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UV spectrophotometric estimation of ofloxacin by area under curve methods in bulk and pharmaceutical dosage form

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ABSTRACT

Simple and precise UV spectrophotometric method by area under curve [AUC] - have been developed and validated for the estimation of ofloxacin in bulk and its tablet formulation. The standard and sample solutions of ofloxacin were prepared in 0.1 N hydrochloric acid. Ofloxacin was estimated area under curve (AUC) method the zero order spectrum of ofloxacin was measured in between 288 nm to 298 nm. Beer's law was obeyed in the concentration range of 1 to 10 µg / ml with coefficient of correlation value 0.9995. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation were of 0.03723% method respectively. The proposed method was successfully applied for the determination of ofloxacin in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed method is simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Ofloxacin, UV spectroscopy, Area under curve method.

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 is official 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 HPLC [5,6], UPLC [7] titrimetric [9]spectrophotometric methods [10,11] for its determination.

This proposed work presents simple, accurate and reproducible UV spectrophotometric methods for determination of ofloxacin in tablet dosage form.

MATERIALS AND METHOD

Instrument and reagents

Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with spectral band width of 0.5 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software.

Reference standard of ofloxacin was obtained from reputed firm with certificate of analysis.
Hydrochloric acid was used of AR grade.

Preparation of standard drug solutions

100 mg standard ofloxacin was weighed accurately and transferred to a 100 ml volumetric flask and sonicated with 30 ml of 0.1N hydrochloric acid for 15 minutes. The volume was made up to the mark with 0.1N hydrochloric acid to give a stock solution of ofloxacin of concentration 1000 µg /ml. From this solution, 10 ml of solution was pipetted out and transferred into 100 ml volumetric flask. The volume was made up to mark with 0.1N hydrochloric acid to give a working standard solution of concentration 100 µg/ml.

Estimation from tablets

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of ofloxacin was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1N hydrochloric acid to give concentration as 100 µg /ml. Such solution was used for analysis.

Experimental

Method : Area under curve (AUC) method

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 were calculated by UV probe 2.42 software. In this method, 10 µg/ml solution of ofloxacin was scanned in the spectrum mode from 288 nm to 298 nm. From zero order spectrum the AUC calculation was done. The AUC spectrum was measured between 288 nm to 298 nm (Fig. 1).

Fig. 1. Area under curve spectrum of ofloxacin (10 µg/ml) showing area from 288 nm to 298 nm

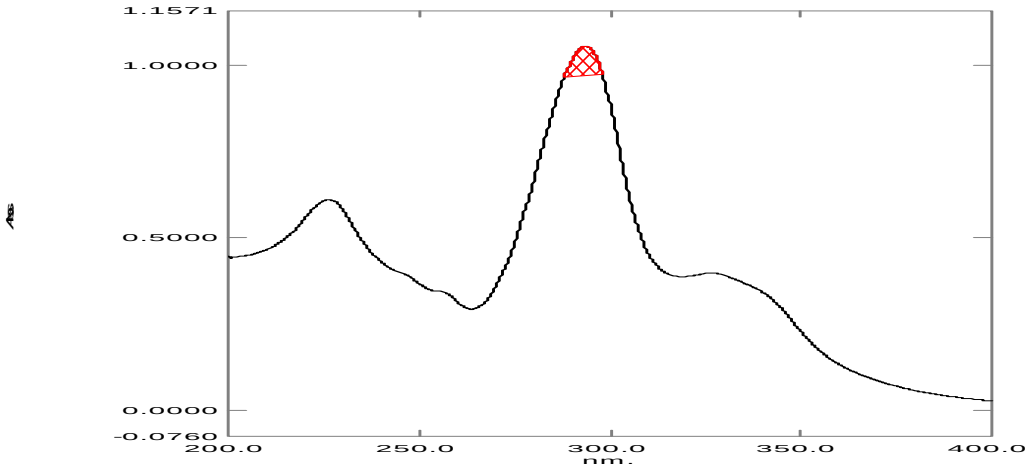
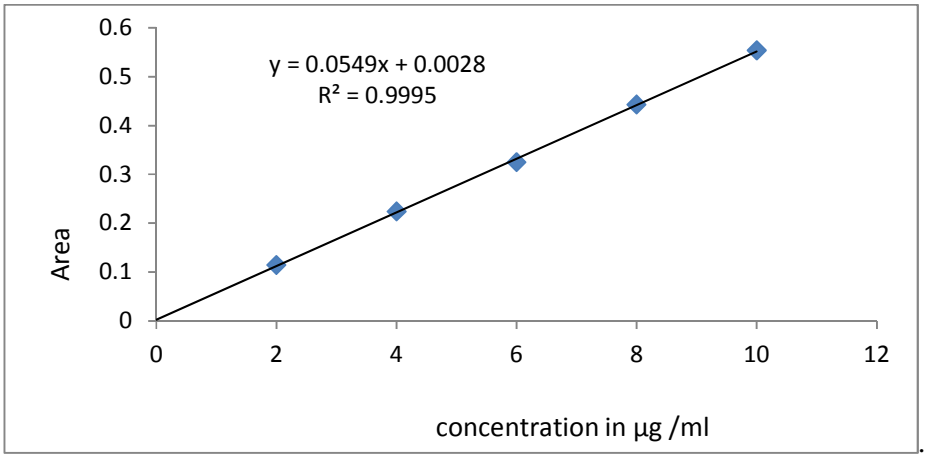


Fig. 2. Calibration curve for ofloxacin by area under curve spectroscopy



Into series of 10 ml graduated flask, varying amount of standard solutions of ofloxacin was pipette out and volume was adjusted with absolute alcohol. Solutions were scanned between 400 nm to 200 nm in spectrum mode. The AUC calculations were done and the calibration curve for ofloxacin was plotted in the concentration range of 1 to 10 µg/ml (Fig. 2).

Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	Area under curve (AUC) method
Detection Wavelength (nm)	288-298
Beer Law Limits (µg/ml)	1-10
Correlation coefficient(r^2)	0.9995
Regression equation (y=b+ac)	
Slope (a)	0.0549
Intercept (b)	0.0028

Validation

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table (2).

Table 2: Results of recovery of ofloxacin for area under curve (AUC) method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	2.0025	100.1253	0.00428	0.213745
2	2	3.9719	99.29825	0.00573	0.144257
2	4	5.9526	99.21053	0.004297	0.072192
2	6	7.9360	99.20113	0.020051	0.252651
		mean	99.4588	0.00859	0.170712

Precision

The method precision was established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical method in six replicates. The values of relative standard deviation lie well within the limits indicated the sample repeatability of the method. The results obtained are tabulated in table 3.

Table 3: Precision- method precision

Experiment no.	Weight of ofloxacin taken in mg	Weight of ofloxacin found in mg
1	10	9.983926
2	10	9.996428
3	10	9.99107
4	10	9.99107
5	10	9.989284
6	10	9.989284
	Standard deviation	0.00372
	%RSD	0.03723

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 10 mg of ofloxacin was transferred to 100 ml of volumetric flask. A 30 ml of 0.1 N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1 N hydrochloric acid to give concentration as 100 µg /ml. Such solution was used for analysis.

Solution was scanned between 400 nm to 200 nm in spectrum mode. The area under curve of resulting solutions was measured at between 288 nm to 298 nm by using as blank as 0.1 N hydrochloric acid. The area under curve of final solutions was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 288 nm to 298 nm. Similarly area under curve of the same solution was read on 1st, 2nd and 5th day. The amount of ofloxacin was estimated by comparison with standard at 288 nm to 298 nm, table 4.

Table 4: Summary of validation parameter for intra-day and inter-day

Sr. no.	Parameters	Area under curve (AUC) method
(A)	Intra-day precision (n=3) Amount found ± % RSD	99.55% 0.5446
(B)	Inter-day precision (n=3) Amount found ± % RSD	98.865% 0.765
(C)	Ruggedness Analyst to analyst(n= 3) %RSD	0.723

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 5.

Table 5: Values of results of LOD and LOQ

parameters	**Area under curve (AUC) method**
Limit of Detection (µg/ml)	0.01466
Limit of Quantification (µg/ml)	0.0444

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of ofloxacin sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of ofloxacin was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULT AND DISCUSSION

The area under curve UV-spectroscopic method is useful for routine analysis of ofloxacin in bulk drug and formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Ofloxacin has the absorbance maxima in the areas were measured between 288 nm to 298 nm . The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 10 µg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of ofloxacin in pharmaceutical formulation.

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