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ESTIMATION OF OFLOXACIN IN BULK AND TABLET DOSAGE FORM BY NANODROP SPECTROPHOTOMETRIC METHOD

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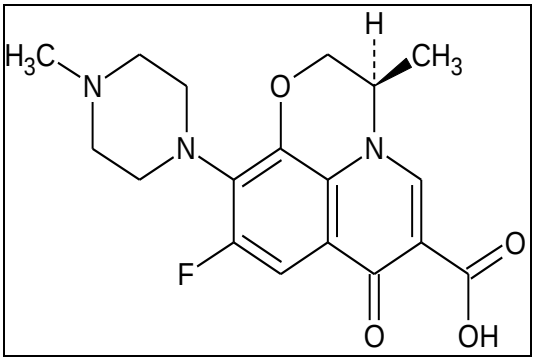
ABSTRACT

Ofloxacin is a fluoroquinolone antibiotic considered to be a second-generation fluoroquinolone. Ofloxacin is administered by oral, intravenous or topical (eye and ear drop) route. It is used as antibacterial for the treatment of various diseases like chronic bronchitis, pneumonia, urethritis, cervicitis, urinary tract infections, prostatitis, uncomplicated urethral and cervical gonorrhea. Ofloxacin is a pale yellow or bright yellow, crystalline powder. It is slightly soluble in water, soluble in glacial acetic acid, slightly soluble or soluble in methylene chloride, slightly soluble in methanol. A simple Nanodrop spectrophotometric method was developed for the determination of Ofloxacin in pure and its pharmaceutical tablet dosage form. Ofloxacin exhibiting λ max at 296 nm in mobile phase (0.05 M Phosphate buffer: Acetonitrile) in ratio of 65:35 and obeyed linearity in the concentration range of 1- 150 ppm. The proposed method was statistically validated.

INTRODUCTION: The scope of developing and validating analytical methods is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. Chemically Ofloxacin is fluoroquinolone. It is a second-generation fluoroquinolone antibiotic drug and is used as antibacterial for treatment of various diseases.



Ofloxacin: (*RS*) - 9- Fluoro- 3- methyl- 10- (4- methylpiperazin- 1- yl) - 7- oxo 2, 3- dihydro- 7 *H*- pyrido [1, 2, 3- *de*]- 1, 4 benzoxazine- 6- carboxylic acid

Tablet formulations containing 100 and 200 mg Ofloxacin in coated and dispersible form are available in the market. Literature survey revealed that various analytical methods such as HPLC, HPTLC and UV Spectrophotometry are used for simultaneous estimation of Ofloxacin with various drugs combination. No Nanodrop spectrophotometric method has been reported for estimation of Ofloxacin in single component formulation. Hence, an attempt has been made to develop new Nanodrop Spectrophotometry method for its estimation in pharmaceutical formulations with good accuracy, simplicity, precision and economy.

The Nanodrop ND-1000 is a full-spectrum spectrophotometer (UV and visible spectrum, 220-750 nm) for measuring the absorbance the sample droplet is held in place by surface tension when it is slightly compressed between the pedestal and the sample arm; this generates the defined pathway of 1 mm. The spectrum measurement is then performed with two optical fibers installed in the pedestal (emitting light of a Xenon lamp) and the sample arm (spectrometer with linear CCD array). Quantification is performed based on the spectrum measurement at the defined pathway of 1 mm. Unlike traditional spectrophotometers, the Nanodrop does not require cuvettes or capillaries. Instead, the sample is pipette directly onto the measurement pedestal.

EXPERIMENTAL:

Instrumentation: Spectral and absorbance measurements were made on Nanodrop spectrophotometer. Denver TB-215D balance was used for weighing the samples. Commercially available tablets of Ofloxacin were procured from the local market and estimated.

Chemicals and Reagents:

Solvents and reagents	Manufacturers
Potassium dihydrogen ortho phosphate (AR grade)	Qualigens fine chemicals
Acetonitrile	Qualigens fine chemicals
Water (Milli Q grade)	Millipore water filter
Ofloxacin standard (99.23%)	UPDPL, Lucknow

Apparatus/Instruments:

Name	Model	Manufacturer/Supplier
Nanodrop spectrophotometer	ND-1000	Nanodrop technologies Inc. USA
pH / ion analyzer	pH 510	Eutech instruments
Micropipettes	DH-43394	Thermo-scientific
Millipore water purification unit	BM5SN3112A	Millipore (India) Pvt. Ltd
Eppendorf and micro-pipette tips	-	From Axygen

OPTIMIZATION:

Scanning and determination of maximum wavelength (λ_{max}): In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, different solutions of the drug (40 ppm and 60 ppm) in mobile phase (0.05 M Phosphate buffer: ACN) were scanned using Nanodrop spectrophotometer within the wavelength region of 220 – 700 nm against mobile phase as blank. The resulting spectra are shown below (Fig. 1, 2 and 3) and the absorption curve showed characteristic absorption maxima at 296 nm for Ofloxacin.

METHOD:

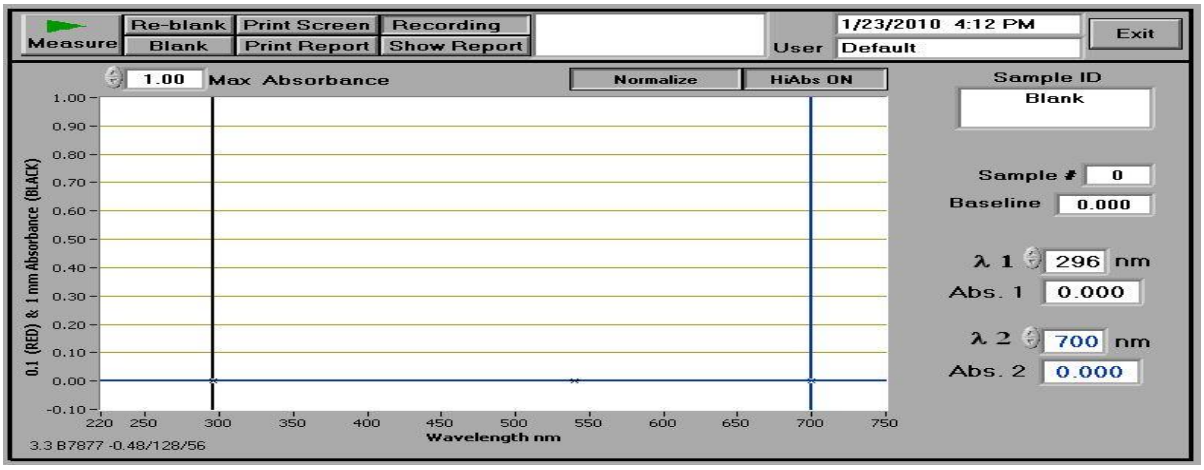
Preparation of 0.05 M Phosphate buffer solution: 680.45 mg of anhydrous Potassium dihydrogen ortho phosphate dissolved in 60 ml Millipore distilled water and volume made up to 100 ml by Millipore distilled water in 100 ml volumetric flask and pH of buffer is 4.56.

Preparation of mobile phase (100 ml): Mix 65 ml of 0.05 M Phosphate buffer and 35 ml of Acetonitrile to made mobile phase of Phosphate buffer: ACN in ratio of 65: 35 of pH 5.04.

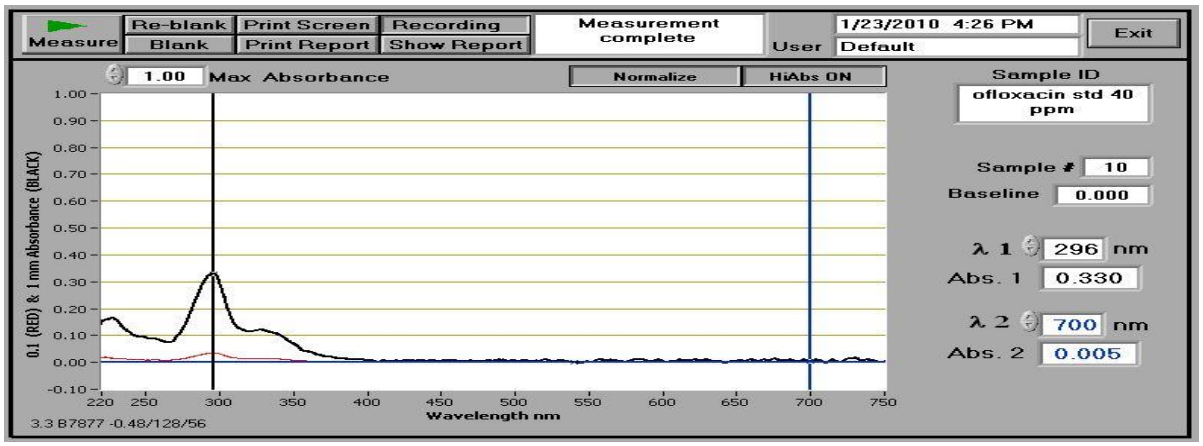
Preparation of Standard Stock Solutions: Standard stock solution was prepared by dissolving 10 mg of Ofloxacin in 10 ml of mobile phase to get concentration of 1mg/ml (1000 ppm) solutions.

Preparation of working standard solutions and construction of standard graph: The prepared stock solution was further diluted with mobile phase (pH-5.04) to get working standard solutions of 1, 2, 4, 6, 8, 10, 12, 20, 40, 60, 80, 100, 120 and 150 ppm of Ofloxacin to construct Beer's law plot for pure drug, the absorbance was measured at λ max 296 nm, against mobile phase (pH-5.04) as blank. The results are shown in table (1). The standard graph was plotted by taking concentration of drug on X-axis and absorbance on Y-axis and is shown fig 4. The drug has obeyed Beer's law in the concentration range of 1-150 ppm. The linearity curve data is shown in table (2).

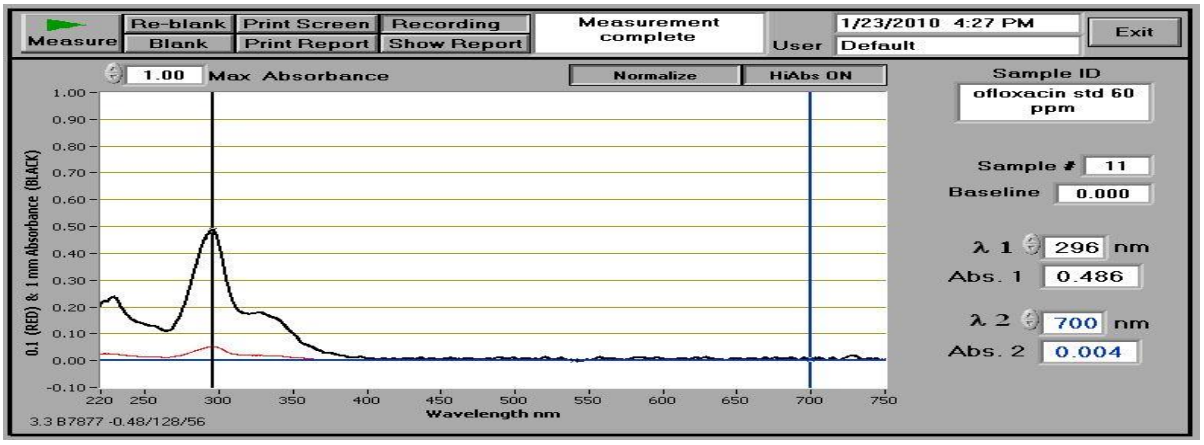
Preparation of sample stock solutions and working sample solutions: Ten tablets were accurately weighed and average was calculated. The tablets were then crushed to obtain fine powder. An accurately weighed quantity of tablet powder equivalent to about 10.0 mg of Ofloxacin was transferred to 10 ml volumetric flask, add 5 ml of mobile phase and shaken for 10 min. The volume was made up to the mark with mobile phase and required dilutions were made from sample stock solution. The recovery study from formation is shown in table (3).



(Fig. 1) Nanodrop spectrum of blank



(Fig. 2) Nanodrop spectrum of Ofloxacin std. 40 ppm



(Fig. 3) Nanodrop spectrum of Ofloxacin std. 60 ppm

Table 1: Linearity table of Ofloxacin in Working Standard

Concentration (ppm)	Absorbance
1	0.002
2	0.010
4	0.028
6	0.045
8	0.065
10	0.076
12	0.095
20	0.175
40	0.330
60	0.486
80	0.649
100	0.834
120	0.976
150	1.227

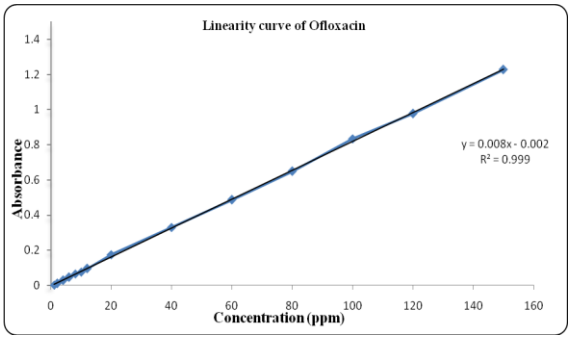


Fig. 4: Linearity curve of Ofloxacin in Working Standard

Table 2: Linearity curve data

Beer's Law limit (ppm)	1-150
Correlation coefficient (R^2)	0.999
Regression equation (y*)	y= 0.008x-0.002
Slope (m)	0.008
Y-Intercept (c)	0.002

Table 3: Recovery from the formulation

Formulation	Labeled amount (mg)	Nanodrop Spectrophotometry method*		
		Mean ± s. d (amount mg recovered)	% Drug recovered	% RSD
Zenflox^R-100-DT (tablets)	100	99.95±1.07	99.95±1.07	1.07

* Each value is average of three determinations ± standard deviation.

VALIDATION:

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of Ofloxacin within the linearity range were taken and added to the pre-analyzed formulation of concentration 10 ppm. From that percentage recovery values were calculated. The results were shown in Table (4). The response obtained for the various concentrations is plotted and observed to be linear (correlation coefficient – 0.999 for Ofloxacin). The graphical representation of accuracy studies is depicted in Figure 5.

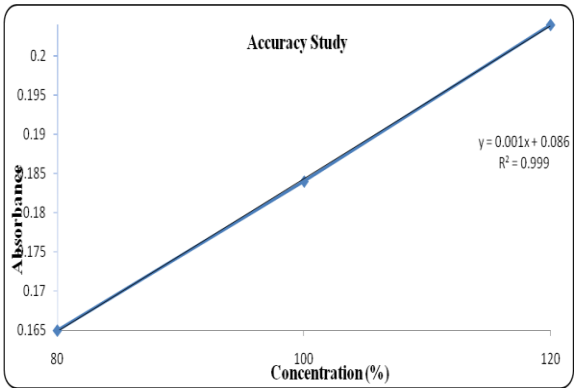


Fig. 5: Accuracy study curve

Table 4: Accuracy Readings

Sample ID	Concentration (µg/ml)		% Recovery of Pure drug	Statistical Analysis
	Pure drug	Formula -tion		
S_1 : 80 %	8	10	96.25	Mean=98.96
S_2 : 80 %	8	10	95	SD=3.847
S_3 : 80 %	8	10	105	
S_4 : 80 %	8	10	102.5	%RSD=3.88
S_5 : 80%	8	10	100	
S_6 : 80%	8	10	95	
S_7 : 100 %	10	10	94	Mean=97.5
S_8 : 100 %	10	10	101	SD=3.86
S_9 : 100 %	10	10	94	
S_{10} : 100 %	10	10	101	%RSD=3.96
S_{11} : 100 %	10	10	93	
S_{12} : 100 %	10	10	102	
S_{13} : 120 %	12	10	95.83	Mean=98.195
S_{14} : 120 %	12	10	104.17	SD=3.80
S_{15} : 120 %	12	10	95	
S_{16} : 120 %	12	10	94.17	%RSD=3.87
S_{17} : 120 %	12	10	102.5	
S_{18} : 120 %	12	10	97.50	

% Recovery = amount recovered/amount introduced X 100

Table 5: Accuracy studies

Concentration (%)	Absorbance (mean)
80	0.165
100	0.184
120	0.204
Correlation coefficient (R^2)	0.999
Slope (m)	0.001
Y-intercept (c)	0.086

Precision: The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From this absorbance, mean, standard deviation and % RSD was calculated. The readings are shown in table 6.

Table 6: Precision readings

Concentrations (ppm)	Absorbance	Statistical analysis
10	0.102	
10	0.097	Mean = 0.10033
10	0.100	
10	0.100	SD = 0.00002134
10	0.103	
10	0.100	%RSD = 0.02127

RESULTS AND DISCUSSION: From the optical characteristics of the proposed method, it was found that Ofloxacin obeys linearity within the concentration range of 1-150 ppm. From the results shown in Table (6) it was found that the % RSD is less than 2, which indicates that the method has good reproducibility. From the results shown in accuracy Table (4), it was found that the percentage recovery values of pure drug from the pre-analyzed solution of formulation were in between 97.5 – 98.96, which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

CONCLUSION: The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the

commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Ofloxacin in pure samples and pharmaceutical formulations.

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REFERENCES:

1. British Pharmacopoeia, vol.1 & 2, The British Pharmacopoeia Commission, London, 2009; 4317-4321.
2. United State Pharmacopoeia 30- NF 25, 2007; 2793.
3. Mathur SC, Kumar Y, Murugesan N, Rathode YKS and Sethi PD: Spectrophotometric determination of Ofloxacin in Pharmaceutical formulations. Indian Drug 1992; 29: 376-377.
4. Panzade PD and Mahadik KR: Simultaneous estimation of Ofloxacin and Tinidazole in tablet dosage form. Indian Drugs 2001; 38: 368-370.
5. Kasture VS, Bhagat AD and Puro NC: Spectrophotometric method for simultaneous estimation of Ofloxacin and Ornidazole in tablet dosage form. Indian Drugs. 2004; 41: 51-53.
6. Nagori BP, Shrivastava B, Sharma V and Rajput AS : Spectrophotometric method for simultaneous estimation of Ofloxacin and Ornidazole in tablet dosage form. Indian Drugs. 2006; 43: 676-678.
7. Halkar UP and Ankalkope PB: Reverse phase high performance liquid chromatographic determination of Ofloxacin and Tinidazole in tablets. Indian Drugs. 2000; 37: 585-588.
8. Kamble NS and Venkatachalam A : High performance liquid chromatographic determination of Ornidazole and Ofloxacin in solid dosage form. Indian Drugs. 2005; 42: 723-725.
9. Wankhede SB, Nanda RK, Prakash A and Chitlange SS : Simultaneous Spectrophotometric estimation of Ofloxacin and Satranidazole in tablet dosage form. J Pharm Res. 2008; 7: 92-94.
10. Priyabrata Pattanayak, Yogendra Prasad Chaudury, Rajesh Sharma, Prithwiraj Mohapatra and Dillip Kumar Setty : Simultaneous Spectrophotometric Estimation of Nitazoxanide and Ofloxacin in Combined Tablet Dosage Form. Research J. Pharm. and Tech. 2(2): April.-June. 2009 ;291-293.
11. Bhusari KP and Chaple DR: Simultaneous Spectrophotometric Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form. Asian J. Research Chem. 2(1): Jan.-Mar. 2009; 60-62.
12. Jitendra D. Fegade, Harshal P. Mehta, Rajesh Y. Chaudhari, Vijay R.Patil: Simultanious Spectrophotometric Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form. International Journal of ChemTech Research, April-June 2009, Vol.1, No.2, 189-194.