ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

SECOND AND THIRD ORDER DERIVATIVE SPECTROPHOTOMETRIC ESTIMATION OF OFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Sivakumar Ch.V*, Chandrakala Gunturu, Meera Indrakanti
Instrumental Methods of Analysis, Department of Biotechnology, ICFAI University,
Dehradun-248 197, Uttrakhand.
Email: siva.bioteck@gmail.com

Received on 14-07-2011 *Accepted on 22-07-2011*

Abstract

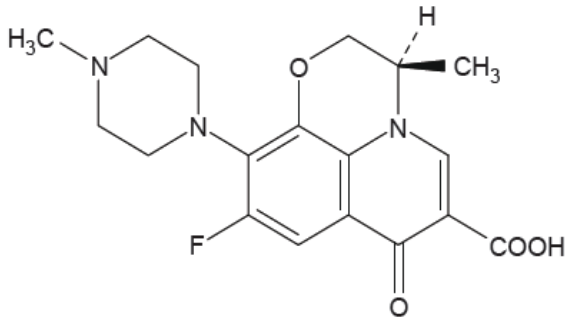
In the present study two simple, accurate, economic and repeatable derivative spectrophotometric methods were proposed for the determination of ofloxacin in bulk drug and in pharmaceutical form. Quantitative determination was carried out using second order derivative values measured at 289 nm and third order derivative values measured at 295 nm. Calibration graphs prepared at their wavelengths of determination were linear in the concentration range of ofloxacin using 2-30 µg/ml and 5-35 µg/ml for second and third order derivative spectrophotometric methods. The correlation in two methods is 0.998 with regression equation of Y=0.00045x-0.00017 and Y= 0.000072x-0.00003, where Y denotes amplitude of peak and x is the concentration of sample in µg/ml. The proposed methods have been extensively validated as per ICH guidelines. The developed spectrophotometric methods in this study are specific, sensitive, reproducible, precise, and can be routinely utilized for the analysis of pharmaceutical formulations.

Keywords: Ofloxacin, second order derivative spectrophotometry, third order derivative spectrophotometry.

Introduction

Ofloxacin[1] (OFL), ((±)-9-fluoro-2, 3dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid) (Fig.1.1a), is a fluoroquinolone derivative which is mainly used as

antibacterial for the treatment of urinary tract infection and sexually transmitted diseases. Ofloxacin is official in USP[3] and BP[2].



Literature survey reveals that few analytical methods were reported which include HPLC[6], HPTLC and spectrophotometric methods. There is no simple, accurate spectrophotometric estimation method available for Ofloxacin in dosage form. Derivative spectrophotometry is an analytical technique of great effectiveness for determination of both qualitative and quantitative information from spectra composed of unresolved bands and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of spectral curve[8]. In the present communication we have developed two simple, sensitive, economic, easy, rapid second and third order derivative methods for quantitative estimation of Ofloxacin in dosage form.

Experimental

A Perkin Elmer model Lambda 25 double beam UV/Vis spectrophotometer with spectral band width of 1 nm, wavelength accuracy 0.5 nm and a matched pair of 10 mm quartz cells was used for measuring absorbance of the solutions. Sartorius arium 611 UF system is used for preparation of ultra pure distilled water. Sartorius BS 124S analytical balance with 0.1 mg accuracy, Ofloxacin was a gift sample from Roorkee Research Labs., (Roorkee, India). Sodium chloride of analytical grade solution was used.

The standard stock solution of Ofloxacin was prepared by dissolving 100 mg of pure powder accurately weighed in 0.1 N NaOH. Initially 50-60 ml of 0.1N NaOH was added stand for 10 mins to ensure complete

soluble. The solution was filtered by using whatmann filter paper and made upto 100 ml total volume with 0.1N NaOH. The final concentration was appeared to 1000µg/ml stock solution. Further dilution was done by taking 10 ml of stock solution in 100 ml volumetric flask and the volume make upto mark with 0.1N NaOH and final concentration was 100µg/ml.

The average tablet weight was calculated using 10 tablets of oflac (400 mg of ofloxacin) then finely ground, homogenized and portion of powder accurately weighed and transferred in to 50 mL and diluted to mark with 0.1N NaOH. The mixture was allow standing for at least 20-30 min for dissolution and filtered through a whatman No 42 paper. Approximate dilutions were done with solvent and second-, third-order derivative spectra were recorded.

Derivative spectrophotometric method

Aliquots of standard stock solution were pipette out and diluted with 0.1N NaOH to get respective concentration solutions for second and third order derivative respectively. Solutions were scanned in the spectrum program from 360-220nm wavelength range on Perkin Elmer Lambda 25 spectrophotometer. Second order derivative spectra were obtained n=2 a sharp peak at 289 nm and calibration curve were found to be linear in the concentration range of 2-30 µg/ml. Third order derivative spectra were obtained at n=4 a sharp peak was obtained at 295 nm and calibration curve were found to be linear in the concentration range of 5-35 µg/ml.

Validation of methods:

Linearity

For both the methods, 5 point calibration curves were plotted on different days. The results obtained were used to calibrate the equation of the line by using linear regression by the least squares regression method. The linearity ranges of both spectrophotometric methods were found to be good. The statistical parameters are given in Table 1.

Specificity

Assessment of second and third order derivative spectrum of Ofloxacin in standard and drug dosage form solutions shows that wavelength of maximum and minimum absorbance did not change at their respective wavelengths. So

the derivative spectrophotometric methods are able to access ofloxacin in presence of excipients and hence, methods can be considered specific.

Recovery Studies

The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to analyzed sample solution at three different concentration levels (80 %, 100 %, and 120 %) within the range of linearity. The basic concentration level of sample solution selected, for spiking of the drugs standard solution, was 2 µg mL^{-1} of ofloxacin for both the methods are represented in Table 2.

Precision and Accuracy of the method

The precision of the proposed methods were determined by repeatability Interday (within-day) and Intraday (between-day). Three different concentrations 5, 10, 15 ug/mL are chosen and analyzed five times in the day for Interday precision and once for intraday precision. The results are reported in terms of standard deviation and relative standard deviation.

Robustness and Ruggedness:

Robustness of the methods was checked by changing the wavelength range and slit width, but results show that these variables significantly cannot affected the absorbance of the drµgs indicating the robustness of the methods.

Ruggedness of the methods was analyzed by different analysts maintained the similar operational conditions using similar homogenous slot of drug aliquots.

Results and Discussion

Ofloxacin being soluble in 0.1N NaOH, all the solutions were prepared in the same solvent. The desired different concentration solutions were prepared, scanned and transformed to second and third order derivative modes. Respective wavelengths were used for subsequent analysis. The linearity was found in the concentration range of 2-30 µg/ml in second derivative and 5-35 µg/ml forthird order derivative mode. The optical characteristics such as absorption maxima, Beer's law limit, were calculated. The regression analysis using method of least squares was

made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations and results will be summarized in table-1.

Table: 1. Results of Regression of Ofloxacin by the proposed methods.

Method	Range (µg/mL)	Linear Regression	R2
Zero-order spectrophotometric method	2-30	y = 0.069x - 0.036	0.99
Second-order Spectrophotometric method	2-30	Y=0.0004x0.0002	0.99
Third-order Spectrophotometric method	5-35	Y=0.00007x0.00003	0.99

Based on the five calibration curves

The suitability of these methods for pharmaceutical dosage forms was tested and confirmed by results that the other ingredients and exicipients present in pharmaceutical dosage forms did not interfere in the estimation when some commercial dosage form (T1, T2) were analyzed by this method.

Table: 2 Results of Precision and accuracy of ofloxacin by the proposed methods.

Method	**Wavelength (nm)**	**Interday (µg/mL) Added**	**Found +SD**	**Accuracy**	**Precision RSD %**	**Interday (µg/mL) Added**	**Found +SD**	**Accuracy**	**Precision RSD %**
Second order Spectrophotometric method	289	2	2.01+ 0.1	0.18	2.1	2	1.99+0.12	0.25	1.22
		4	4.02+0.09	1.27	1.98	4	4.01+ 0.06	0.72	1.78
Third order spectrophotometric method	295	6	6.02+ 0.06	1.01	1.56	6	6.02+0.51	0.26	2.16
		8	8.03+ 0.1	1.22	2.11	8	7.99+0.21	0.55	1.91

RSD= Relative Standard Deviation, Precision= (%relative error) (found-added)/addedx100. Average of six determinants

Table: 3 Results of recovery values of ofloxacin in pharmaceutical formulations.

Method	**Wavelength (nm)**	**Added g/mL**	**Found µg/mL**	**Recovery %**	**RSD %**
First-Order spectrophotometric method	289	5	4.98+0.119	99.6	2.27
		10	9.97+0.311	99.7	3.18
Second-Order spectrophotometric method	295	5	4.93+0.145	98.6	2.95
		10	9.99+ 0.114	99.9	2.54

Table: 4 Results of ofloxacin values from pharmaceutical formulations.

Commerical Preparations	Method	Labelled amount (mg)	% Labelled Amount Found (mg)	% RSD
Esoflox 200	Second order spectrophotometric	200	99.6 %	1.21
	Third order spectrophotometric	200	100.1 %	1.91

Average of six determinations

The method was validated for various parameters such as precision, recovery, intraday and Interday analysis, accuracy, linearity.

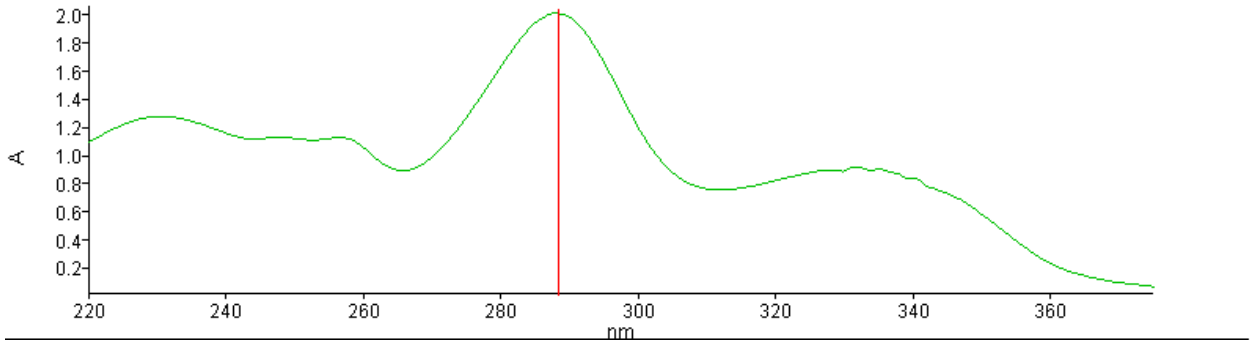


Fig 1: Zero-order absorption spectra of Ofloxacin.

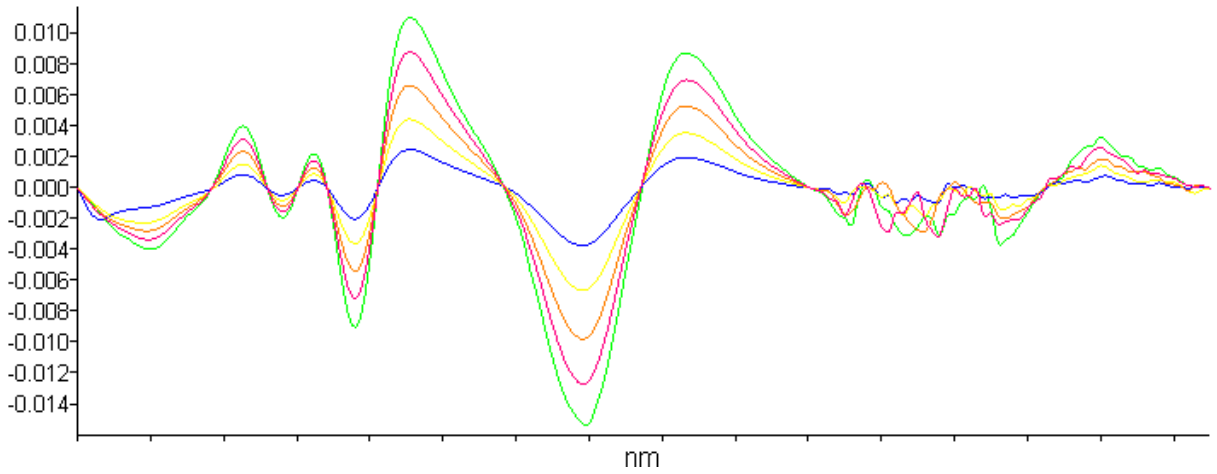


Fig 2: second order spectrum of ofloxacin standards.

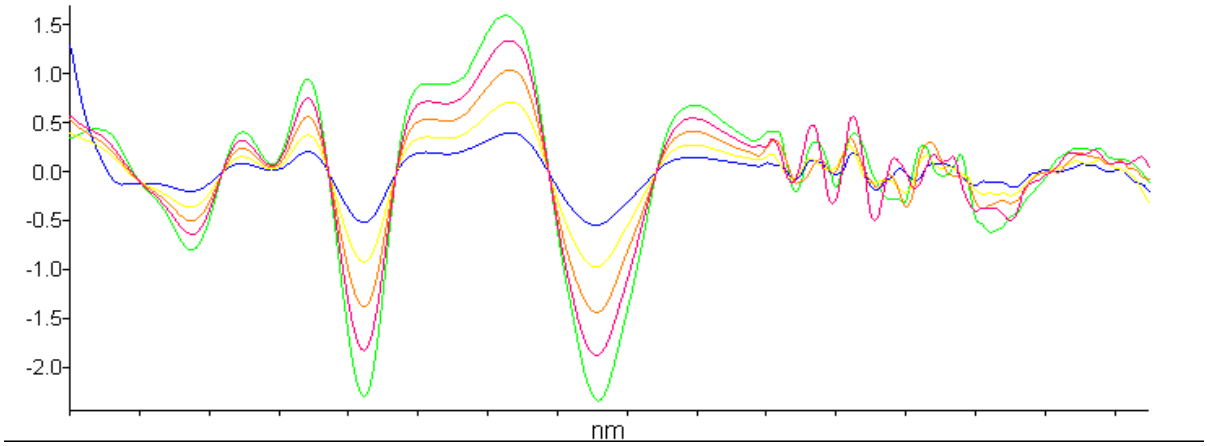
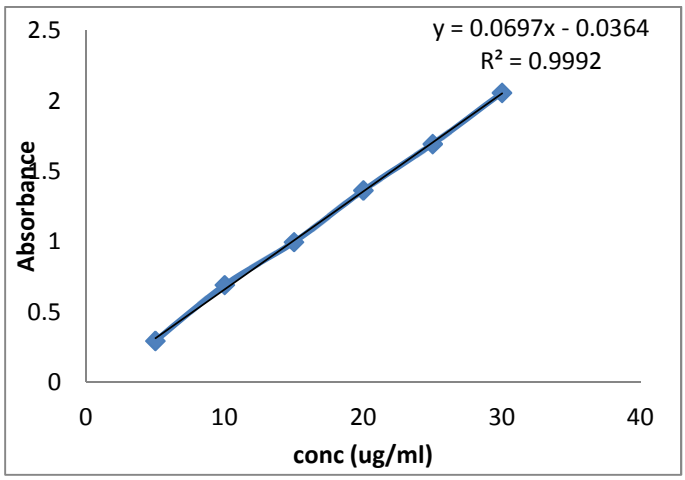
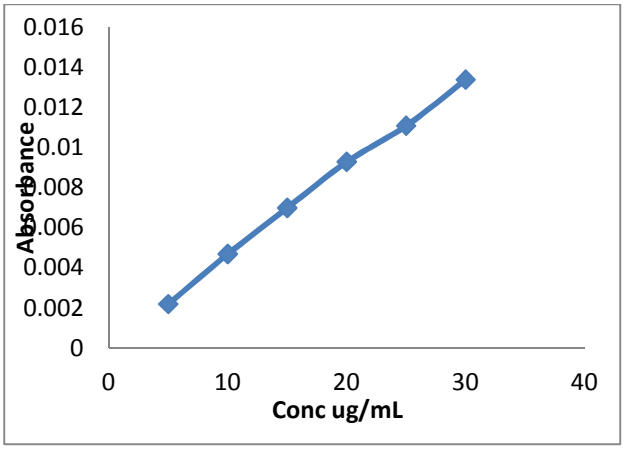


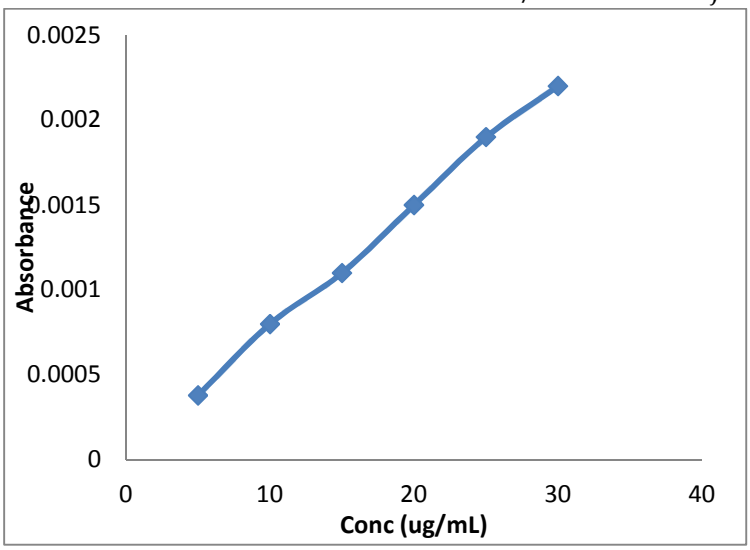
Fig 3: Third order spectrum of ofloxacin standards



Graph 1: Zero order Calibration curve of ofloxacin



Graph 2: Second order Calibration curve of ofloxacin



Graph 3: Third order calibration curve of ofloxacin.

Conclusion

The proposed methods are simple, precise, accurate, economical, easy, and rapid for the determination of Ofloxacin in pharmaceutical dosage forms. Analysis of authentic pharmaceutical formulations containing Ofloxacin was done no interference from the common additives and excipients. It can easily and conveniently be used for regular quality control analysis. It can be concluded that these methods can be used for the quantitative analysis of combined dosage forms in small laboratories with accuracy.

Acknowledgements:

The authors are thankful to M/s Roorkee Research Laboratories, Roorkee for providing gift samples of ofloxacin drug.

Reference

1. Budavari S. Eds. In. The Merck Index. Merck & Co., Inc; Whitehouse Station, NJ. 2001; 13 Ed: pp 1213.
2. British pharmacopoeia. Licensing division HMSO, Norwich. 2003; pp357.
3. United States Pharmacopoeia. United States Pharmacopoeial Convention, Inc. Rockville, 2004; pp1335.
4. European Pharmacopoeia. EDQM, Council of Europe, Strasbourg, France. 2005; 5th Ed: pp 2131.

5. Hesham salem. Spectroflurometric, atomic absorption spectrometric and spectrophotometric determination of some fluroquionlones. American Journal of Applied Sciences. 2005; 2: pp719-729.
6. Mathur SC, Kumar Y, Murugesan N, Rathode YKS and Sethi PD. Spectrophotometric determination of ofloxacin in pharmaceutical formulation . Indian drugs. 1992; 29: pp376-377.
7. Arjekar AP, Kapadia US, Raj SV and Kunjir SS. 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 pp 261-266.
8. Yilmaz B. Determination of Metoprolol in Pharmaceutical preparations by Zero, First, Second, and Third order derivative Spectrophotometric Method. International Journal of Pharma and Biosciences. 2010; 1, pp1-15.
9. Bhusari KP, Chaple DR. Simultaneous Spectrophotometric Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form. 2009; 2 (1), pp 60-62.
10. ICH Harmonised Tripartite Guideline, 2005. Validation of Analytical Procedures: Text and Methodology Q2 (R_1).

Corresponding Author:
Sivakumar Ch.V*,
Email: *siva.bioteck@gmail.com*