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Reverse Phase High Performance Liquid Chromatography method for Simultaneous Determination of Ofloxacin and Ornidazole in Pharmaceutical Dosage

Rajan V. Rele* and Swapnil A. Sawant
Central Research Laboratory, D.G. Ruparel College, Matunga, Mumbai 400016, India
drvinraj@gmail.com

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Abstract

For validation of ofloxacin and ornidazole from its combined pharmaceutical formulations a reverse phase high pressure liquid chromatography method is proposed. Resolution was carried out on Waters Symmetry shield C8 column. A mobile phase composed of acetonitrile and buffer (18:82% (v/v). Wavelength 280 nm was used for detection. The ICH guidelines was applied for study of different parameters. The proposed method is easily and successfully applied to analyze ofloxacin and ornidazole from pharmaceutical combined formulations.

Keywords: Ofloxacin, Ornidazole, Ortho phosphoric acid, Acetonitrile, Ammonium acetate.

Introduction

Ofloxacin is a synthetic broad spectrum antibacterial agent. It is 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[1], USP[2], and EP[3]. Spectrophotometric[4,5] and HPTLC[6], HPLC[7-11] methods were reported in literature for their simultaneous estimation ofloxacin and ornidazole in combined formulations. A reverse phase high performance liquid chromatographic method has proposed for assay of ofloxacin and ornidazole in combined formulations.

Materials and Methods

Chemicals and Regientes: Pure ofloxacin and ornidazole standards were used during analysis with certificate of analysis. Analytical grade quality of ammonium acetate, ortho phosphoric acid and acetonitrile were used.

A HPLC grade, Millipore water was used for preparation of solutions. A acetonitrile and buffer of pH 3.0 (18:82 % , v/v) was used as diluent for preparation of standard and sample solutions .

Instrumentation: i. The HPLC system used was La Chrome Hitachi with, ii. D 7200 separation module as auto sampler, iii. D-7400 as UV detector.

Preparation of solutions: Stock solutions of 200 µg /ml of ofloxacin and 500 µg /ml of ornidazole were prepared respectively. From stock solutions, 20 µg /ml of ofloxacin and 50 µg /ml of ornidazole as working standard solutions were prepared respectively.

Sample preparation: For Average weight determination of each tablet, twenty tablets were used. A 2 mg of ofloxacin and 5 mg of ornidazole powder blend was weighted accurately. It was dissolved with 5 ml of diluent and further diluted to 10 ml to give 200 µg /ml of ofloxacin and 500 µg /ml. of ornidazole respectively. Such solution was further diluted to get 20 µg /ml of ofloxacin and 50 µg /ml. of ornidazole respectively. Such solution is used working sample solution.

Chromatographic condition: A reverse phase Waters Symmetry shield C8 with 5 µ particle size (15 x 4.6 mm i.d.) column used was for resolution. A buffer was made up of 0.01 M ammonium acetate and the ortho-phosphoric acid was used to adjust pH as 3.0. A mobile phase composed was acetonitrile and buffer (18:82 % (v/v). Wavelength 280 nm was used for detection. The flow rate of 1.5 ml /min was adjusted to mobile phase and injection volume was set at 5.0 µl. (Figure-1).

Method Validation: System suitability: It was studied by injecting standard solutions in six replicates. The Retention time, area, asymmetry and resolution were calculated. Such different Parameters were represented in Table-1.

Specificity: The blank, standard ofloxacin and ornidazole were injected to prove specificity. Specificity of method gives ability of method to resolve the gradients. The Figure 1 and 2 represents recorded chromatogram of the standard and sample respectively.

Linearity: A calibration curve was obtained throughout the concentration range under the given experimental conditions. It is graph of area of peak (y) v/s % of standard injected. (x). Table-2 represents regression data.

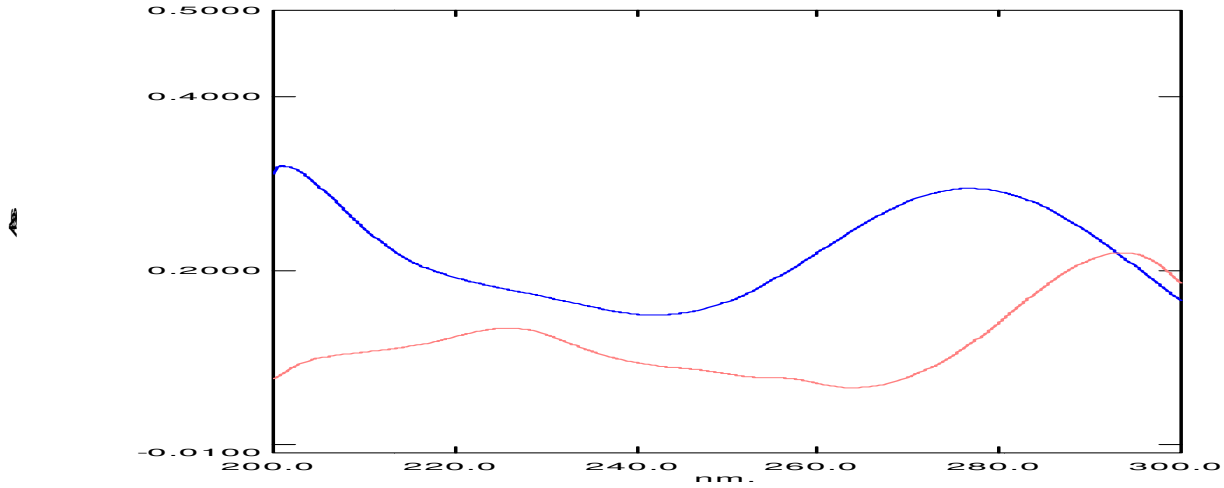

Figure-1
Ultra-violet spectra of ofloxacin and ornidazole

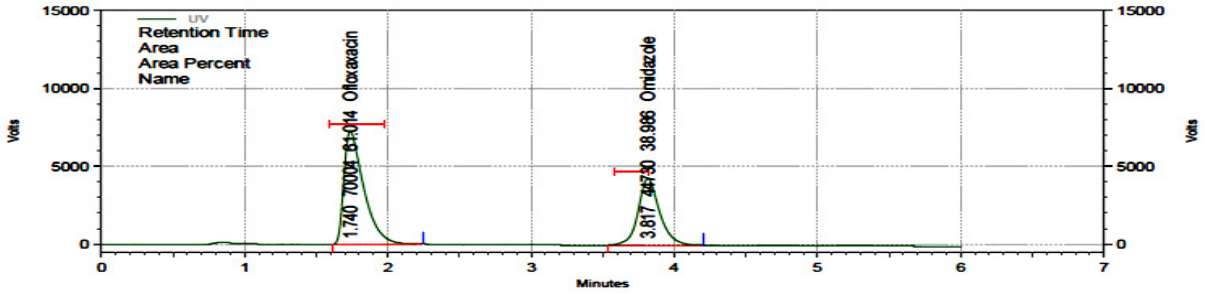

Figure-1
Chromatogram of standard ofloxacin and ornidazole

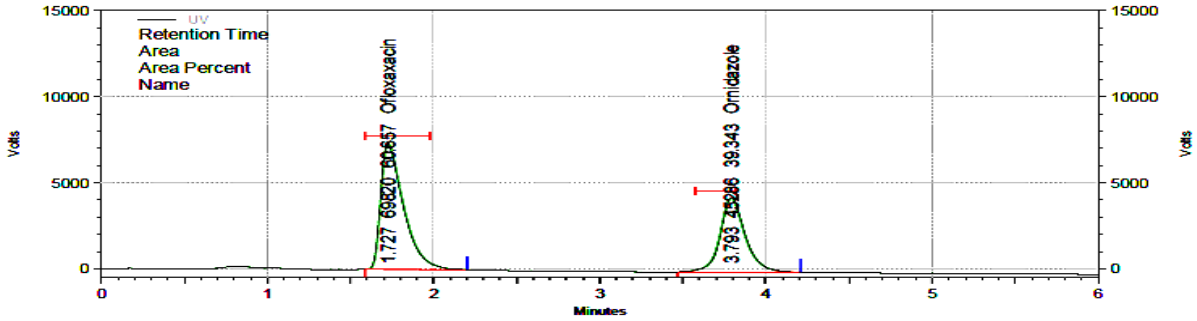

Figure-2
Chromatogram of sample ofloxacin and ornidazole

Table-1
Parameters of system suitability

Drug	RT	Observed Area	Factor of Asymmetry	Resolution
Ofloxacin	1.8	69748	1.90	-
ornidazole	3.9	44006	1.13	8.02

Accuracy: The accuracy of the proposed was determined on synthetic mixture containing 80 %, 100 % and 120 % known amount of drug. The percentage recovery by the assay was reported for accuracy. The values recovery are formulated on Table-3, 4.

Precision: The five replicates were carried out for precision of the proposed method of each ofloxacin and ornidazole. The coefficient of variance values were lies within the limits. The Table-5 is for tabulated for result.

Table-2
Regression analysis values

Parameters	Ornidazole	Ofloxacin
Coefficient of Correlation	0.9980	0.9999
y	2871	530.8
m	47373	70983

Y= mx +c

Table-3
Evaluation data for accuracy of ofloxacin

Level	sample no.	Ofloxacin in µg/ml	Peak area	Added ofloxacin in µg/ml	Recovered ofloxacin in µg/ml	% recovered
80%	1	2.25	56642	16.8	17.05	101.51
	2	2.1	55717	16.8	16.78	99.85
	3	2.3	55838	16.8	16.81	100.07
100%	1	2.35	69761	21	21.00	100.02
	2	2.28	69584	21	20.95	99.76
	3	2.22	70215	21	21.14	100.67
120%	1	2.2	85127	25.2	25.63	101.71
	2	2.24	84940	25.2	25.57	101.48
	3	2.25	84402	25.2	25.41	100.84

Table-4
Evaluation data for accuracy of ornidazole

Level	Sample no.	Ornidazole in µg/ml	Peak area	Added ornidazole in µg/ml	Recovered ornidazole in µg/ml	% recovered
80%	1	5.15	35798	40.8	41.49	101.68
	2	5.19	35763	40.8	41.45	101.59
	3	5.22	35135	40.8	40.72	99.80
100%	1	5.12	44190	51	51.21	100.42
	2	5.25	43936	51	50.92	99.84
	3	5.26	43828	51	50.79	99.60
120%	1	5.24	53232	61.2	61.69	100.80
	2	5.18	53926	61.2	62.50	102.12
	3	5.05	53198	61.2	61.65	100.74

Table-4
Statistical data of method of precision of ofloxacin and ornidazole

	Ofloxacin			Ornidazole		
Test	**Weight of test**	**Area obtained**	**% recovery**	**Weight of test**	**Area obtained**	**% recovery**
1	2.05	68200	99.25	5.05	43277	99.32
2	2.09	69632	99.40	5.09	43843	99.83
3	2.06	69579	100.77	5.06	44023	100.83
4	2.08	69648	99.90	5.08	44298	101.06
5	2.07	69503	100.17	5.07	43795	100.11
6	2.08	69857	100.20	5.08	43849	100.04
	Mean Assay		**99.95**		**Mean Assay**	**100.20**
	SD		**0.561**		**SD**	**0.647**
	COV		**0.561**		**COV**	**0.645**

Robustness: The robustness study was determined by variations in method parameters as per ICH guidelines. The typical variations involves study of change in flow rate by $\pm$ 0.2 ml /min, mobile phase by $\pm$ 0.2 % and change in wavelength by ± 5 nm. The nature of robustness of the method is indicated by analysis of date under such conditions.

Method Application: For Average weight determination of each tablet, twenty tablets were used. A 2 mg of ofloxacin and 5 mg of ornidazole powder blend was weighted accurately. It was dissolved with 5 ml of diluent and further diluted to 10 ml to give 200 µg /ml of ofloxacin and 500 µg /ml. of ornidazole respectively. Such solution was further diluted to get 20 µg /ml of ofloxacin and 50 µg /ml. of ornidazole respectively. This solution is used working sample solution.

A 10.0 µl sample solution was injection volume was used for analysis. For the Sample solution peak area was calculated and compared with that of standard peak area. The table no. 4, 5 represents assay results.

Results and Discussion

The retention time of ofloxacin and ornidazole were 1.8 and 3.9 respectively in the proposed method. The linearity equations were given as Y=70983 X+ 530.8 and Y= 47373 X+2871 respectively where X is amount of ofloxacin or ornidazole in µg /ml as concentration and Y represents peak area. The coefficient of co-relation was less than 1. It exhibits excellent correlation between areas and concentration of in the ranges. The ofloxacin shows RSD as 0.561 while ornidazole showed RSD as 0.645 (limit % RSD < 2.0%). The mean recovery was about 100%. The accuracy of the method is observed with high percentage recovery. The use of buffer and acetonitrile (82:18%, v/v) gave peak with good resolution. The robustness studies indicated that there was no effect on the drug study. No interfering peaks were observed during run time indicted that excipient did not interfere the assay of formulation.

Conclusion

The low values of statistical data for proposed method reports the method is accurate and reproducible. The method of standard addition was used for recovery study. The results are given in Table 3-5. It gives accuracy and reproducibility of the above method.

Thus RP-HPLC method is strongly recommended in assay of ofloxacin and ornidazole in their combined formulations.

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