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ORIGINAL RESEARCH ARTICLE

A Validated UV-Spectrophotometric Method for the Estimation of Ofloxacin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT:

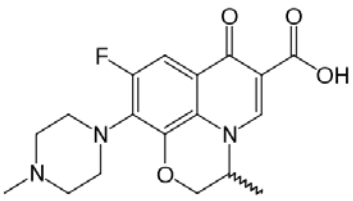
A new, simple, precise and accurate method for the estimation of Ofloxacin in bulk and pharmaceutical dosage forms has been developed. 0.1N HCl was chosen as the solvent system. The λmax was found to be 293nm. The responses were linear in the range of 02-20µg/ml. The regression equation of the calibration graph and correlation coefficient were found to be y = 0.121x - 0.015 and 0.999 respectively. Validation of the method was done in order to demonstrate accuracy, precision, interday and intraday assay, robustness, and ruggedness, of the proposed method. The %RSD values for both intraday and interday precision were less than 1%. The recovery of the drug from the sample was ranged between 97.792% and 100.49%. Commercial tablets containing 200mg of Ofloxacin (Oflxcin and Acoflox) were analyzed by the proposed method and the results were well within the claimed limits.

Key words: Ofloxacin, Robustness, Ruggedness, Validation

INTRODUCTION:

Ofloxacin (**Fig.1**) is a fluoroquinolone derivative with potent activity against a broad spectrum of bacteria. Chemically, it is (±)-9-fluoro-2, 3-dihydro-3-methyl-10- (4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine -6-carboxylic acid[1]. It is mainly used as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases. Ofloxacin is official in USP[2] and BP[3], but not in IP. The assay procedure mentioned in these pharmacopoeias uses non aqueous titration for estimation of Ofloxacin. Literature surveys reveal different spectrophotometric methods[4,5], atomic absorption spectrometric[4], spectroflurometry[4], HPLC[6,7] and microbiological method[8] for its determination. Thus an attempt was made to develop new, simple, accurate and validated method for determination of Ofloxacin by UV spectrophotometric method.

Fig 1: Chemical structure of Ofloxacin



MATERIALS AND METHODS

Chemicals & Reagents: Analytically pure Ofloxacin was obtained as a gift sample from Glenmark Pharmaceuticals, Hyderabad (India). Commercial tablet formulations were purchased from the local market. All chemicals and reagents used were of Analytical Grade, obtained from Merck.

Instruments: A SHIMADZU double beam UV/Visible recording spectrophotometer (Model: 1700) with 2 nm spectral bandwidth was employed for all spectrophotometric measurement using 10mm matched quartz cell and Borosil glass wares were used for the study. Calibrated electronic single pan balances Sartorius CP 225 D, pH Meter (LABINDIA), Enertech Fast Clean Ultrasonicator were also used during the analysis.

Standard Stock Solution and Working Standard Solutions: The standard stock solution of Oflaxacin was prepared by transferring accurately weighed 10 mg of drug to 10 ml volumetric flask and dissolving it with 0.1N HCl to get a concentration of 1000 µg/ml. The solution was diluted accordingly to get a concentration of 100µg/ml and was kept as the stock solution. The prepared stock solution was diluted with 0.1N

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HCl solution to get working standard solutions of concentrations 02-20 µg/ml.

Determination of λmax: The standard solution of Ofloxacin (10 µg/ml) was scanned in the wavelength region of 200-400 nm and the λmax was found to be 293 nm. (**Fig.2**)

Preparation of calibration curve: The working standard solutions of Ofloxacin were scanned in the UV region and the absorbances were observed against 0.1N HCl solution as blank at 293nm. Finally the calibration curve was plotted between concentration (x-axis) and absorbance (y-axis).

Assay of tablet dosage form: 10 tablets of brand OFLXCIN (manufactured by Bombay Tablet Mfg. Co, Mumbai) containing 200mg of Ofloxacin were weighed, average weight determined and finely crushed to powder. An accurate weight equivalent to 10mg of the drug was transferred to 100ml volumetric flask. The drug was extracted 4 times by adding solvent in potions, 20 ml each time and the volume was made upto the mark by using solvent. It was then diluted (within the linearity range), absorbances of the sample solution were recorded at determined λmax and the concentration of the drug in sample was found out. Similarly, the assay of ACOFLOX (manufactured by Acme Pharmaceuticals, Ahmedabad) containing 200mg of Ofloxacin was carried out.

Validation

Accuracy: The accuracy of the proposed method was tested by recovery studies at 80%, 100%, and 120% by adding a known amount of pure drug to the pre-analyzed formulation of concentration 10µg/ml.

Precision: The precision of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (10µg/ml) within the Beer's range and finding out the absorbance by the proposed method.

Intraday Assay: The intraday assay of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (10µg/ml) within the Beer's range and finding out the absorbance by the proposed method at 3 different time period of the same day.

Interday Assay: The interday assay of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (10µg/ml) within the Beer's range and finding out the absorbance by the proposed method on 3 different days.

Robustness: The robustness of the method was carried out by changing the solvent system to Glacial Acetic acid.

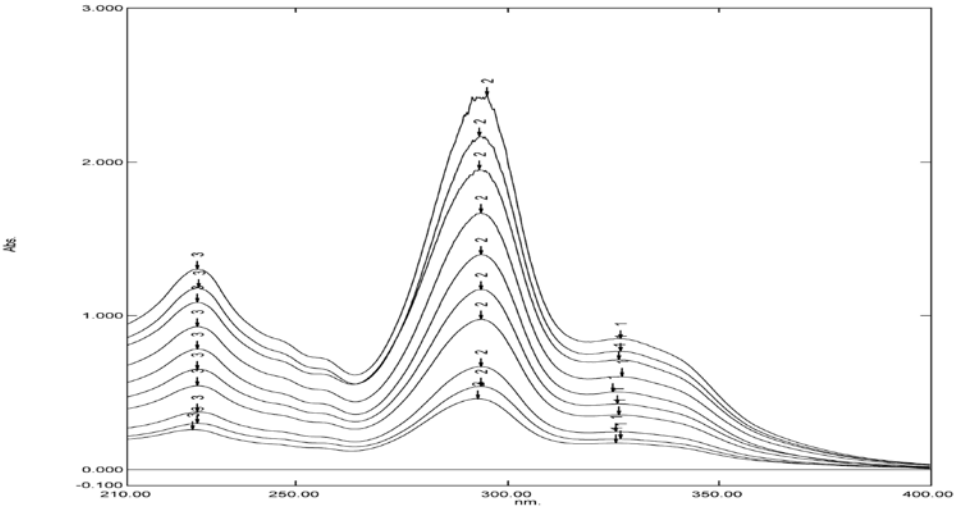
Ruggedness: In order to determine the ruggedness of the proposed method, the method was carried out simultaneously by two analysts.

RESULTS AND DISCUSSION

The regression equation of the calibration curve was found to be y=0.121x-0.015. The calibration curve is shown in (**Fig 3**).and represented in (**Table 1**)**.** The assay results of the commercial formulations are shown in (**Table 2**).

The method was found to be accurate and precise which was evident from its low %RSD values.(**Table 3 & 4**). Similarly the %RSD for Intraday and Interday Assay were found to be 0.001497 and 0.00387 respectively.(**Table 5 & 6**). The %RSD for Robustness was found to be 0.0112 and 0.0062 for the proposed method by taking 0.1N HCl solution and glacial acetic acid respectively (**Table 7**) while the %RSD for Ruggedness was found to be 0.040 and 0.0129 respectively when performed by two analysts separately.(**Table 8**). The limit of detection and limit of quantification were found to be 1.60 mg and 4.878mg respectively

Fig 2: Overlay Spectra of Ofloxacin showing λmax at 293nm



3: Calibration curve of Ofloxacin at 293nm

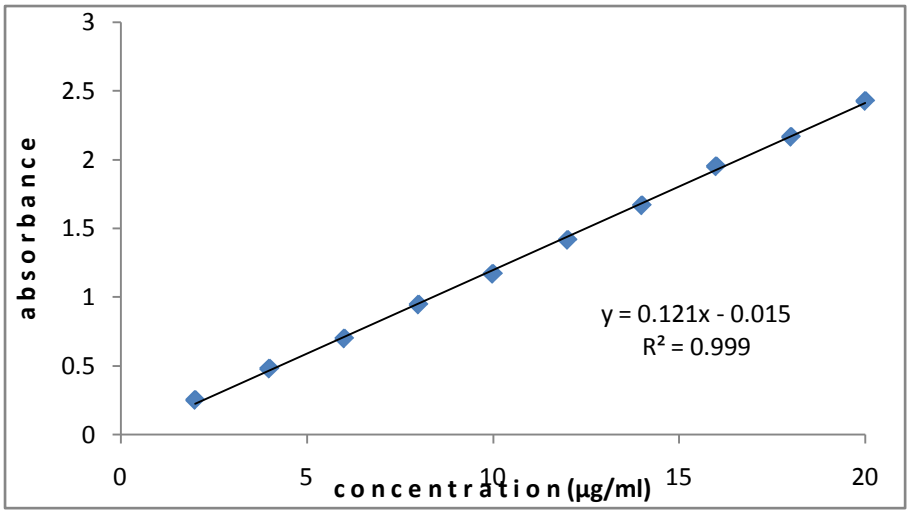


Table 1: Linearity table of Ofloxacin in 0.1N HCl solution

Concentration (µg/ml)	Absorbance
2	0.252
4	0.482
6	0.741
8	0.947
10	1.172
12	1.419
14	1.670
16	1.952
18	2.165
20	2.429

Table 2: Assay results of the marketed formulations

Formulation	Label Claimed(mg)	Observed amount(mg)	Assay Result (%)
OFLXCIN (Bombay Tablet Mfg. Co)	200	197.268	98.634
ACOFLOX (Acme Pharmaceuticals, Ahmedabad)	200	198.586	99.793

Table 3: Statistical analysis for ACCURACY of the proposed method

Samples	Concentration (µg/ml)		%Recovery	Statistical Analysis
	Pure	Formulation		
S1: 80%	8	10	98.02	Mean: 097.79
S1: 80%	8	10	97.77	S.D: 0.2946
S1: 80%	8	10	98.14	S.D: 0.3031
S2: 100%	10	10	101.48	Mean: 100.53
S2: 100%	10	10	99.25	S.D: 1.1510
S2: 100%	10	10	100.86	S.D: 1.1449
S3: 120%	12	10	101.40	Mean: 100.50
S3: 120%	12	10	101.79	S.D: 1.895
S3: 120%	12	10	98.33	%RSD: 1.8855

Table 4: Statistical analysis for PRECISION of the proposed method

Concentration	Absorbance	Amount Present	Statistical Analysis
10	1.226	10.0082	
10	1.229	10.033	Mean: 10.016
10	1.226	10.0082	
10	1.228	10.024	S.D: 0.01161
10	1.226	10.0082	%RSD:0.010869
10	1.229	10.033	
10	1.226	10.0082	
10	1.226	10.0082	

Table 5: Statistical analysis for INTRADAY ASSAY of the proposed method

S No.	Concentration (µg/ml)	Absorbance			Statistical Analysis
		Sampling 1	Sampling 2	Sampling 3	
01	10	1.228	1.228	1.226	Mean:10.00826
02	10	1.225	1.224	1.226	
03	10	1.226	1.225	1.228	S.D:0.00154
04	10	1.227	1.226	1.224	
05	10	1.224	1.2266	1.225	%RSD:0.001497
06	10	1.228	1.228	1.224	

Table 6: Statistical analysis for INTERDAY ASSAY of the proposed method

S No.	Concentration (µg/ml)	Absorbance			Statistical Analysis
		DAY 1	DAY 2	DAY 3	
01	10	1.223	1.235	1.237	Mean:10.0760
02	10	1.239	1.229	1.235	
03	10	1.234	1.237	1.234	S.D:0.003982
04	10	1.235	1.238	1.235	
05	10	1.238	1.235	1.228	%RSD:0.00387
06	10	1.234	1.237	1.234	

Table 7: Statistical analysis for ROBUSTNESS of the proposed method

ANALYST-I				ANALYST-II			
Conc. (µg/ml)	Abs	Calculated amount (mg)	Statistical Analysis	Conc. (µg/ml)	Abs	Calculated amount (mg)	Statistical Analysis
10	1.226	10.0082	Mean: 10.013	10	1.228	10.024	Mean:10.021
10	1.228	10.033		10	1.227	10.016	
10	1.229	10.024	S.D: 0.001506	10	1.227	10.016	S.D:0.00686
10	1.226	10.008		10	1.229	10.033	
10	1.226	10.008	%RSD:0.0112	10	1.228	10.024	%RSD:0.0062
10	1.225	10		10	1.227	10.016	

Table 8: Statistical analysis for RUGGEDNESS of the proposed method

0.1N HCl				Glacial Acetic Acid			
Conc. (µg/ml)	Abs	Calculated amount (mg)	Statistical Analysis	Conc. (µg/ml)	Abs	Calculated amount (mg)	Statistical Analysis
10	1.227	10.10744	Mean:10.064	10	1.221	9.975207	Mean:9.9820
10	1.227	10.10744		10	1.226	10.00826	
10	1.223	10.09917	S.D:0.0023	10	1.222	9.975207	S.D:0.0142
10	1.226	10.00826		10	1.221	9.966942	
10	1.225	10.00	%RSD:0.040	10	1.223	9.983471	%RSD:0.0129
10	1.223	9.983471		10	1.223	9.983471	

CONCLUSION:

The proposed method was found to be simple, sensitive, precise and rapid for the determination of Ofloxacin from pure and its dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims without interference of excipient and the other additives. Thus the proposed method can be used as an alternative method to the reported ones for the routine analysis of the drug in bulk and pharmaceutical dosage forms and can also be used for dissolution or similar studies**.**

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REFERENCES:

1. Sweetman SC. In Martindale, the Complete Drug Reference. London: Pharmaceutical Press, London. 1999; 32nd ed: pp 233.
2. United State Pharmacopoeia. United State Pharmacopoeial Convention, Inc. 2003; 26th ed: pp 1334.
3. British Pharmacopoeia. H. M. Stationary Press, London. 2007; II, pp 1163.
4. Hesham Salem. Spectroflurometric, atomic absorption spectrometric and spectrophotometric determination of some fluroquinolones. American Journal of Applied Sciences, 2005, 2: 719-729.
5. Mathur SC, Kumar Y, Murugesan N, Rathode YKS and Sethi PD. Spectrophotometric determination of Ofloxacin in Pharmaceutical formulations. Indian Drugs. 1992; 29: 376-377.
6. Immanuel C and Kumar AKH. Simple and rapid high-performance liquid chromatography method for the determination of Ofloxacin concentrations in plasma and urine. J Chromatogr B Biomed Sci Appl. 2001; 760: 91-95.
7. A.P. Arjekar, U.S. Kapadia, S.V. Raj and S.S. Kunjir. Quantitative determination of lomefloxacin, ofloxacin, pefloxacin and enrofloxacin in pharmaceutical dosages, bulk drug and processes monitoring of enrofloxacin by HPLC-RP. Indian Drugs.,1996, 33: 261-266
8. EVL. Silveria and EES Schapoval. Microbiological assay for determination of ofloxacin injection. J. Pharm. Biomed Anal., 2002, 1-2; 91-96.