyl-10-(-methyl-1-piperzinyl)-7-oxo-7H-pyrido- [1, 2, 3 de]1, 4-bonzoxazine-6-carboxylic acid} (fig: 1) is a synthetic fluoroquinolone derivative, which has demonstrated broad-spectrum activity against many pathogenic gram-negative and gram-positive bacteria. The bactericidal action of ofloxacin results from interference with enzyme DNA gyrase that is needed for the synthesis of bacterial DNA. There are various UV spectroscopy methods are available for routine analysis but these methods are limited for lower concentration (nano-gram level). Some HPLC, LC-MS methods are reported for determination of ofloxacin at nano-gram level but all these methods need preparative conditions or consumed various organic solvents and also require high skill to perform but proposed method is simple and sensitive which make it applicable for routine analysis.

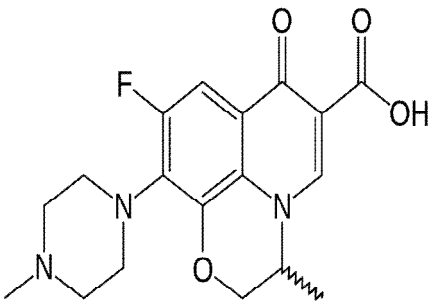

Fig. 1: Structure of ofloxacin

EXPERIMENTAL

Apparatus and materials

All fluorescence measurements were done on Shimadzu RF-5301 PC spectrofluorimeter loaded with in built software and equipped with a 150W xenon lamp, using single quartz cell of 1 cm path length. Relative fluorescence intensity was measured at excitation wavelength (λ_{exc}) of 295nm and emission wavelength (λ_{em}) of 485nm.

Analytically pure Ofloxacin was obtained as a gift sample from IPCA Pharmaceuticals, Mumbai (India). Commercial tablet formulations were purchased from the local market. All chemicals and reagents used were of Analytical Grade.

Analytical Method Development

Different pH media alone and in combination with different organic solvents, in various proportions, were tried. For selection of media the criteria employed was sensitivity of the method, ease of sample preparation, solubility of the drug, cost and applicability of the method for various purposes. Primary stock solution of 100 µg/ml of ofloxacin was prepared in 0.1N hydrochloric acid (pH 1.2). For preparation of different concentrations, aliquots of primary stock solution were transferred into series of 10 ml standard flasks and volume was made with 0.1N hydrochloric acid. Five different concentrations (200, 400, 600, 800, 1000 and 1400 ng/ml) of ofloxacin were prepared for calibration curve. Relative fluorescence intensity was measured at excitation wavelength (λ_{exc}) of 295nm and emission wavelength (λ_{em}) of 485nm (Table-1).

Analytical validation

Linearity

The linearity was calculated by linear regression analysis, using least square regression method. The calibration curve was plotted between the fluorescence intensities of the ofloxacin and concentrations of the calibration standards.

Accuracy and precision

The accuracy of the proposed method was determine using different levels of drug concentrations starting with lower concentration (LC=300 ng/ml), intermediate concentration (IC=500 ng/ml) and higher concentration (HC=1200 ng/ml). All the concentrations were prepared from independent stock solution and analyzed (n = 6). Accuracy was assessed as the percentage relative standard deviation (RSD %) and mean percentage recovery.

Repeatability was determined by using different levels of drug concentrations (same concentration levels taken in accuracy study), prepared from independent stock solutions and analyzed. Inter-day and intra-day variations were studied to determine intermediate precision of the proposed analytical method. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. The percent relative standard deviation (RSD %) of the predicted concentrations from the regression equation was taken as precision. The % recovery of the added pure drug was calculated as, % recovery = $[(C_t - C_s)/C_a] \times 100$, where C_t is the total drug concentration measured after standard addition; C_s, drug concentration in the formulation sample; C_a, drug concentration added to formulation.

Robustness

Robustness of the proposed method was determined by (a) changing pH of the media by ±0.1 units and (b) stability of drug in the selected medium at room temperature for 8 h. Three different concentrations (LC, IC and HC) were prepared in different pH media and mean percentage recovery was determined.

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

The detection limit (DL) and quantitation limit (QL) of ofloxacin by the proposed method was determined using calibration standards. DL and QL were calculated as 3.3 σ/S and 10 σ/S respectively, where S is

the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation

Interference study

The proposed method was found to be selective for the determination of ofloxacin in the presence of common excipients of formulations.

Estimation from formulations

Three commercial and one in-house tablet formulations were assayed for estimation of ofloxacin by developed and validated method.

RESULT AND DISCUSSION

The statistical analysis of data obtained for the estimation of ofloxacin in pure solution indicated a high level of precision for the proposed method as evidenced by low standard deviation values (Table1). The low values of coefficient of variation (Table 1) further established the precision of the proposed method. The regression plot showed that there was a linear dependence of the fluorescence intensity on the concentration of the ofloxacin over the ranges cited in Table-1. Linear regression analysis of the data gave the following equation: relative fluorescent intensity = 1369 x concentration (in ng/ml) – 166.1 with regression coefficient (r^2) = 0.99

Validation of developed method

All the three concentration levels showed accuracy ranged from -0.22 to 0.065%. The mean % recovery values are nearly 100% with low SD values (<1.0) shows accuracy of method. The accuracy of proposed method was further validated by performance recovery studies of standard addition method. The mean recoveries (%RSD) for lower, intermediate and higher concentrations were found to be 100.12 (0.870), 99.57 (0.564) and 100.25 (0.218). This result revealed the validity and reliability of the proposed method as shown in Table 2.

Precision of the proposed methods was studied by evaluating repeatability and intermediate precision. Repeatability (% RSD) ranged from 0.175% to 0.414%, at all three levels of concentrations. In intermediate precision study excellent %RSD values were found out (<1.5%) in all the cases. This % RSD values shows that these methods have very good repeatability and intermediate precision (Table 3).

LOD and LOQ were found to be 30.25 and 91.20 ng/ml, respectively. Robustness was found to be very high as variation of pH of the selected media by ±0.1 did not have any significant effect on relative fluorescence intensity (Table 4). The mean percentage of recovery (±SD) was found to be 99.58 ((±1.24) to 100.25 (±0.34). The ofloxacin solution in selected medium exhibited no spectrofluorimetric changes for 8 h when kept at room temperature.

The proposed method was evaluated by estimation of ofloxacin in pharmaceutical formulations. The assay values of ofloxacin for different formulations ranged from 99.75% to 101.23% with standard deviation less than 1.25%. Assay values of formulations were very close to the label claim. This indicated that the interference of excipients matrix is insignificant in estimation of ofloxacin by the proposed method (Table 5).

CONCLUSION

The proposed method is quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The methods have wider linear range with good accuracy and precision. Hence, the data presented in the manuscript by spectrofluorimetric method for the determination of ofloxacin in its pure and dosage form demonstrate that the proposed method is accurate, precise, linear, selective and offer advantages of reagent availability and stability, less time consumption and high sensitivity. Thus it can be extended for routine analysis of ofloxacin in pharmaceutical industries, hospitals and research laboratories. Unlike

the gas chromatography and high performance liquid chromatography procedures, the spectrofluorimetric instrument is simple and not of high cost. Moreover the methods are free from interferences by common additives and excipients.

Table 1: Calibration data of Ofloxacin

Concentration	Intensity	SD	%CV
200	1383.67	0.8468	0.4517
400	2563.33	2.0294	0.5288
600	3886.33	3.7138	0.6137
800	5129.00	3.9015	0.4799
1000	6366.33	8.7149	0.8544
1400	8442.67	6.8180	0.4986

Table 2: Accuracy data

Concentration level	% Mean Recovery	% RSD	% Accuracy
LC	100.12 ± 0.812	0.870	0.065
IC	99.57 ± 0.654	0.564	-0.222
HC	100.25 ± 0.125	0.218	-0.095

Table 3: Intermediate Precision data

Concentration level	Inter day Repeatability % RSD			Intraday Repeatability % RSD
	DAY-1	DAY-2	DAY-3	
LC	0.3033	0.4761	0.4700	0.4139
IC	0.3850	0.2524	0.2340	0.1747
HC	0.1571	0.2762	0.0933	0.4067

Table 4: Robustness study

Concentration level	% Mean Recovery ± SD	t_{cal}
LC	99.58 ± 1.24	0.6190
IC	100.25 ± 0.34	0.5980
HC	99.87 ± 0.27	0.0403

t_{tab} value at 95% confidence interval, d.f = 4, two-sided is 2.78

Table 5: Estimation from formulations

Marketed Formulation	Label claim (mg)	% Assay	% RSD
Brand A	200	101.23	1.023
Brand B	200	99.83	0.627
Brand C	200	100.52	0.825
In-house formulation	200	99.75	0.254

REFERENCES

1. Directors of the American Society of Hospital Pharmacists, Drug Information. 88, Bethesda, MD, USA, 1988:415-420.
2. Proceedings of the World Health Organisation Meeting on Use of Quinolones in Food Animals and Potential Impact on Human Health, Geneva, Switzerland, 1998.
3. The European Agency for the Evaluation of Medical/Products. ICH Topic Q2BNote for Guideline on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, 1996.
4. United States Pharmacopoeia, invalidation of Compendial Methods, 26th ed, Pharmacopoeia Convention Inc., Rockville, MD, 2003:2439-2442.
5. S. Bolton, in: Pharmaceutical Statistics: practical and clinical application, 3rd ed, Marcel Dekker, NewYork, 1997:216-264.
6. Miller JC and Miller JN in: Statistics for Analytical Chemistry, 2nd ed, Wiley, NewYork, 1984:83-117.
7. Venugopal K, Movva S and Saha RN. New rapid and sensitive spectrofluorimetric method for the determination of gatifloxacin in bulk and formulations. IJPS. 2006;68:726-730.
8. Patel PU, Subaghia BN, Patel MM and Geetapetla M. Indian J Pharm Sci**.** 2004;93:28- 30.
9. Prema G, Geetha R, Hemanth Kumar AK, Venkatesan P, Chandrasekaran VK and Narayanan PR. Indian J Phamacol**.** 1998;30:263-265.
10. Bakshi M, Singh B, Singh A and Singh S. J Pharm Biomed Anal**.** 2001;26:891-894.