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Research Article

Estimation of Ofloxacin in Bulk and Formulation by Second Order DerivativeUV-Spectrophotometric Methods

Shinde Shivaji[*1], Jadhav Santosh[1], Kharat Rekha[1], Ansari Afaque[2] and Tamboli Ashpak[3]

[1]*Department of Pharmaceutics, Sahyadri College of Pharmacy, Methwade, Sangola-413307, Solapur, Maharashtra, India*
[2]*Department of Pharmaceutics, D.S.T.S Mandals College of pharmacy, Solapur, Maharashtra, India*
[3]*Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola-413307, Solapur, Maharashtra, India*

***Correspondence Info:**
Shinde Shivaji
Department of Pharmaceutics,
Sahyadri College of Pharmacy,
Methwade, Sangola-413307,
Solapur, Maharashtra, India
E-mail: sshivaji43@gmail.com

Abstract

Simple, fast and reliable spectrophotometric methods were developed for determination of Ofloxacin in bulk and pharmaceutical dosage forms. The quantitative determination of the drug was carried out using the second order Derivative Area under Curve method values measured at 295-301nm. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Ofloxacin using 2-10µg/ml (r^2=0.9947) for second order Derivative Area under Curve spectrophotometric method. The proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. The developed methods were successfully applied to estimate the amount of Ofloxacin in pharmaceutical formulations.
Keywords: Ofloxacin, Second order Derivative, Area under Curve (AUC).

1. Introduction

Ofloxacin is a synthetic broad spectrum antibacterial agent. Chemically ofloxacin[1] is a fluorinated carboxy-quinolone. It is a racemate, (±)-9-fluro-2, 3-dihydro-3-methyl 10- (4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. It has official compendial applications in BP[2], USP[3], and EP[4]. The assay procedure mentioned in these pharmacopoeias uses non aqueous titration for estimation of ofloxacin. Literature survey reveals spectrophotometric methods, atomic absorption spectrometry, spectro-flurometry[5,6,7,8], HPLC[9] and microbiological method[10] for its determination. To our knowledge no UV-spectrophotometric method is reported an attempt has been made to develop new Zero Order and Area under Curve Spectrophotometric method for estimation of Ofloxacin in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

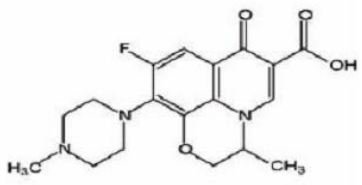

Fig. 1: Chemical structure of Ofloxacin.

2. Materials and Methods

2.1 Derivative Spectrophotometric Methods

Derivative spectrophotometry is a useful means of resolving two overlapping spectra and eliminating matrix interferences or interferences due to an indistinct shoulder on side of an absorption band. Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zeroth order or D^0 spectrum. The absorbance of a sample is

differentiated with respect to wavelength λ to generate first, second or higher order derivative.

[A]= f(λ): zero order

[dA/dλ= f(λ): first order

[$d^2A/d\lambda^2$]= f(λ): second order

The first derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ max of the absorption band. The second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ max of the fundamental band.[11]

2.2 Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 295 to 301 nm.

Area calculation: $(\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are wavelength range start and end point of curve region[12].

2.3 Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.4 Materials

Reference standard of Ofloxacin API was supplied as gift sample by Lupin Laboratory Park, Aurangabad. Methanol was obtained from Research - Lab Fine Chem Industries, Islampur, Mumbai, Maharashtra. Tablet sample with label claim 200 mg per tablet were purchased from local market Pune, Maharashtra, India.

2.5 Method development

2.5.1 Preparation of Standard and Sample Solutions

Stock solution of 10µg/ml of Ofloxacin was prepared in Methanol, Second Order Derivative Area under Curve spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with Methanol in a concentration range of 2, 04, 06, 08, and 10µg/ml with Methanol for Second Order Derivative Area under Curve spectrophotometric methods. Methanol also used as a blank solution.

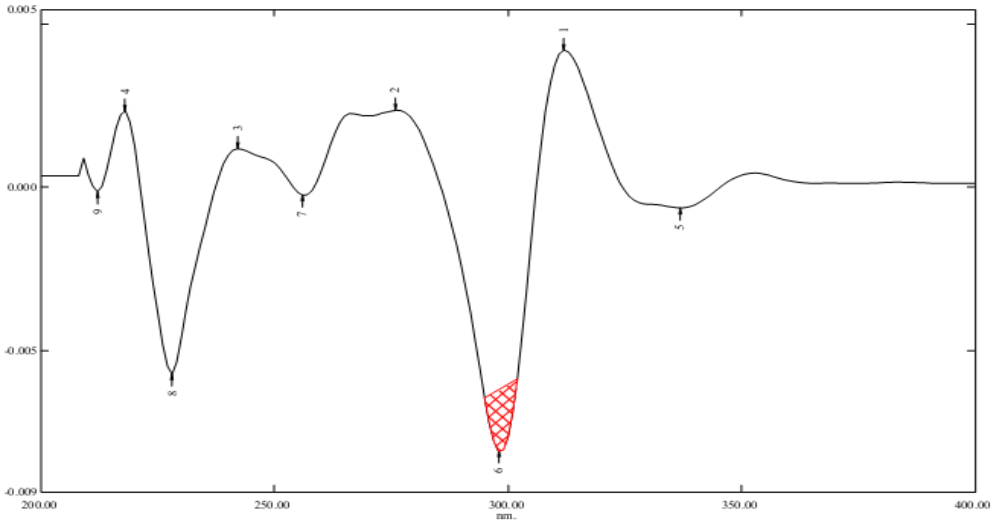

Fig. 2: Second order derivative Area under Curve spectrum of Ofloxacin in Methanol (10µg/ml).

2.5.2 Calibration curve for Ofloxacin

The dilutions were made from standard stock solution to get concentration of 2-10µg/ml respectively. These solutions were scanned from 400 to 200 nm and Second Order Derivative Area under Curve values was integrated in the range of 295-301 nm. The calibration curve was plotted between areas under curve values against concentration.

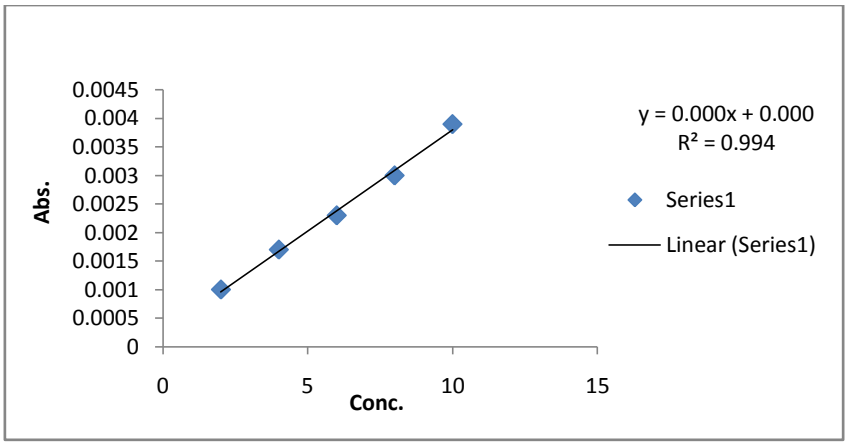

Fig. 3: Linearity of Ofloxacin

2.5.3 Assay of tablet formulation

Twenty tablets each containing 200 mg of Ofloxacin were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Ofloxacin was transferred in 100 ml of volumetric flask. 50 ml of distilled water was added and sonicated for 15 minutes. Then solution was further diluted up to the mark with Methanol. The solution was filtered using Whatmann filter paper no. 41, first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10µg/mL solution with water, subjected for UV analysis using Methanol as blank. This procedure was repeated three times.

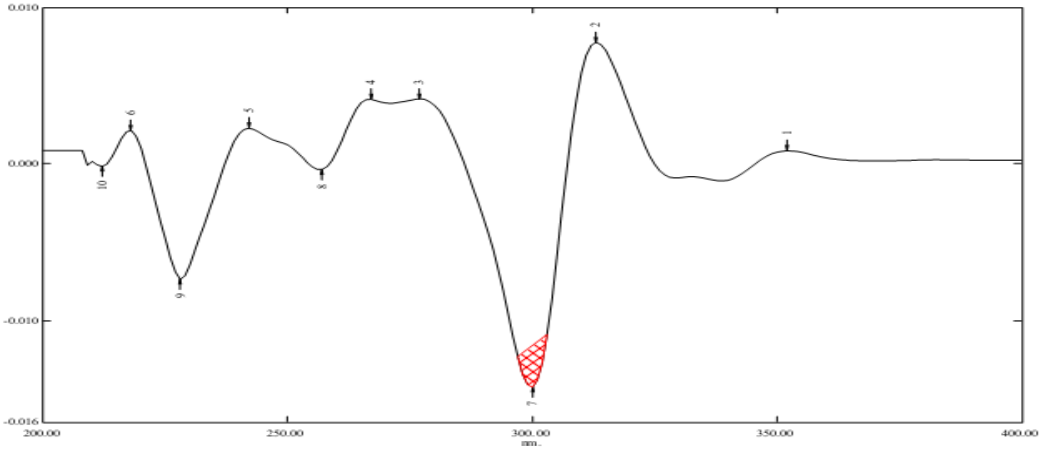

Fig. 4: Second order derivative Area under Curve spectrum of Ofloxacin of dosage form in Methanol (10µg/ml).

Table 1: Assay of tablet dosage form

Sr.No.	Sample Solution Concentration (µg/ml)	Amount found (%)*	Mean % found	%RSD*
1	10	97.05		
2	10	97.12	97.07	0.044
3	10	97.04		

*n=3, % RSD = % Relative Standard Deviation.

2.6 Method validation

The above method was validated for various parameters such as Accuracy, Linearity, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH guideline.

2.6.1 Accuracy

The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 15µg/ml Sample solution. Second Order Derivative Area under curve (AUC) was measured in wavelength range 295-301 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Table 2: Accuracy results for Ofloxacin

Accuracy level	Sample conc (µg/ml)	Std. conc	Total amount. Added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80	10	12	27	99.07		
100	10	15	30	98.98	99.03	0.0462
120	10	18	33	99.04		

2.6.2 Precision

The precision of an analytical procedure expresses the closeness of an agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions intraday precision was studied by integrating area of standard solution of 10µg/ml concentration at six independent series in the same day. Inter-day precision studies were performed by integrating area of standard solution of 10µg/ml concentration on three consequent days. The % RSD were calculated.

Table 3: Precision Study

Parameter	Intra day	Inter-day
Sample sol conc. µg/ml	10	10
AUC (mean)	0.0039	0.004
% RSD	1.49	0.14

2.6.3 Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

$$LOD = 3.3\ \sigma / S$$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$$LOQ = 10\ \sigma / S$$

Where, σ is standard deviation of the response and

S is the slope of the calibration curve.

LOD& LOQ of Ofloxacin was found to be 0.88µg/ml &2.67µg/ml respectively.

Table 4: Summary of validation parameters

Parameter	Result
λ range	295-301
Regression Equation (y=mx+c)	Y=0.0004x+0.0002
Linearity range	2-10µg/ml
Slope	0.0004
Intercept	0.0002
Correlation coefficient (R^2)	0.9947
Limit of Detection (LOD) µg/ml	0.88
Limit of Quantitation (LOQ) µg/ml	2.67
Accuracy (Mean % Recovery)	99.03
Precision (%RSD)	1.49

3. Results and Discussion

The UV visible spectroscopic method for the Ofloxacin by Second order derivative Area under Curve was found to be simple, accurate, economical and reproducible. The drug concentrations were found to be linear in the range of 02-10µg/ml and the correlation coefficient value of 0.9947 indicates that developed method was linear. For Precision the percent relative standard deviation (% RSD) was found to be 1.49 while, intra-day and inter-day precision results in terms of percent relative standard deviation values were found to be 1.49 and 0.14 respectively. Thus developed method is precise. The accuracy of the method was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value is ≤ 2 indicates the accuracy of the method. The Limit of Detection and Limit of Quantitation values were found to be 0.88µg/ml &2.67µg/ml respectively. The result of the analysis for pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for routine quality control analysis of Ofloxacin in bulk and pharmaceutical formulations.

4. Conclusion

The above developed spectroscopic AUC method for the analysis of Ofloxacin by Second order derivative Area under Curve was found to be simple, precise, and accurate, can be used for assay of bulk drug and pharmaceutical dosage formulations.

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