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Research Article

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UV derivative spectrophotometric methods for estimation of ofloxacin in bulk and pharmaceutical dosage form

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ABSTRACT

The objective of the study was to develop simple, accurate, precise and rapid UV first and second order derivative spectrophotometric methods with subsequent validation by using ICH guidelines for the determination of ofloxacin in pharmaceutical dosage form. The proposed first and second order derivative methods involve the measurement of absorbance of drug at 278 nm and 234.6 nm for the estimation of ofloxacin respectively. The linearity of the proposed methods were found in the concentration range of 0.5 – 10 µg /ml (r^2= 0.9995) for first order and 2 – 10 µg /ml (r^2= 0.9953) for second order derivative methods and the percentage mean recovery was found to be 100.069 % and 99.792% respectively. The methods were also statistically validated for its linearity, accuracy and precision. Both intra and inter day variation showed less percentage (%) RSD values indicating high grade of precision of these methods.

Keywords: UV derivative spectrophotometric estimation, ofloxacin, validation.

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.

EXPERIMENTAL SECTION

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.

Method: First order derivative method

For the selection of analytical wavelength, 10 µg/ml solution of ofloxacin was scanned in the spectrum mode from 400 nm to 200 nm by using 0.1 N hydrochloric acid as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 278 nm.

Fig. 1(a): Overlay spectra of first order derivative of ofloxacin in the concentration range of 2 – 10 µg/ ml

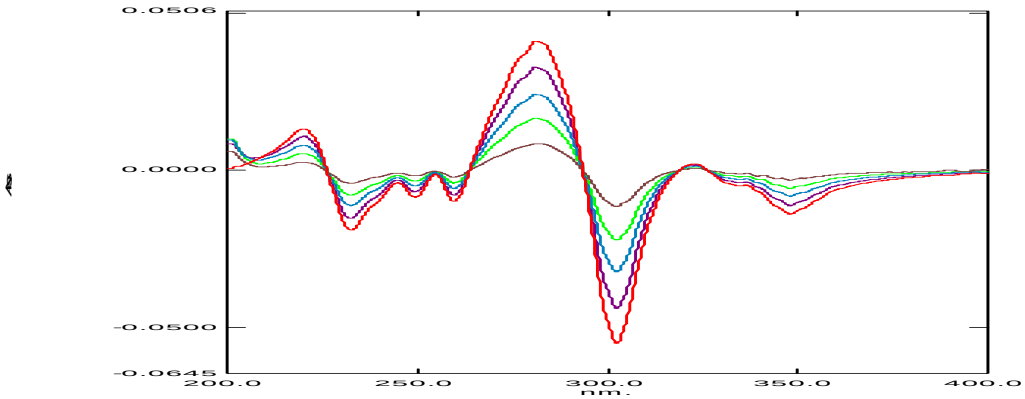
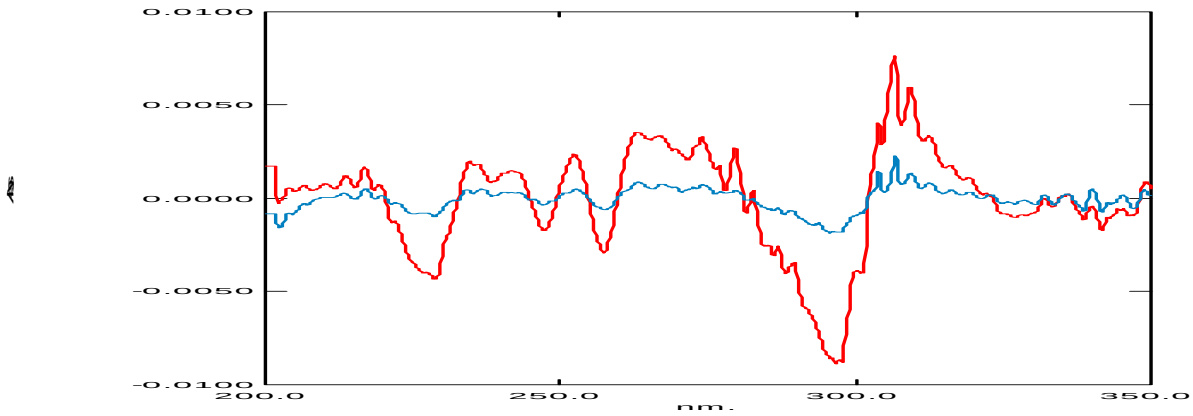


Fig. 1(b): Overlay spectra of second order derivative of ofloxacin in the concentrationrange of 2 and 10 µg/ ml



Method: Second order derivative method

For the selection of analytical wavelength, 10 µg/ml solution of ofloxacin was scanned in the spectrum mode from 400 nm to 200 nm by using 0.1N hydrochloric acid as blank. The second order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 234.6 nm.

Preparation of calibration curves

Series of solutions containing 0.5 – 10 µg/ ml of ofloxacin were used to determine linearity of the proposed method respectively. Solutions were scanned in the spectrum mode and absorbance spectra were converted to first order derivative spectra [Fig. 1(a), 1(b)].

After observing the overlain first order derivative spectra of ofloxacin, wave length selected was 278 nm, where ofloxacin showed considerable absorbance. The calibration curves were plotted of dA/ dλ against concentrations [Fig. 2 (a)].

After observing the overlain second order derivative spectra of ofloxacin wave length selected was 234.6 nm, ofloxacin showed considerable absorbance. The calibration curves were plotted of dA/ dλ against concentrations [Fig. 2(b)].

Fig.2 (a): Calibration curve of ofloxacin in the concentration range of 2-10 µg/ml

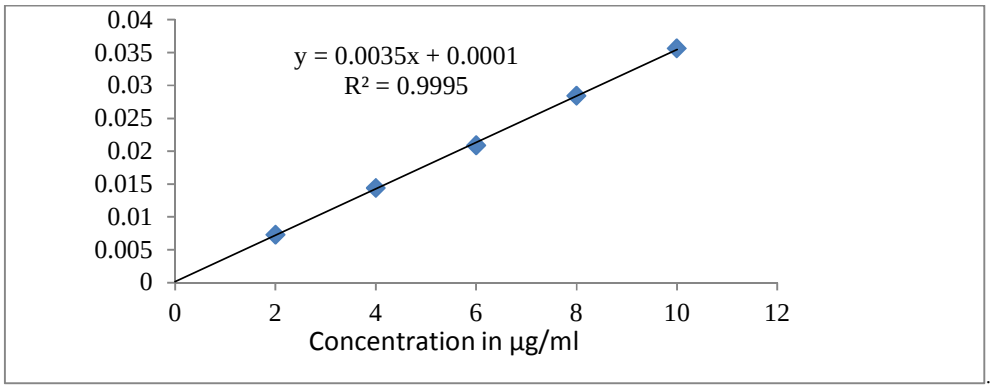
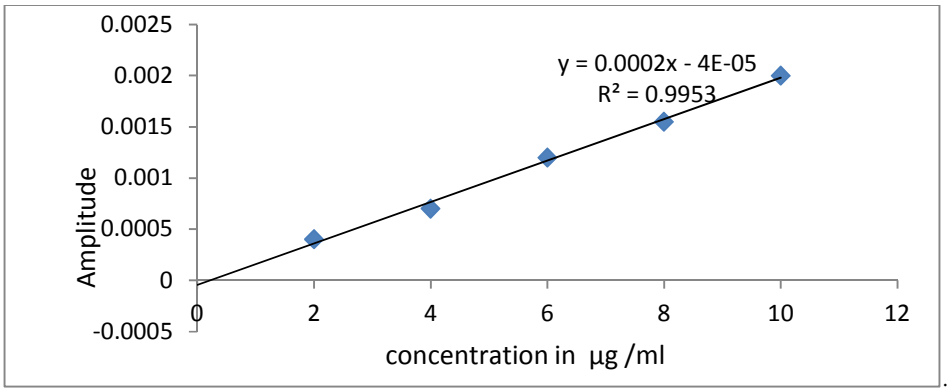


Fig.2 (b): Calibration curve of ofloxacin in the concentration range of 2-10 µg/ml



Results of the analysis are given in table 1.

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

Parameter	First order	Second order
Detection Wavelength (nm)	278	234.6
Beer Law Limits (µg/ml)	0.5-10	2-10
Correlation coefficient(r^2)	0.9995	0.9953
Regression equation (y=b+ac)		
Slope (a)	0.0035	0.0002
Intercept (b)	0.0001	-0.00005

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 of each drug. Such solution was scanned in the range of 200-400 nm against 0.1 N hydrochloric acid as blank. The absorbance spectra were converted to first order and second order derivative spectra. Calculations were done as per the equations. The concentrations of ofloxacin present in tablets were calculated by substituting the values of absorbance in linearity equations.

(a) For first order derivative method, Y = 0.0035x + 0.0001
(b) For second order derivative method, Y = 0.0002x + 0.00005

Method Validation
These methods were validated according to ICH guidelines.

Accuracy
To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for ofloxacin was found in the range of 99.75 % to 100.08 for first order derivative method and 99.623 to 100.29 % for second order derivative respectively. (Table 2(a) and (b)).

Table 2 (a): Statistical evaluation of the data subjected to accuracy for first order derivative method

Level of % recovery	Amount present in µg/ml	Amount added in µg/ml	Amount found in µg/ml	% Recovery	Mean % recovery
80%	2.0	1.6	3.61	100.27	99.75
	2.0	1.6	3.53	98.05	
	2.0	1.6	3.63	100.83	
100%	2.0	2.0	4.02	100.50	100.08
	2.0	2.0	4.03	100.75	
	2.0	2.0	3.96	99.00	
120%	2.0	2.4	4.41	100.23	100.07
	2.0	2.4	4.45	101.13	
	2.0	2.4	4.35	98.86	
% R.S.D.				1.060	

Table 2 (b): Statistical evaluation of the data subjected to accuracy for second order derivative method

Level of % recovery	Amount present in µg/ml	Amount added in µg/ml	Amount found in µg/ml	% Recovery	Mean % recovery
80%	2.0	1.6	3.65	101.38	99.623
	2.0	1.6	3.54	98.33	
	2.0	1.6	3.57	99.16	
100%	2.0	2.0	3.95	98.75	99.66
	2.0	2.0	4.07	101.75	
	2.0	2.0	3.94	98.50	
120%	2.0	2.4	4.44	100.90	100.29
	2.0	2.4	4.38	99.54	
	2.0	2.4	4.42	100.45	
%R.S.D.				1.2924	

Linearity
The linearity of measurement was evaluated by analyzing different concentration of the standard solutions of ofloxacin in the range of 0.5-10 µg/ml for first order and 2 to 10 µg/ml for second order derivative respectively.

Precision
The method precision was established by carrying out the analysis of tablets powder blend containing 200 mg of ofloxacin. The assay was carried out for the drugs by using proposed analytical method in six replicates. The values of relative standard deviation were well within limits 0.6459 % and 1.154 % for ofloxacin respectively indicating the sample repeatability of the methods. The results obtained are tabulated in table 3**.**

Table 3: Statistical evaluation of the data subjected to method of precision

Sr. No.	Sample No.	% Assay	
		First order derivative method	Second order derivative method
1	1	100.5	98.75
2	2	100.75	101.75
3	3	99.00	98.50
4	4	100.02	99.870
5	5	100.37	99.92
6	6	100.65	99.967
Mean % assay		100.215	99.792
%R.S.D.		0.6459	1.154

Intra-day precision was estimated by assaying tablets powder blend containing 200 mg of ofloxacin. The assay was carried out for the drugs by using proposed analytical method in six replicates. The results were average for statistical evaluation.

Inter-day precision was estimated by assaying tablets powder blend containing 200 mg of ofloxacin for three consecutive days (i.e. 1^{st}, 3^{rd} and 5^{th} days). The statistical validation data for intra and inter day precision is summarized in table 4.

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

Sr. No.	Parameters	First order derivative	Second order derivative
1	Intra-day precision (N=3)amount found ± % R.S.D.	99.60% 0.24847	99.35% 0.03646
2	Inter-day precision (N=3)amount found ± % R.S.D.	98.484 0.13607	98.785% 0.1768

Both intra- day and inter-day precision variation found to be less in % RSD values. It indicates high degree of precision of the method.

RESULT AND DISCUSSION

The developed first and second order derivative spectrophotometric methods for determination of ofloxacin in tablet formulation was found to be simple and convenient for the routine analysis of drug. The proposed method is accurate, precise and reproducible. It is confirmed from validation data as given in tables 1 to 4. The % RSD was found to be less than 1, which indicates validity of method. Linearity was observed by linear regression equation method for ofloxacin in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity figure 2 (a) and 2 (b).

The assay results obtained by proposed method is shown in table 2 are in good agreement. Hence proposed method can be used for routine analysis pharmaceutical dosage form. Methods are simple, accurate, precise, reliable, rapid, sensitive, reproducible and economical. It is validate as per ICH guidelines.

CONCLUSION

The proposed methods are simple, precise, accurate and rapid for the determination of ofloxacin pharmaceutical dosage form. The methods do not require any ratio of first and second order derivative. The amplitude of first and second order derivative can be directly used to assay of formulation. This method can be adopted as an alternative to the existing methods. It can be easily and conveniently adopted for routine quality control analysis.

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