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Research article

Estimation of ofloxacin in bulk and formulation by derivative UV-spectrophotometric methods

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ABSTRACT

Simple, fast and reliable spectrophotometric methods were developed for determination of Ofloxacin in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in Methanol. The quantitative determination of the drug was carried out using the zero/0th, first, and second order method values measured at 298nm, 300nm and 300nm respectively. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Ofloxacin using 2-10µg/ml (r^2=0.9938, r^2=0.9992, r^2=0.9945) for zero,first and second order spectrophotometric method. All 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, UV visible spectrophotometry, Zero, first and second order derivative spectrum.

INTRODUCTION

Ofloxacin is a synthetic broad spectrum antibacterial agent. Chemically ofloxacin 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 is official in BP, USP, and EP. 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, HPLC and microbiological method for its determination. Hence an attempt has been made to develop new Zero, first and second Order Spectrophotometric methodsmethod for estimation of Ofloxacin in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy [1].

MATERIALS AND METHODS

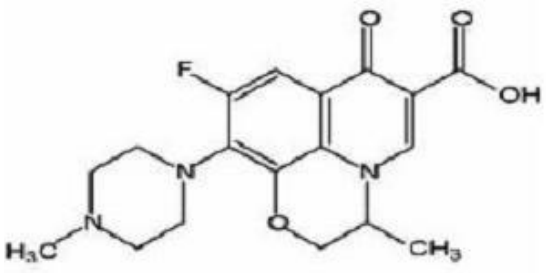
Derivative Spectrophotometric Methods

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 D0 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

[d2A/dλ2]= f(λ): second order

Figure 1: Chemical structure of Ofloxacin



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. 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.

MATERIALS

Reference standard of Ofloxacin API was supplied as gift sample by Marksan Pharmaceutical Ltd., Verna, and Goa.Methanol was getting from Research - Lab Fine Chem Industries, Islampur,Mumbai, Maharashtra.Tablet sample with label claim 200 mg per tablet were purchased from local market Pune [2].

Method development

Preparation of Standard and Sample Solutions

Stock solution of 10µg/ml of Ofloxacin was prepared in Methanol, for zero, first and second order spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with Methanol in a concentration range of 02, 04, 06, 08, and 10µg/ml with Methanol for zero order and area under the curve spectrophotometric methods. Methanol was used as a blank solution.

Assay Procedure

Twenty tablets each containing 200mg of Ofloxacin were weighed crushed to powder and average weight was calculated. Powder equivalent to 10mg of Ofloxacin was transferred in 100ml of volumetric flask. A 50 ml of Methanol 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. Appropriate dilutions were made with Methanol from stock solution for zero, first and second order spectrophotometric methods [3].

RESULTS AND DISCUSSION

The zero,first and second order method values spectra for Ofloxacin were recorded at the wavelength of 298nm, 300nm, 300nm respectively.

Linearity and Range

Under the experimental conditions described, the graph obtained for zero, first and second order method spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y=0.0933x +0.0049(r2=0.9938) at 298 nm for zero order derivative spectrophotometry, y=0.001x-0.0011(r2=0.9992) at 300nm for first order derivative spectrophotometry and y= 0.0005x + 0.0008 (r2=0.9945) at 300nm for second order derivative spectrophotometry. The range was found to be 2-10µg/ml for allzero, first and second order spectrophotometric methods [4].

Accuracy

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels. Accuracy results for Ofloxacin.

10µg/ml standard solution. For Zero, first and second order derivative were measured in wavelength range at 298, 300 and 300nm respectively and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level [5].

Precision

To determine the precision of the method, Ofloxacinsolutions at a concentration of 10µg/ml were analysed each three times for all zero, first and second order spectrophotometric methods. Solutions for the standard curves were prepared fresh everyday [6].

Sensitivity

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.

The LOD and LOQ were found to be 0.94µg/ml and 2.87µg/ml for zero order derivative, 0.35µg/ml &1.06µg/mlfor first order derivative and 0.89µg/ml &2.70µg/ml for second order derivative respectively.

Analysis of the Marketed Formulation

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.86%, 99.32% and 99.40% forzero, first and second order derivative spectrophotometric methods respectively. It may therefore be inferred that degradation of Ofloxacin had not occurred in the marketed formulations that were analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Ofloxacin in pharmaceutical dosage form [7, 8].

CONCLUSION

No UV/ zero, first and second order spectrophotometric methods have been described for the determination of Ofloxacin. Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Ofloxacin. The developed methods can be concluded as accurate, sensitive and

precise and can be easily applied to the pharmaceutical formulation.

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