Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(9):169-175

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Stability Indicating High Performance Liquid Chromatography Method for Determination of Ofloxacin in Bulk Drug and Pharmaceutical Dosage Form

Rajan V Rele* and Sandip P Patil

Central research laboratory, D.G. Ruparel College, Matunga, Mumbai 400016, India

---

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 water symmetry C8 (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 (85:15 % v/v). The buffer was mixtures of 1 % tri-ethyl amine adjusted the pH 2.6 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. All the peaks of degradation products were resolved from the active pharmaceutical ingredient with significantly differ retention time. As the method could effectively separate the drug from its degraded products, hence above method can be also used for study of stability indicating parameters. The method has been successfully used to assay of pharmaceutical dosage form i.e. tablets with good recoveries.

Keywords: Ofloxacin; Tri-ethylamine; Acetonitrile; Ortho-phosphoric acid; HPLC; Forced degradation

---

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] , UPLC [8], titrimetric [9] spectrophotometric methods [10-12] 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. Tri-ethyl amine, 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 (85:15 % 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

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.

Preparation of 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 water symmetry C8 (15 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer and acetonitrile (85:15 % v/v). The buffer was mixtures of 0.1 M tri-ethyl amine adjusted the pH 2.6 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. (Figure 1) The injection volume of the standard and sample solution was set at 1.0 µl.

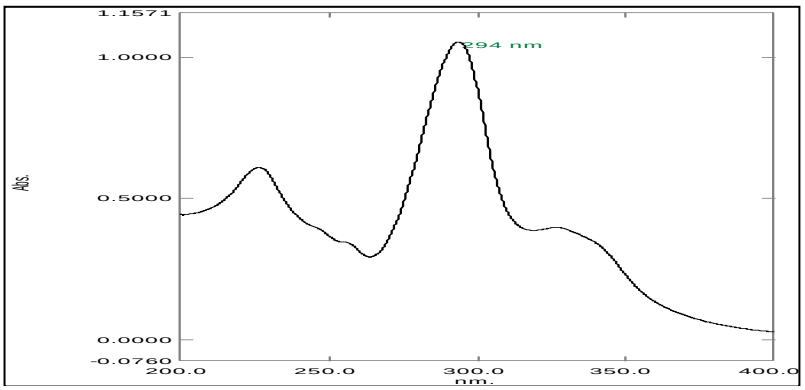

Figure 1: UV spectra of ofloxacin

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
3.37	3573056	100%	3270	1.6152

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 figures 2 and 3 respectively.

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 2.

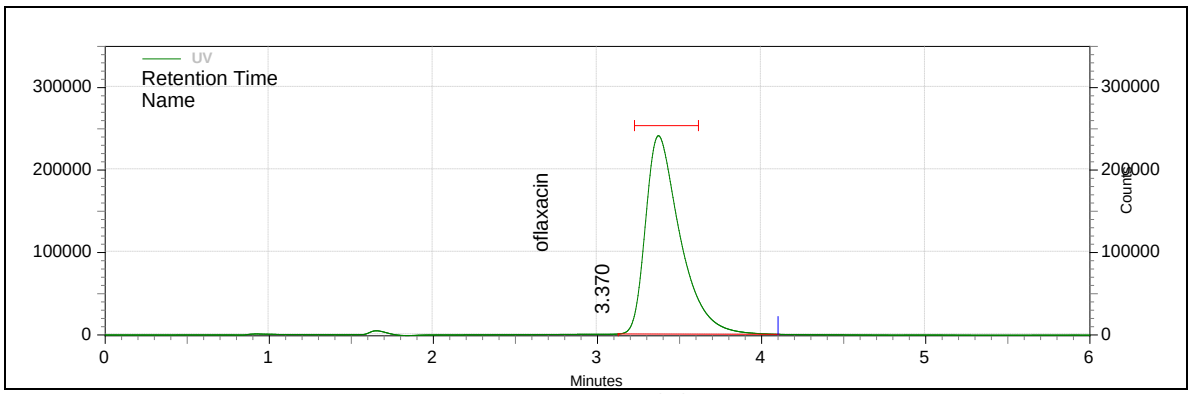

Figure 2: Typical chromatogram of ofloxacin (standard)

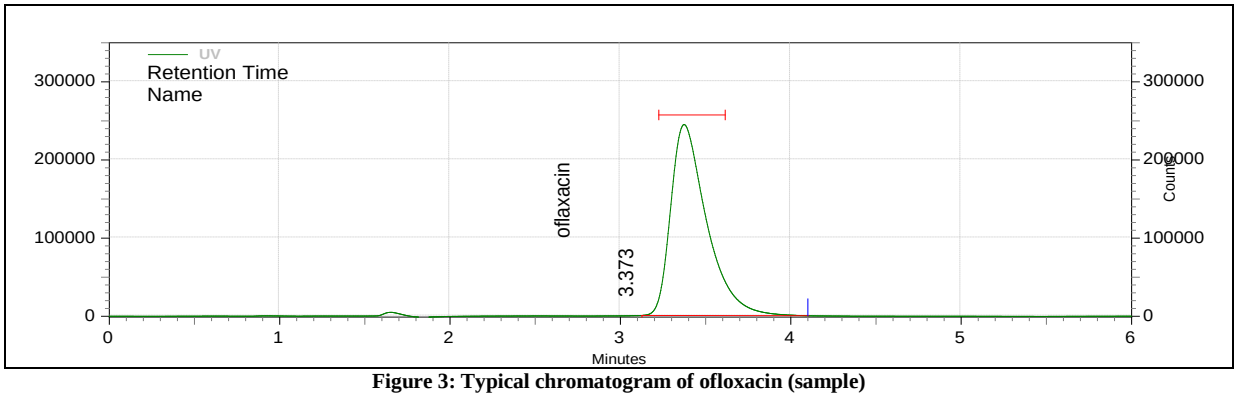

Figure 3: Typical chromatogram of ofloxacin (sample)

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 3.

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

Parameters	Ofloxacin
Correlation Coefficient (r)	0.9956
% Intercept (y)	255926
Slope (m)	151949

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	2.12	2821202	16.64	16.48	99.02	**99.20**
	2	2.11	2826729	16.64	16.51	99.22	
	3	2.08	2830843	16.64	16.53	99.36	
100%	1	2.09	3588919	20.8	20.96	100.78	**100.56**
	2	2.07	3577048	20.8	20.89	100.44	
	3	2.12	3577352	20.8	20.89	100.45	
150%	1	2.11	4231593	24.96	24.71	99.02	**99.66**
	2	2.14	4266904	24.96	24.92	99.84	
	3	2.12	4278622	24.96	24.99	100.12	
					Mean recovery of all level		**99.81**

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 4.

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

Test	wt of test	Area	% Assay
Test solution -1	2.12	3513101	100.54
Test solution -2	2.11	3506342	99.88
Test solution -3	2.14	3517112	101.61
Test solution -4	2.13	3516610	101.12
Test solution -5	2.12	3517112	100.66
Test solution -6	2.12	3518610	100.70
	Mean Assay		**100.75**
	SD		**0.581**
	RSD		**0.577**

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 ± 0.2 ml /min
Variation in mobile phase composition by ± 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 4. It indicates the amount of ofloxacin in the product meets the requirement.

Degradation studies
Acid degradation studies: To 1ml of stock solutions of ofloxacin, 1ml of different concentration of hydrochloric acid such as 0.5 N and 0.1 N were added and refluxed for 2 hrs at 60°C. The resultant solutions were diluted with diluent to obtain 20 µg /ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of samples.

Base degradation studies: To 1ml of stock solution of ofloxacin 1ml, of 0.1N sodium hydroxide was and added and refluxed for 2 hrs at 60°C. The resultant solution was diluted with diluent to obtain 20 µg/ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies: To 1ml of stock solution of ofloxacin 1ml 10% hydrogen peroxide was and added and refluxed for 2 hrs at 60°C. The resultant solution was diluted with diluent to obtain 20 µg /ml and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry heat degradation studies: The Standard drug solution was placed in oven at 105°C for 6 hours. The resultant solution was diluted with diluent to obtain 20 µg/ml and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photo stability degradation studies: The Standard drug solution was exposed to UV light (254 and 302 nm) by keeping the beaker in UV chamber for 24 hrs. The resultant solution was diluted with diluent to obtain 20 µg /ml and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies: The Standard drug solution was refluxed in water bath for 6 hrs at 60°C. The resultant solution was diluted with diluent to obtain 20 µg/ml and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

The percent of drug degraded in the presence of acidic, alkali, oxidative, dry heat, photostability and neutral conditions were studied. The amount of drug recovered or degraded is calculated by comparing the area of the standard with that of the area of the degraded sample. The results are furnished in table 5.

Table 5: Result of forced degradation study

S No	Test	Weight of sample	Area of Sample	Area of Standard	% Assay	Mean Assay
1	Acid hydrolysis 0.5 N	2.21	3868185	4239203	91.6627	91.1402
		2.19	3859014	4239203	90.6178	
2	Acid hydrolysis 0.1 N	2.23	3370051	4239203	80.58134	80.141
		2.2	3378675	4239203	79.70072	
3	Base hydrolysis 0.1N	2.19	3751472	4239203	88.09251	88.337
		2.2	3755155	4239203	88.58164	
4	H2O2-10%	2.19	3637707	4239203	85.42107	85.0934
		2.17	3639312	4239203	84.67831	
5	254nm	2.21	3685524	4239203	87.33427	87.3342
		2.19	3681003	4239203	86.43775	
6	302nm	2.24	3258225	4239203	78.25683	78.0489
		2.23	3255452	4239203	77.84116	
7	Thermal	2.22	3461907	4239203	82.40651	82.5882
		2.23	3461586	4239203	82.77004	
8	Neutral	2.2	4200194	4239203	99.07982	99.1199
		2.21	4184579	4239203	99.16016	

RESULTS

In the proposed method, the retention time of ofloxacin was 3.37 min. The linearity was in the range of 10-30 µg/ml. The regression equation of the linearity was given as Y = 151949X + 255926 where X is concentration of ofloxacin in µg /ml. and Y is corresponding peak area. The coefficient of co-relation was 0.9956. The result shows that an excellent correlation between peak area and concentration of ofloxacin in the range indicated. The relative standard deviation for method precision was 0.577 (limit % RSD < 2.0%). The mean recovery of the ofloxacin was 100.75. The high percentage recovery indicates that the proposed method is highly accurate. The use of buffer and acetonitrile (85:15 % (v/v) gave peak with good resolution. The robustness studies indicated that there was no effect on the drug study. No interfering peaks were found in the chromatogram of the formulation within the run time indicted that excipients used in the formulation did not interfere the estimation of drug. 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 (Table 4). 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. (Table 3) The

stability indicating nature of the proposed method was established by performing force degradation, which provided degradation behavior of ofloxacin under various conditions. The results of force degradation were given in table 5.

DISCUSSION

The proposed stability indicating reverse phase HPLC method is useful to separate various degradants in alkaline, acidic, oxidative, neutral and thermal conditions (table 6).

Table 6: Percentage recovery in forced degradation

S No	Test	% Recovery
1	Acid hydrolysis 0.5 N	91.1402
2	Acid hydrolysis 0.1 N	80.141
3	Base hydrolysis 0.1N	88.337
4	H2O2, 10%	85.0934
5	254 nm	87.3342
6	302 nm	78.0489
7	Thermal	82.5882
8	Neutral	99.1199

The drug undergoes degradation in different conditions as per table 6. This can be successfully used for validation of drug as well as for determining stability of drug in various conditions as per ICH guidelines.

CONCLUSION

Thus the proposed RP-HPLC method is used for estimation of ofloxacin from active pharmaceutical ingredient. It is more economical, 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 and degradation of drug in various conditions as per ICH guidelines.

ACKNOWLEDGMENT

Authors express sincere thanks to the principal, Dr. Tushar M. Desai, of D.G. Ruparel College, Mumbai for encouragement and providing laboratory facilities.

REFERENCES

[1] Budavari S. Eds. In. *The Merck Index. Merck & co.,Inc; Whitehouse Station*, NJ., **2001**, 1213 and 1229.
[2] British pharmacopoeia. Licensing division HMSO, Norwich, **2003**, 357.
[3] United States Pharmacopoeia. United States Pharmacopoeia Convention, Inc. Rockville, **2004**, 1335.
[4] European Pharmacopoeia. EDQM, Council of Europe ,Strasbourg, France. **2005**, 5th Ed, 2131.
[5] P. V. Rege. and Ramesh Mapari, , International Journal of Pharma and Bio Sciences, 2011, 2(4) ,51-58,.
[6] Anjay Kumar Natubhai Patel, A. Anton Smith, S. Amuthalakshmi and V. N. Gandhia, R. Manavalan, , J. Curr. Chem. Pharm. Sc, 2011, 1(1), 59-64.
[7] Rajan V. Rele, Prathamesh Tiwante, Der Pharmacia Lettre, 2015, 7 (10),188-192
[8] Goswami Smita L, Patel Nirav B, Desai Hemant T, Suresh Jain, , International Journal of Pharmacy and Pharmaceutical Sciences, 2012, 4(4), 275-278.
[8] Kanakapura Basavaiah Vinay, Hosakere Doddarevanna Revanasiddappa, Okram Zenita Devi, Pavagada Jagannathamurthy Ramesh, Kanakapura Basavaiah, Brazilian Journal of Pharmaceutical Sciences, 2011,47, (2), 251-260.
[9] Arun Kumar Dash, T. Siva Kishore, Loya Harika, Umadevi Kothapalli, Kothakota Vandana, Kishant Kumar Pradhan, International Journal of Pharmaceutical & Biological Archives, 2011, 2(4),1157-1161.

[10] P.J. Ramesh ,K. Basavaiah, O. Zenita Devi, K.B. Vinay, , Chemical Industry & Chemical Engineering Quarterly, 2010,16 (4).
[11] Rajan V. Rele, Der Pharmacia Sinica, 2015, 6(9), 39-43
[12] Rajan V. Rele,Journal of Chemical and Pharmaceutical Research, 2015, 7(9),817-822