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Determination of ofloxacin in bulk drug and pharmaceutical dosage form by high performance liquid chromatography method

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ABSTRACT

Rapid and accurate reverse phase high performance liquid chromatography method is described for determination of ofloxacin from the bulk drug and pharmaceutical dosage form. It was observed that Polaris C18 (15 x 4.6 mm i.d.) with 5 µ particle size column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of buffer and acetonitrile (80:20 % v/v). The buffer was mixtures of 0.01 M ammonium acetate adjusted the pH 3 with ortho-phosphoric acid. The detection was carried out at wavelength 294 nm. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution with the linear range 10-30 µg/ ml. The method has been successfully used to assay of pharmaceutical dosage form i.e. tablets with good recoveries.

Key words: Ofloxacin, Ammonium acetate, acetonitrile, orthophosphoric acid, HPLC.

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 METHODS

Chemical and reagents

Reference standard of ofloxacin was obtained from reputed firm with certificate of analysis. Ammonium acetate, acetonitrile and ortho-phosphoric acid were used of analytical grade and HPLC grade water was used from Millipore. Standard and sample solutions were prepared in diluent [mixture of buffer and acetonitrile (80:20 % v/v)].

Instrumentation

The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZChrom Elite software.

A SHIMADZU analytical balance(0.01 mg) was used.

Preparation of Standard preparation

Standard solution

A 20 mg of standard ofloxacin was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 2 minutes. The volume was adjusted up to the mark with diluent to give concentration as 2000 µg /ml. The working standard solution was prepared by diluting 1 ml of 2000 µg /ml solution to 10 ml with diluent to get concentration 200 µg /ml.

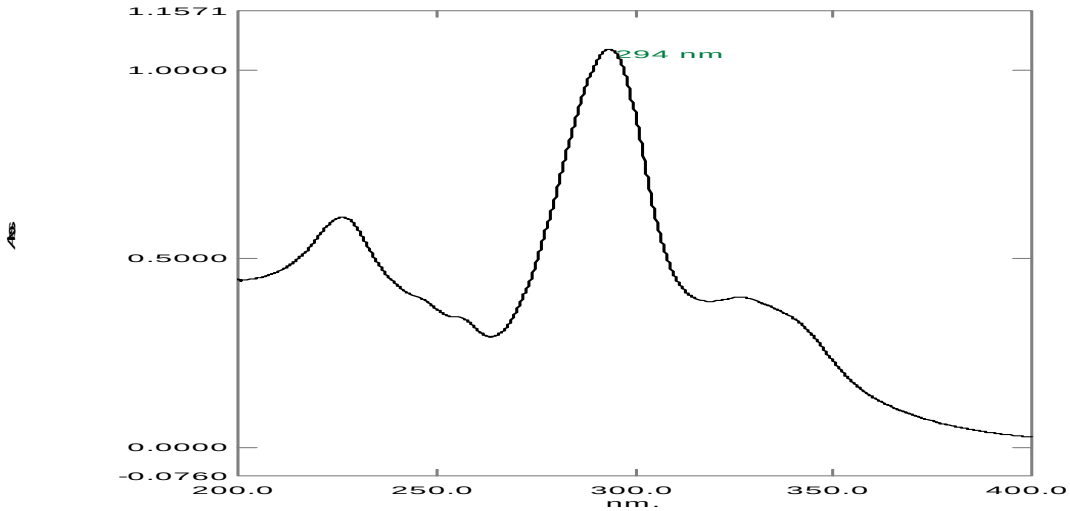
Sample preparation

Twenty tablets were weighed accurately and average weight of each tablet was determined. About 2 mg of ofloxacin sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 200 µg /ml.

Chromatographic condition

Chromatographic separation was performed at ambient temperature on a reverse phase Polaris C18 (15 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer and acetonitrile (80:20 % v/v). The buffer was mixtures of 0.01 M ammonium acetate adjusted the pH 3 with ortho-phosphoric acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 294 nm. (Fig. no.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: UV spectra of ofloxacin



Method validation

System suitability

System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), symmetry, area and % area were determined. The results are shown in table 1 which indicates good performance of the system.

Table 1: System suitability parameters evaluated on standard solution of Ofloxacin

Retention Time	Area	Area %	USP Plate Count	Symmetry
3.603	2952005	100	1932	1.66

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard ofloxacin was injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

Figure 2: Typical chromatogram of ofloxacin (standard)

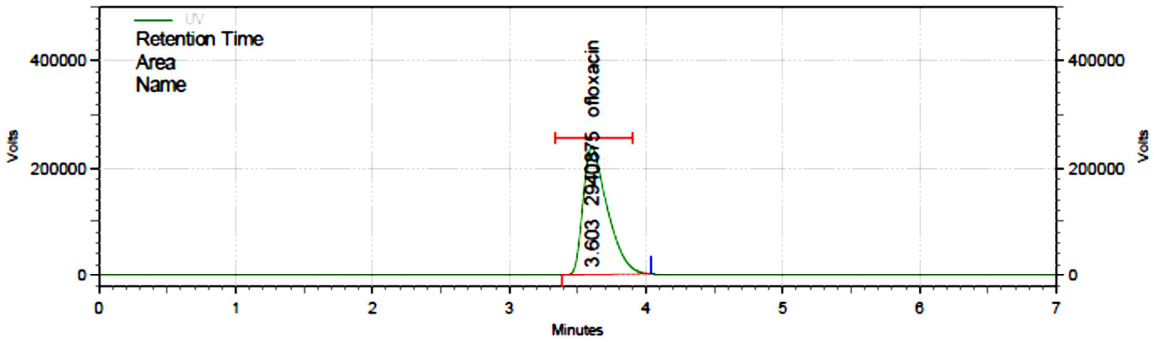
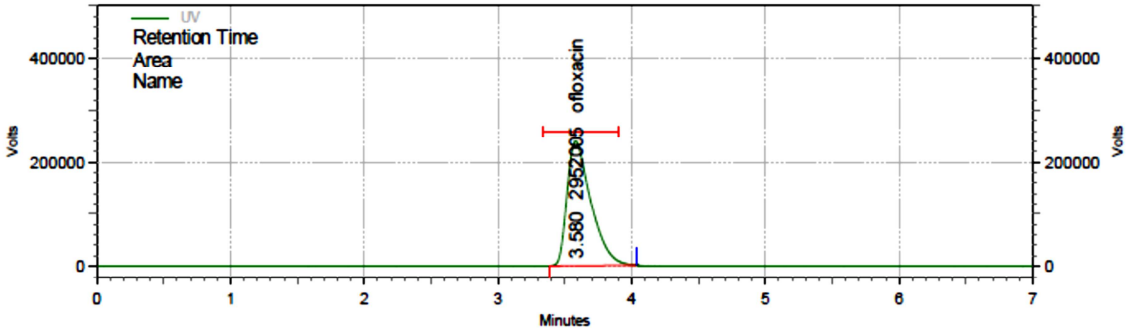


Figure 3: Typical chromatogram of ofloxacin(sample)



Linearity

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in table no. 2.

Table 2: Statistical evaluation of the data subjected to regression analysis

Parameters	ofloxacin
Correlation Coefficient (r)	0.9999
% Intercept (y)	19374
Slope (m)	28848

Accuracy

The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 80 %, 100 % and 120 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table no.3.

Table 3: Statistical evaluation of the data subjected to accuracy of ofloxacin

level	test	wt in mg	area	quantity added in µg/ml	quantity recovered in µg/ml	% recovery	mean recovery
80%	1	2.26	2343094	18.08	18.01	99.63	**99.52**
	2	10.45	2340012	18.08	17.99	99.50	
	3	10.3	2338099	18.08	17.98	99.42	
100%	1	10.11	2944870	22.6	22.64	100.18	**100.03**
	2	10.21	2939847	22.6	22.60	100.01	
	3	10.13	2937037	22.6	22.58	99.91	
120%	1	10.26	3530215	27.12	27.14	100.08	**99.96**
	2	10.26	3525166	27.12	27.10	99.93	
	3	10.28	3522472	27.12	27.08	99.86	
					Mean recovery of all level		**99.84**

Precision

The method precision was established by carrying out the analysis of ofloxacin. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. The results of the same are tabulated in the table no. 4.

Table 4: Statistical evaluation of the data subjected to method precision of ofloxacin

Test	wt of test	Area	% assay
Test solution -1	10.31	2940875	99.37
Test solution -2	10.41	2932322	100.04
Test solution -3	10.45	2927390	100.26
Test solution -4	10.39	2937486	100.03
Test solution -5	10.36	2930033	99.48
Test solution -6	10.4	2925786	99.72
	Mean Assay		**99.82**
	SD		**0.349**
	RSD		**0.349**

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:
Variation in the flow rate by $\pm$ 0.2 ml /min
Variation in mobile phase composition by $\pm$ 2 %
Variation in wavelength ± 5 nm

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Method application

A sample equivalent to 2 mg of ofloxacin sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml diluent was added and sonicated for 10 minutes to dissolve it. Further volume was made up to the mark with the diluent to give 200 µg /ml. From this solution 1.0 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in table no. 4. It indicates the amount of ofloxacin in the product meets the requirement.

RESULTS AND CONCLUSION

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. Thus the proposed RP-HPLC method is used for estimation of ofloxacin from active pharmaceutical ingredient. It is more precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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