Research Article www.ijcps.com

International Journal of Chemical and Pharmaceutical Sciences
2014, Mar., Vol. 5 (1)

ISSN: 0976-9390
IJCPS

Analytical method development and validation for the estimation of ofloxacin in tablet dosage form by reverse phase high performance liquid chromatography

[1]Ashok K. Bera, [2]Amit K. De and [3]Biswajit Pal*.

[1] Shri Ritam Vidyapith, 293/1 Raja Rammohan Roy Road, Kolkata, India.

[2] R & D Division, Dey's Medical Stores (Mfg) Ltd., 62 Bondel Road, Kolkata, India.

[3] Department of Chemistry, St. Paul's C. M. College, 33/1 Raja Rammohan Roy Sarani, Kolkata, India.

***Corresponding Author:** E-Mail: palbiswajit@yahoo.com

ABSTRACT

The current work is concerned with the development and validation of a simple, rapid and precise chromatographic technique for the estimation of ofloxacin, a popular fluoroquinolone, from marketed tablet dosage forms. The liquid chromatographic separation was carried out using Xterra column (4.6 × 150 mm, 5µm, C_{18}) with a mobile phase composed of phosphate buffer (5.3 mM) and acetonitrile in the ratio of 60:40 (v/v) adjusted to pH 3.5, following an isocratic elution protocol. The flow rate was 0.5 ml/min and effluents were monitored at 295 nm. The retention time was 2.31 min. The method was validated with respect to linearity, range, accuracy, precession, limit of detection (LOD) and limit of quantification (LOQ) as per USP/ICH guidelines. The method showed good linearity for all the drugs in the range of 0.01 to 0.50µg/ml. The mean recovery of ofloxacin was found to be 99.78%. The result predicts the simplicity, accuracy and the precision of the developed method suitable for routine analysis of marketed formulations containing Ofloxacin.

Keywords: Ofloxacin, RP-HPLC, Marketed formulations, Solid dosage forms.

1. INTRODUCTION

Ofloxacin is a second generation synthetic fluorinated carboxyquinolone with broad-spectrum of pharmacological action [1-3]. Chemically, ofloxacin has three condensed 6-membered rings made up of a fluorinated carboxyquinolone with a benzoxazine ring and structurally related to nalidixic acid [4]. The chemical name of ofloxacin is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido [1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid [5-7]. The chemical structure of the compound is shown in figure 1.

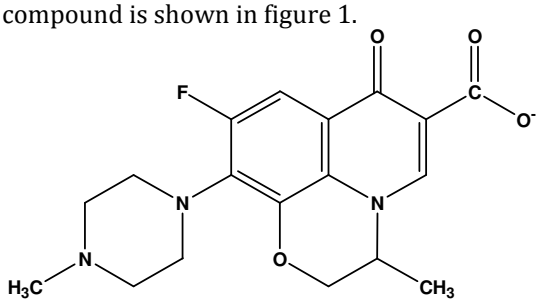

Figure -1: Chemical structure of ofloxacin.

Ofloxacin is used clinically in the treatment of certain infections such as pneumonia, bronchitis, gastro intestinal and infections of the skin, urinary tract, reproductive organs caused by *Haemophilus influenza, Streptococcus pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *E. Coli* bacteria [8-13]. It is also administered along with nitro-imidazoles for the treatment of diarrhea symptoms resulting form amoebiosis mainly in the tropical regions. This antimicrobial drug is active against most pathogenic Gram-negative, Gram-positive bacteria, and some anaerobes through destruction of bacterial Topoisomerase IV and DNA gyrase enzymes required for the synthesis of bacterial DNA [14, 15]. Ofloxacin contains two ionizable functional groups, one is the carboxylic acid group and the other one is the basic piperazinyl group and the antimicrobial activity [16] of this drug depends on both the carboxylic acid and the carbonyl groups. The drug presents an appreciable pharmacokinetic profile. On oral administration

the drug is extensively absorbed from the gastrointestinal tract and the peak plasma concentration is achieved within 1-3hours [17]. Fixed solid oral dosage forms of this drug are available in the market and extensive literature review presents a large number of techniques including spectrophotometry and chromatography for the estimation of this drug from these formulations [18-22]. However, in most cases these techniques are more time consuming and less cost effective. These techniques mostly used a C18 column and a mobile phase consisting of phosphate buffer and methanol at flow rates 1ml/min and above. In this study we present a simple, rapid, precise and accurate method for the estimation of ofloxacin from formulations available in the market. In this study we used a mobile phase consisting of phosphate buffer and acetonitrile at a flow rate of 0.5ml/min and a total run time of only 5 minutes. This reduced run time and flow rates did not affected the peak resolution, accuracy and precession of the developed analytical technique. This makes the method suitable for routine analysis of formulations containing ofloxacin.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Potassium dihydrogen phosphate and dipotassium hydrogen phosphate of AR grade, acetonitrile and phosphoric acid of HPLC grade were purchased from Merck Ltd., Mumbai. Water (HPLC grade) was obtained by using Aurium 611 UV water purification system of Sartorius, Germany. Reference standard of ofloxacin was obtained from Central Drug Laboratory, Kolkata. The pharmaceutical dosage form used in the study was Euflox tablet (Lupin) having of label claim of ofloxacin 200 mg from the retail shop.

2.2. Chromatographic conditions

Analysis was carried out using Waters Alliance e2695 separation module with Waters 2489 dual lambda absorbance detector. A C_{18}, 5 µm X terra column having 150 mm × 4.6 mm id in gradient mode was used for separation. The mobile phase consists of 5.3 mM phosphate buffer (pH 3.5 ± 0.1) and acetonitrile in the ratio of 60: 40. All solutions including mobile phase, were sonicated for 15 minutes and filtered through a 0.45 µm membrane filter (Millipore) before use. The mobile phase was delivered through a rheodyne injector with a 10 µl loop at a flow rate of 0.5 ml /min. The eluent was monitored by UV detection at 295 nm. All data were analyzed by using Empower 3 software.

2.3. Procedure

A standard stock solution containing 0.5 mg/ml of ofloxacin was prepared in 10 ml HPLC grade water by addition of one drop of conc. HCl followed by sonication for 10 minutes and the final volume was made by mobile phase. Final working standard solution was made by diluting stock solution to 0.02 mg/ml of ofloxacin in mobile phase. Twenty tablets each containing 200 mg (label claim) of ofloxacin were individually weighed, mean weight was determined and triturated to obtain homogeneous mixture. A quantity of powder mass equivalent to 12.5 mg of ofloxacin was weighed accurately and transformed to a 25 ml volumetric flask. About 15 ml of HPLC grade water and 1 drop of conc. HCl were added to the volumetric flask and sonicated for 10 minutes and volume was made up to the mark by mobile phase. The solution was then filtered through Whatman no. 1. Aliquots of filtered solution were diluted with mobile phase in order to obtain solution with final concentration of 0.02 mg/ml of ofloxacin (theoretical value). The contents of standard and sample solution were then filtered through 0.45 µm syringe filter. Chromatograms standard solution (six replicates) was recorded. A typical chromatogram of ofloxacin was presented in figure 2. The retention time of ofloxacin was 2.13 min. The amount of ofloxacin in commercial formulation was calculated by comparing area of the sample solution with that of standard solution.

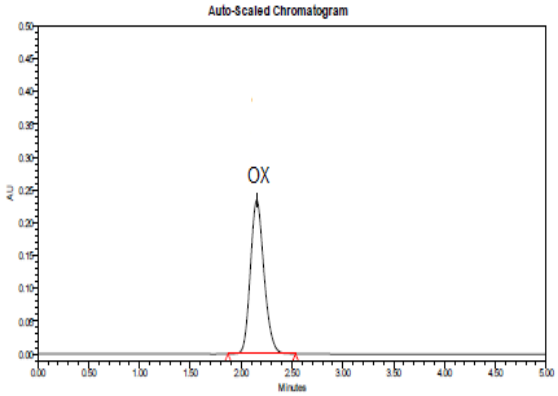

Figure - 2: Representative chromatogram for Ofloxacin (retention time = 2.130).

2.4. Method validation

The proposed method was validated as per International Conference on Harmonization (ICH) guidelines [23, 24] with respect to linearity and range, precision, accuracy, LOD and LOQ, robustness and ruggedness.

2.5. Linearity and range

Linearity of the proposed method was established by least squares linear regression analysis of the calibration curve. The calibration curve was constructed by plotting average peak

area against concentration of standard ofloxacin solution ranging from 0.01 to 0.50 µg/ml and correlation co-efficient was determined.

2.6. Precession

The precession of the proposed method was ascertained from the peak area response obtained by making six replicate injections of standard solution of ofloxacin. The percentage RSD with respect to the peak area, peak retention and amount were calculated.

2.7. Accuracy

Accuracy of developed method was confirmed by doing recovery study as ICH norms [23, 24]. A known quantity of the pure drug was added to the pre-analyzed samples at three different concentration levels 90%, 110%, and 120% by replicate analysis (n=3). % Recovery and % RSD were calculated for each concentration.

2.8. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) decide about sensitivity of a method. LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. LOD and LOQ were calculated by using the values of slopes and intercepts of the calibration curves.

2.9. Ruggedness and robustness

The ruggedness of the developed method was established by determining ofloxacin in different instruments like Waters HPLC (515 pump, 600 pump; 2487 dual λ absorbance detector), Merk Hitachi HPLC (La Chrome pump L-7100, L-7400 UV detector) by different operators, using similar type columns of different make.

Robustness of the developed method was also determined by making slight variation in chromatographic condition like temperature, mobile phase as well as its pH.

3. RESULTS AND DISCUSSION

To develop the proposed RP-HPLC method, chromatographic conditions were optimized to get best resolution and peak shape. Properly selected column, wavelength and mobile phase gave a well resolved, sharp peak for ofloxacin with retention time 2.13 min (Figure 2). The system suitability was evaluated by making six replicate injections of the standard preparation and the peak response was recorded at optimized chromatographic conditions (Table 1).

Table -1: Sample Suitability Parameter

Parameters	Ofloxacin
Wavelength of the max absorbance (nm)	295
Retention Time (mins)	2.13
Theoretical Plate	12312
LOD (µg/ml)	0.09
LOQ (µg/ml)	0.26

The calibration curves were linear over the concentration range of 0.01 to 0.05 µg/ml for ofloxacin. Correlation coefficient was found to be 0.99 (Table 2). Precision of the proposed method was measured in terms of repeatability of application and measurement data. Repeatability was carried out using six replicates of standard solutions of ofloxacin.

Table - 2: Linearity Parameters

Parameters	Ofloxacin
Linearity range (µg/ml)	0.0-0.50
Correlation coefficient	0.99
Slope	1423
Intercept	1175

Table - 3: Precision Parameters

Parameters	Ofloxacin
Peak Area	0.60
Peak RT	0.10
Amount (mg/tablet)	0.16

Table - 4: Accuracy Parameters

Tablet Formulation	Drug	Labelled Amount of Drug (mg/tab)	Amount mg/tab found	% label claim (n =6)	% RSD	Recovery Studies (n = 3)				
						Total Amt. after spiking (mg)	Amt recovered (mg) Mean ± SD	% Recovery	% Mean Recovery	% RSD
Euflox Tab (Lupin)	Ofloxa-cin	200	200.01	100.00	0.99	180	180.01±2.09	100.00	99.78	0.54
						220	218.79±3.09	99.45		
						240	239.77±1.76	99.90		

Precision study showed very low percent relative standard deviation with respect to peak area, peak retention time and amount. The values were 0.60, 0.10 and 0.00 respectively (Table 3). Accuracy of the method was calculated by percent mean recovery studies (n=3). The mean recovery observed was 99.78%.

The %RSD with respect to accuracy of recovery test was 0.54 (Table 4) which showed that the method was free from interference of the excepients used in the formulations.

The method was satisfactory with respect to ruggedness and robustness. The limit of detection (LOD) and the limit quantification (LOQ) were found to be 0.09 and 0.26 µg/ml respectively (Table 1).

4. CONCLUSION

Thus the above developed RP-HPLC method for the determination of ofloxacin is simple, precise, accurate and economic. Statistical analysis proves that the method is reproducible and selective for the analysis of ofloxacin in pharmaceutical formulations. More over the shorter duration of analysis for ofloxacin makes the reported method suitable for routine quality control analysis work.

Acknowledgments

The authors gratefully acknowledge Dey's Medical, Kolkata for providing necessary facilities for carrying out this study.

5. REFERENCES

1. Nelson JM, Chiller TM, Powers JH and Angulo FJ. Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. **Clin Infect Dis.** 2007; 44 (7): 977–980.
2. Martindale PK. **The Complete Drug Reference.** Edited by The Pharmaceutical Press, London, 1999; 32nded: pp.233, 289.
3. Susan BS. **The Merck Index.** Edited by Merk and co. Whitehouse Station, NJ, 2001; 13th ed: pp. 1213 and 1229.
4. Incilay SS and Ayla T. **Anal Lett.** 2003; 36: 1163-1181.
5. Sweetman SC. **Martindale, The Complete Drug Reference**, 32nd Edition. Pharmaceutical Press, London, 1999; p.233.
6. Maryadele JO' Neil. **The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals**. 14th edition. New Jersey, Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station, 2006;p.1170.
7. Gao X, Liu Y, Li H, Bian J, Zhao Y, Cao Y, Mao Y, Li X, Xu Y, Ozaki Y and Wu J. A cooperative hydrogen bonding system with a C—H···O hydrogen bond in ofloxacin. **J Mol Structure.** 2013; 1040: 122-28.
8. Sweetman SC. **Martindale, The Complete Drug Reference.** 32nd Edition. Pharmaceutical Press, London, 1999; pp.165-66.
9. CorradoML. The clinical experience with ofloxacin in the treatment of sexually transmitted diseases. **Am J Obstet Gynecol.** 1991; 164: 1396-1399.
10. Perletti G, Marras E, Wagenlehner FM and Magri V. Antimicrobial therapy for chronic bacterial prostatitis. **Cochrane Database Syst Rev.** 2013; 8: CD009071. doi: 10.1002/14651858.CD009071.pub2.
11. Lipsky BA, Tack KJ, Kuo CC, Wang SP and Grayston JT. Ofloxacin treatment of Chlamydia pneumonia (strain TWAR) lower respiratory tract infections. **Am J Med.** 1990; 89(6):722-724.
12. Perez-Gonzalvo ME, Mosquera-Pestana JA, Ramos D, Apilanez J, Borja J, Arnau C, Garcia-Barbal J and Marin-Pardo J. Ofloxacin versus trimethoprim - sulfamethoxazole in the treatment of patients with acute exacerbation of chronic bronchitis. Study of Ofloxacin in Lower Respiratory Tract Infections Research Group. **Clin Ther.** 1996; 18(3): 440-447.
13. Ishiura Y, Fujimura M, Saito M, Shibata K, Nomura M, Nakatsumi Y and Matsuda T. Additive effect of continuouslow - dose ofloxacin on erythromycin therapy for si nobronchial syndrome. **Respir Med.**1995; 89(10): 677-84.
14. Mouton Y and Leory O. Ofloxacin. **Int J Antimicrob Agent.** 1991; 1: 57-74.
15. Kasabe J, Shitole VV, Waghmare VV and Mohite V. Simultaneous estimation of metronidazole and ofloxacin in combined dosage form by reverse phase high performance liquid chromatography method. **Int J Chem Tech Resear.** 1, 2009; 1:1244-1250.
16. Monk JP. Ofloxacin: A review of its antimicrobial activity. pharmacokinetic properties and therapeutic use. **Drugs**.1987; 33: 348-391.

17. Fuhrmann V, Schenk P, Mittermayer C, El Menyawi I, Ratheiser K and Thalhammer F. Single-dose pharmacokinetics of ofloxacin during continuous venovenous hemofiltration in critical care patients. **Am J Kidney Dis.** 2003; 42(2):310-314.
18. Shervington LA, Abba M, Hussain B and Donnelly J. The simultaneous separation and determination of five quinolone antibiotics using isocratic reversed-phase HPLC: application to stability studies on an ofloxacin tablet formulation. **J Pharm Biomed Anal.** 2005; 39:769-775.
19. Amin SA, Dessouki HA and Agwa IA. Ion-pairing and reversed phase liquid chromatography for the determination of three different quinolones: Enrofloxacin, lomefloxacin and ofloxacin. **Arabian J Chem.** 2011; 4:249–57.
20. Sharma S, Sharma MC and Sahu NK. Simultaneous determination of nitazoxanide and ofloxacin in pharmaceutical preparations using UV-spectrophotometric and high performance thin layer chromatographic methods. 2012; Article in press.
21. Patel DM, Soneji JA, Patel PB and Patel CN. Development and validation of a method for simultaneous estimation of ofloxacin and ornidazole in different dissolution media. **Pharm Methods.** 2012; 3:102-105.
22. Wankhede SB, Prakash A, Kumari B and Chitlange SS. Simultaneous spectrophotometric estimation of norfloxacin and ornidazole in tablet dosage form. **Indian J Pharm Sci.** 2009; 71: 325-328.
23. United States Pharmacopeia. **USP Convention, Rockville,** MD, 2008; p 1225**.**
24. ICH (Q2, R1).Note for guidance on validation of analytical methods: Definitions and terminology. **International conference on harmonisation,** 1994; pp 9-13.