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Simultaneous determination of ofloxacin and ornidazole in pharmaceutical dosage by reverse phase high performance liquid chromatography

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for simultaneous determination of ofloxacin and ornidazole from combined dosage form i.e. tablets. The separation of drug was achieved on Zorbax Eclipse C18 (250 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 a mixture of buffer and acetonitrile (65:35 % (v/v)). The buffer was 0.03 M disodium hydrogen phosphate solution adjusted the pH 3.2 with ortho-phosphoric acid. The detection was carried out at wavelength 284 nm. The mixture of buffer of pH 3.2 and acetonitrile (65:35% v/v) was used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze ofloxacin and ornidazole from combined dosage form i.e. tablets.

Keywords: Ofloxacin, Ornidazole, Acetonitrile, disodium phosphate, ortho phosphoric acid.

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 spectrophotometric methods [5, 6, 7, 8], atomic absorption spectrometry [5], spectro-flurometry [5], HPLC[9] and microbiological method [10] for its determination.

Ornidazole [1] is a 5-nitro-imidazole derivative used as anti-infective agent. It is not official in any Pharmacopoeia. Literature survey reveals that ornidazole is estimated by voltametry [11] and HPLC [12] methods for its determination in dosage forms and biological fluids. Ofloxacin and ornidazole in combined tablet dosage form is available in the market, has gained increasing acceptance in diarrhoea, bacterial and protozoal infections.

Spectrophotometric [13, 14] and HPTLC [15] , HPLC[16-20] methods have been established for their simultaneous estimation ofloxacin and ornidazole in tablet dosage form. This proposed work presents simple, accurate and reproducible reverse phase high performance liquid chromatographic method for simultaneous determination of ofloxacin and ornidazole in tablet dosage form.

Chemical and reagents

Reference standard of ofloxacin and ornidazole were obtained from reputed firm with certificate of analysis. Disodium hydrogen phosphate, acetonitrile and ortho phosphoric acid were used of analytical grade and the HPLC grade water was used from Millipore. Standard and sample solutions were prepared in diluent [mixture of buffer of pH 3.2 and acetonitrile (65:35 % (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 EZ Chrom Elite software.

A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of Standard preparation

Standard solution

A 2 mg of standard ofloxacin and 5 mg of ornidazole and were weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent [mixture of buffer of pH 3.2 and acetonitrile [65:35 % (v/v)] was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 200 µg /ml of ofloxacin and 500 µg /ml of ornidazole respectively. The working standard solution was prepared by diluting 1 ml of 200 µg /ml of ofloxacin and 500 µg /ml. of ornidazole solution to 10 ml with diluent to get concentration 20 µg /ml of ofloxacin and 50 µg /ml of ornidazole respectively.

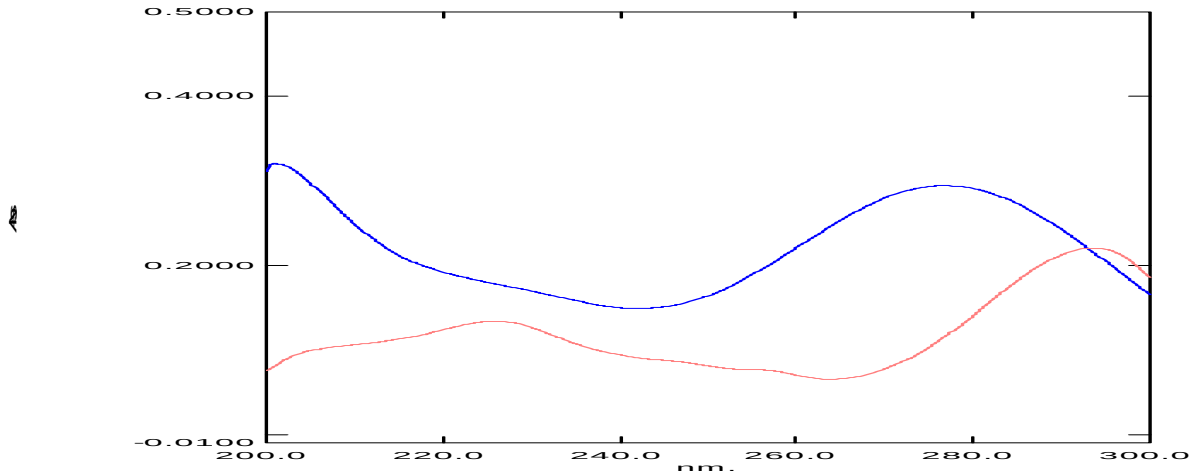
Sample preparation

Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 2 mg of standard ofloxacin and 5 mg of ornidazole were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 200 µg /ml of ofloxacin and 500 µg /ml. of ornidazole respectively. The working standard solution was prepared by diluting 1 ml of 200 µg /ml of ofloxacin and 500 µg /ml of ornidazole solution to 10 ml with diluent to get concentration 20 µg /ml of ofloxacin and 50 µg /ml. of ornidazole respectively.

Chromatographic condition

Chromatographic separation was performed on a reverse phase Zorbax Eclipse C18 (250 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer of pH 3.2 and acetonitrile [65:35 % (v/v)]. The buffer was 0.03M disodium hydrogen phosphate adjusted the pH 3.2 with ortho-phosphoric acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 284 nm. (Fig.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: Overlay UV spectra of ofloxacin and ornidazole



Method validation

System suitability

System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), asymmetry, resolution 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 and ornidazole

Name	Retention Time	Area	USP Plate Count	Asymmetry	Resolution
Ofloxacin	2.580	271113	1689	1.53669	-
ornidazole	4.397	171898	3370	1.41333	6.5575

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 and ornidazole (standard)

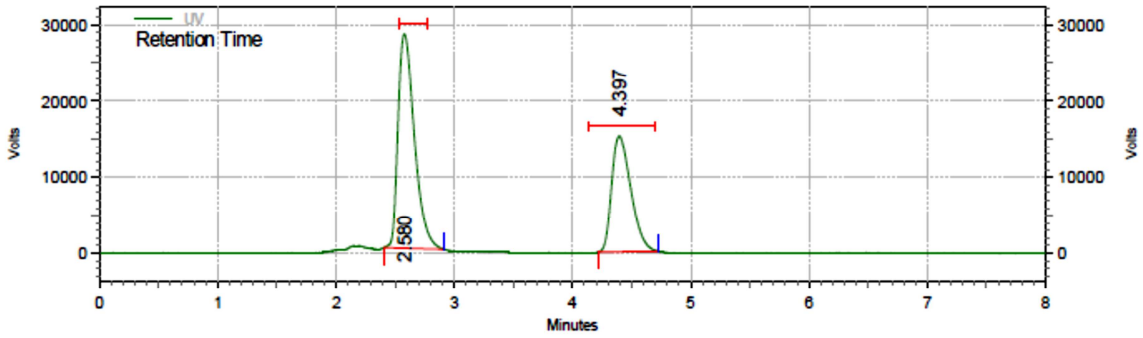
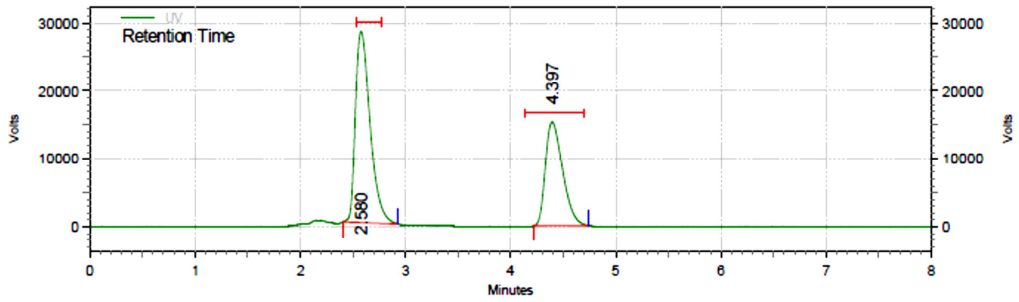


Figure 3: Typical chromatogram of ofloxacin and ornidazole (sample)



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	Ornidazole	Ofloxacin
Correlation Coefficient (r)	0.9987	0.9961
% Intercept (y)	8084.6	29372
Slope (m)	165653	246572

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, 4.

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	2.05	218093	16.56	16.64	100.48	**100.75**
	2	2.06	218754	16.56	16.69	100.78	
	3	2.07	219219	16.56	16.72	101.00	
100%	1	2.08	270862	20.7	20.66	99.83	**100.10**
	2	2.03	271868	20.7	20.74	100.20	
	3	2.06	272032	20.7	20.75	100.26	
120%	1	2.09	323873	24.84	24.71	99.47	**99.35**
	2	2.10	323350	24.84	24.67	99.31	
	3	2.05	323208	24.84	24.66	99.27	

Table 4 : Statistical evaluation of the data subjected to accuracy of ornidazole

level	test	wt in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	5.05	136140	40.48	39.95	98.70	**99.31**
	2	5.08	136996	40.48	40.21	99.32	
	3	5.02	137800	40.48	40.44	99.90	
100%	1	5.10	172830	50.6	50.72	100.24	**100.12**
	2	5.07	172078	50.6	50.50	99.80	
	3	5.06	172946	50.6	50.76	100.31	
120%	1	5.09	206033	60.72	60.47	99.58	**99.65**
	2	5.10	206044	60.72	60.47	99.59	
	3	5.03	206474	60.72	60.60	99.80	

Precision

The method precision was established by carrying out the analysis of ofloxacin and ornidazole. 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, 5.

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

Test	wt of test	Area	% assay
Test-1	2.07	271113	99.92
Test-1	2.08	271997	99.77
Test-13	2.07	270874	99.83
Test-4	2.06	271088	100.40
Test-5	2.06	270452	100.16
Test-6	2.08	272510	99.95
	Mean Assay		**100.01**
	SD		**0.235**
	RSD		**0.234**

Table 5: Statistical evaluation of the data subjected to method precision of ornidazole

Test	wt of test	Area	% assay
Test-1	5.06	171898	99.70
Test-1	5.07	172386	99.79
Test-13	5.06	172361	99.97
Test-4	5.08	172604	99.72
Test-5	5.06	171368	99.39
Test-6	5.07	173065	100.18
	Mean Assay		**99.79**
	SD		**0.266**
	RSD		**0.267**

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 ± 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
Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 2 mg of standard ofloxacin and 5 mg of ornidazole were weighted accurately and transferred in 10 ml volumetric flask to give concentration as 200 µg /ml of ofloxacin and 500 µg /ml. of ornidazole respectively. The working standard solution was prepared by diluting 1 ml of 200 µg /ml of ofloxacin and 500 µg /ml. of ornidazole resolution to 10 ml with diluent to get concentration 20 µg /ml of ofloxacin and 50 µg /ml. of ornidazole respectively. 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, 5. It indicates the amount of ofloxacin and ornidazole in the product meets the requirement.

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 and ornidazole 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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