International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 367-374, Jan-Mar 2010

Simultaneous Determination and Validation of Ofloxacin and Ornidazole in Combined Dosage Pharmaceutical Formulation

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ABSTRACT: A modified simple, selective, rapid, precise reversed phase high performance liquid chromatography method has been developed and validated for the simultaneous estimation of ofloxacin and ornidazole. This combination is used as various G.I tract infections worldwide. The method was carried out on a Xterra ™ ® RP18, 5 µm (4.6 X 250mm) column with a mobile phase consisting of acetonitrile : mixed phosohate buffer [0.21012% (w/v) KH_2PO_4 and 0.10852% (w/v) K_2HPO_4, in HPLC grade water and pH is adjusted to 3.0 with 10% potassium hydroxide solution.] in the ratio 40 : 60. The flow rate was 1.0 ml/min and the effluent was monitored for ofloxacin at 294 nm and for ornidazole at 305 nm (Waters 2487 dual absorbance detector). The validation of the proposed method was also carried out in terms of linearity, accuracy, precision, symmetry factor, plate count, regression, and recovery. The retention time of ofloxacin and ornidazole was 2.84 min and 4.39 min respectively. Percentage recoveries for Ofloxacin and Ornidazole were 99.54% to 97.60% and 99.77% to 98.47% respectively. In conclusion this method can be used for routine quality control analysis due to its simplicity and accuracy.
Key words: RP-HPLC; Ofloxacin; Ornidazole.

INTRODUCTION

Ofloxacin (OFL) is a fluoroquinolone derivative. Chemically, it is (±)-9-fluoro-2, 3-dihydro-3-methyl-10- (4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]- 1,4-benzoxazine -6-carboxylic acid.[1] It is mainly used as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases. Ofloxacin is characterized by a good pharmacokinetic profile. Following oral administration, there is rapid and extensive oral absorption from the gastrointestinal tract achieving peak serum concentration within 1 – 3 h and levels in excess of 100g/ml in the urine and bladder[2-5]. It is used in the treatment of urinary tract, prostate, skin, urinary and respiratory tract infections. It is also used to treat certain sexually transmitted diseases. Ofloxacin is used as an antibacterial agent in the treatment of infections caused by a wide range of both Gram-positive and Gram-negative bacteria as well as Chlamydia infections[6-8].

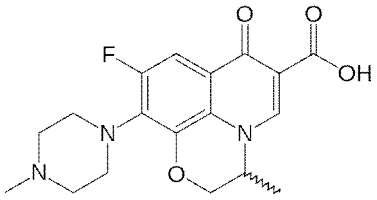

Figure1. Chemical structure of ofloxacin

Ornidazole is a 5-nitroimidazole derivative and is used in the treatment of susceptible protozoal infections and also in anaerobic bacterial infections. It has been used for amebic liver abscesses, duodenal ulcers, giardiasis, intestinal lambliasis and vaginitis[9-11]. Ornidazole has recently been used with success in patients with active Crohn's disease6. It is more ef-fective against amebiasis than metronidazole, which is the most commonly used nitroimidazole derivative in therapy[12-14]. Ornidazole has also been preferred for surgical prophylaxis because of its longer elimination half-life

and excellent penetration into lipidic tissues versus other nitroimidazole derivatives[15, 16].

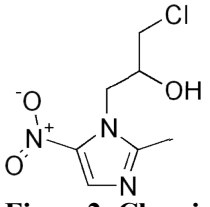

Figure2. Chemical structure of ornidazole

Ornidazole is used in combination with Ofloxacine in the tretment of PLD and in intra-abdominal infection. In this present communication we reports two simple, accurate, and most economical methods for simultaneous estimation of ornidazole and ofloxacin in combained dosage forms.

Fixed dose combination of Ornidazole 500 mg and Ofloxacin 200 mg is available in the tablets form in the market. The present work describes a simple, precise and accurate reversed phase HPLC method for the simultaneous estimation of Ornidazole and Ofloxacin in combined dosage form. The method was validated according to procedures and acceptance criteria based on FDA guidelines[17] and recommendations of ICH[18].

MATERIALS AND METHODS

Materials

Acetonitrile (HPLC grade) was procured from Thomas Baker (Chemicals) Pvt. Ltd., Mumbai. Anhydrous potassium dihydrogen phosphate (AR grade) and dipotassium hydrogen phosphate (AR grade) were purchased from Merck India Ltd., Mumbai, India. Water (HPLC grade) was obtained from aurium® 611UV water purification system of Sartorius, Germany. Working standard of Ornidazole and Ofloxacin was obtained from Granules India Ltd and Umedica Laboratories Pvt. Ltd respectively as a gift sample.

Chromatograpic conditions

A Waters® HPLC (515 pumps) system was used for analysis. The method was carried out on Xterra™® RP-18 (250 mm × i.d-4.6 mm, pore-5 µm) column as a stationary phase and mobile phase consisting of Acetonitrile : Buffer (0.21012% solution of anhydrous potassium dihydrogen phosphate and 0.10852% (w/v) dipotassium hydrogen phosphate of in water, pH: 3.0) in the ratio 40:60 (v/v). The mobile phase was filtered through 0.45 µm membrane and degassed. The flow rate was 1.0 ml/min. Rheodyne injector and 20µl loop was used for the injection of samples. Detection was done for Ornidazole at 305 nm and for Ofloxacin at 294 nm (Waters 2487 dual detector). Empower2 software provided by Waters was used throughout this experiment.

Preparation of Standard Solution

For Assay

Solution A

0.0103g working standard of Ofloxacin (99.15%) was taken in a 25 ml of volumetric flask and to it 12.5ml of water and 1.0 drop of concentrated HCl were added and sonicated well. The volume was then made up to 25ml with water.

Solution B

From solution A 1 ml of aliquot was taken in a 25 ml volumetric flask and volume was made up to the mark by the mobile phase. The solution was labeled 100%.

Solution C

0.0252g of Ornidazole (99.61%) was taken was taken in a 25 ml of volumetric flask and to it 12.5ml of water and 1.0 drop of concentrated HCl were added and sonicated well. The volume was then made up to 25ml with water.

Solution D

From solution C 1 ml of aliquot was taken in a 25 ml volumetric flask and volume was made up to the mark by the mobile phase. The solution was labeled 100%.

For Linearity

Solution E

0.8ml of solution A was taken in a 25ml volumetric flask and volume was made up to 25ml with mobile phase. The solution was labeled 80%.

Solution F

1.2ml of solution A was taken in a 25ml volumetric flask and volume was made up to 25ml with mobile phase. The solution was labeled 120%.

Solution G

0.8ml of solution C was taken in a 25ml volumetric flask and volume was made up to 25ml with mobile phase. The solution was labeled 80%.

Solution H

1.2ml of solution C was taken in a 25ml volumetric flask and volume was made up to 25ml with mobile phase. The solution was labeled 120%.

Preparation of Sample Solution

For Assay

Solution I

Twenty tablets (average weight/tablet: 1.0693g) were taken and finely powered. 0.0522 g of the powder was accurately weighed equivalent to 1.0101 mg of Ornidazole and 0.41314 mg of Ofloxacin respectively in a 25 ml volumetric flask and the volume was made up to 25 ml by the mobile phase. 1ml of aliquot was taken in a 25 ml volumetric flask and made up to the mark with mobile phase.

Solution J

From solution I 1ml of aliquot was taken in a 25 ml volumetric flask and made up to the mark with mobile phase.

For Recovery and Accuracy
Three separate 25 ml volumetric flask were taken.
Solution K
0.8 ml of solution I was taken in a 25 ml volumetric flask and the volume was made with mobile phase.
Solution L
1 ml of solution I was taken in a 25 ml volumetric flask. To it 0.1 ml of solution A and 0.1 ml of solution C was added and the volume was made with mobile phase.
Solution M
1 ml of solution I was taken in a 25 ml volumetric flask. To it 0.2 ml of solution A and 0.2 ml of solution C was added and the volume was made with mobile phase.

Assay Method
With the optimized chromatographic conditions (room temperature 25^{o}C), a steady baseline was recorded. In the first phase standard Ornidazole solution was injected six times. This procedure was repeated for the sample solution obtained from the formulation. Retention time of Ornidazole was 2.128. The peak area of the standard and sample solution was obtained from the chromatogram.
In the second phase six replicate standard Ofloxacin solutions were injected. This procedure was repeated for the sample solution obtained from the formulation. Retention time of Ofloxacin was 4.077. The peak area of the standard and sample solution was obtained from the software. The concentrations of the drugs were calculated using following formula.

$$\text{Concentration of drugs (mg/tablet)} = \frac{\text{Sample area} \times \text{standard concentration}}{\text{Standard area} \times \text{sample concentration}} \times \text{Dilution Factor}$$

Method Validation
Accuracy of the method was studied by recovery experiments. To the powdered tablets formulation (500 mg of Ornidazole and 50 mg of Ofloxacin), and working standard drugs were added at the level of 7.62 %, 15.24 % and 22.86 % of the actual assay value. The extraction of drugs was followed using sample preparation procedure and those were analysed. The method validation result sheet of Ornidazole and Ofloxacin was given in Table -1. The percentage recovery of spiked sample was calculated and presented in Table-2.1 – Table-2.3. Precision of the method was demonstrated by repeatability studies. This was done by injecting consecutively the standard solution for six times and passing them through the assay procedure.
Linearity and range of the method was determined by analysing mixed standard containing 0.1002 g of Ornidazole and 0.0122 g of Ofloxacin respectively. The calibration curve was plotted using peak area *vs.* concentration of standard solution; the values are presented in Table-2 and Table-3. The limit of detection (LOD) and limit of quantification (LOQ) of the method was determined by injecting progressively low concentration of the standard solutions with the optimized chromatographic conditions.

Limit of detection (LOD) and Limit of quantitation (LOQ)
The LOD and LOQ were separately determined based on the standard calibration curve. The residual standard deviation (RSD) of the regression line or the standard deviation of Y– intercept of regression lines may be used to calculate LOD and LOQ. LOD = 3.3 ×D/S and LOQ = 10 × D/S, where D is the standard deviation of the Y intercepts of regression line and S is the slope of the calibration curve.

RESULTS AND DISCUSSION
The chromatograms of mixed sample solutions are presented in Fig. 3 and 4. The accuracy of the method was determined by recovery studies were carried out and the percentage of recovery was calculated. From the data obtained, recoveries for the standard drugs were considered accurate. The precision procedure was satisfactory. The concentration range from 0.808 – 1.211 mg/ml of Ornidazole and 0.3305 - 0.4948 mg/ml of Ofloxacin were examined by the assay procedure and the calibration curves were plotted (Fig. 5 and 6). The calibration curve shows linear response over the range of concentration used in the assay procedure. The calibration curve shows linearity, which justifies the use of single point calibration and the proximity of maximum points to the calibration line demonstrated that the method has accurate linearity to the concentration to the analyte. The retention time of ofloxacin and ornidazole was found to be 2.83 and 4.39 min. respectively. The limit of detection (LOD) for Ornidazole and Ofloxacin was found to be 5µg/ml and 6µg/ml respectively (Table – 5). The limit of quantification (LOQ) for Ornidazole and Ofloxacin was found to be 15µg/ml and 18 µg/ml, respectively (Table – 5). The ruggedness of the method was determined by carrying out the experiment of different instruments like Waters HPLC 600 pumps, Merck Hitachi HPLC Lachrom pump-L-7100, Merck Hitachi UV Lachrom detector L-7400 etc. by different operators using different columns of similar type like Lichrocart® column(15 cm, i.d 4.6 mm, particle size 5 µm) of Waters. Robustness of the method was determined by making slight changes in the chromatographic conditions. After that there is no interference due to excipients. The system suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry (Table – 4). Experimental results reveal that, the present developed RP-HPLC method is simple, accurate,

selective,precise, rugged, robust, linear and rapid for the estimation of Ornidazole and Ofloxacin in combination form of dosage formulation. Hence this method can be applied for the quality control of raw materials, formulations and dissolution studies.

CONCLUSION

It is concluded that the proposed methods in the present investigation are simple, sensitive, accurate and precise and can be successfully applied for the routine estimation of ornidazole and ofloxacine in combined dosage forms.

Table1. Method validation result sheet of Ornidazole (500mg) and Ofloxacin (200mg)

No	Parameters	Experiment	Result	Limit	Reference
1.	Plate Count 5 sigma method	w.r.t. Ornidazole peak.	3217.68	>2000	Manufacturer
		w.r.t. Ofloxacin peak.	3988.95	>2000	Manufacturer
2.	Symmetry factor	For Ornidazole peak.	1.28	0.8-1.5	B.P 2007
		For Ofloxacin peak.	1.21	0.8-1.5	B.P 2007
3.	Resolution	w.r.t. Ornidazole and Ofloxacin peak.	6.29	>1.5	B.P 2007
4.	% RSD (Precision) Six replicate injection	Ornidazole peak area.	0.3	Not more than 2.0	B.P 2007
		Ornidazole peak R.T.	0.1		
		Ornidazole amount.	0.3		
		Ofloxacin peak area.	0.8	Not more than 2.0	B.P 2007
		Ofloxacin peak R.T	0.3		
		Ofloxacin amount.	0.8		
5.	Regression (R^2)	For Ornidazole	1	Not more than 1.0	Statistics
		For Ofloxacin	1		
6.	Recovery difference in soln. K (%)	For Ornidazole	0.2266	-	-
		For Ofloxacin	0.4503		
7.	Recovery difference in soln. L (%)	For Ornidazole	0.313	-	-
		For Ofloxacin	1.2353		
8.	Recovery difference in soln. M (%)	For Ornidazole	1.5306	-	-
		For Ofloxacin	2.3917		
9.	Accuracy for Soln. K (%)	For Ornidazole	99.7734	-	-
		For Ofloxacin	99.5497		
10.	Accuracy for Soln. L (%)	For Ornidazole	99.687	-	-
		For Ofloxacin	98.7647		
11.	Accuracy for Soln. M (%)	For Ornidazole	98.4694	-	-
		For Ofloxacin	97.6083		
12.	Accuracy (%RSD) Three different concentration solution	For Ornidazole	0.7343	Not more than 2.0	B.P. 2007
		For Ofloxacin	0.9901		

Table2. results of analysis of formulation and recovery studies

Name	Actual concentration in sample HO-140(F) (mg)	Concentrat-ion in Soln. K (mg)	less Amount %	Concentration in Soln. L (mg)	Excess Amount %	Concentration in Soln. M (mg)	Excess Amount %
Ornidazole	1.0101	0.808	20.0079	1.1105	9.9396	1.2109	19.8792
Ofloxacin	0.41314	0.33048	20.008	0.45399	9.888	0.49484	19.775

Table3. results of analysis of formulation and recovery studies

Name	Average assy in sample HO-140(F) (mg)	Average assy from Soln. K (mg)	less Amount %	Average assy from Soln. L (mg)	Excess Amount %	Average assy from Soln. M (mg)	Excess Amount %
Ornidazole	517.313	414.9816	19.7813	570.3513	10.2526	628.069	21.4098
Ofloxacin	211.5875	170.2063	19.557	235.1223	11.123	258.4903	22.167

Table4. results of analysis of formulation and recovery studies

Name	Recovery difference in Soln. K (%)	Accuracy for Soln. K (%)	Recovery difference in Soln. L (%)	Accuracy for Soln. L (%)	Recovey difference in Soln. M (%)	Accuracy for Soln. M (%)	Accuracy % RSD
Ornidazole	0.2266	99.7734	0.313	99.687	1.5306	98.4694	0.7343
Ofloxacin	0.4503	99.5497	1.2353	98.7647	2.3917	97.6083	0.9901

Horno® (Dey's Medical Stores (Mfg.) Ltd., 62, Bondel Road, Kolkata-700019) each tablet contain Ornidazole 500mg and Ofloxacin 200mg.

Table5. System suitability studies

Parameters	Ornidazole	Ofloxacin
Theoretical plate count 5 sigma method	3217.68	3988.95
Resolution factor	6.29	6.29
Symmetry factor	1.28	1.21
LOD (ng/mg)	5.00	6.00
LOD (ng/mg)	15.00	18.00

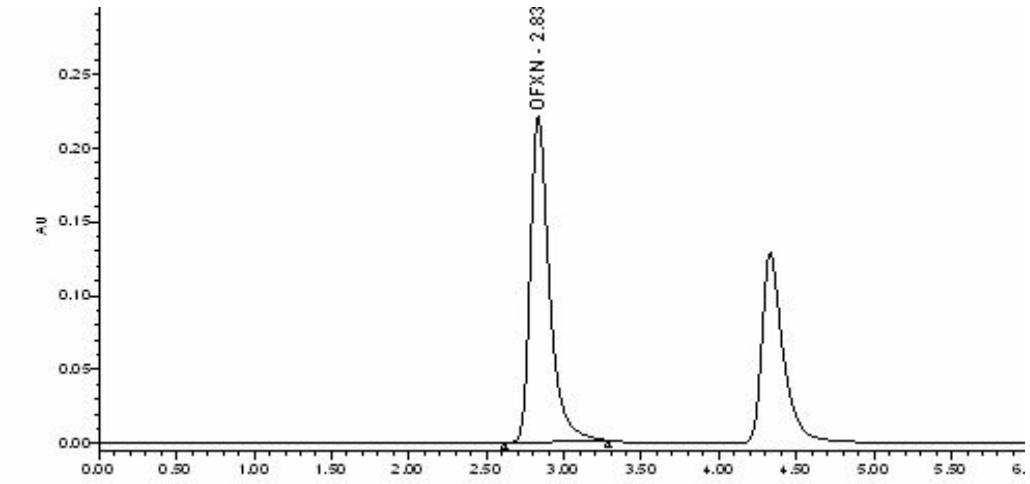

Figure3. A typical HPLC chromatogram of Ofloxacin in mixed sample at 294 nm.

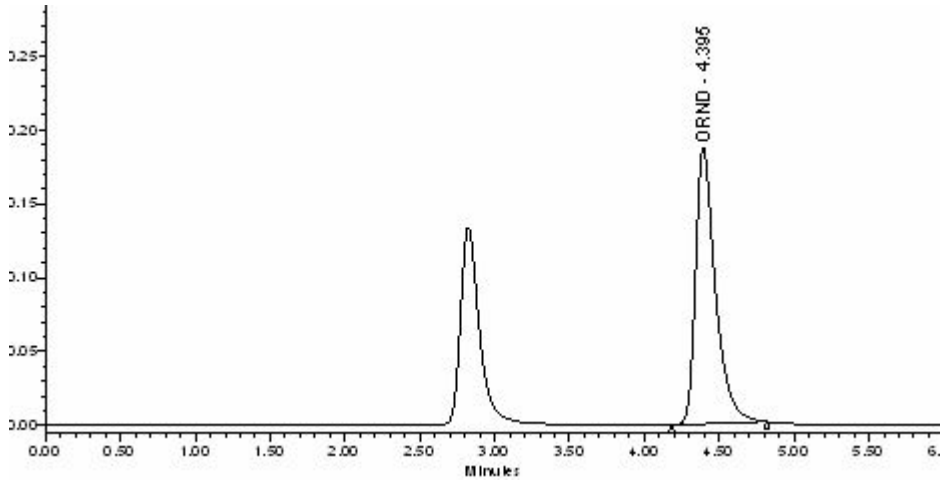

Figure4. A typical HPLC chromatogram of Ornidazole in mixed sample at 305 nm.

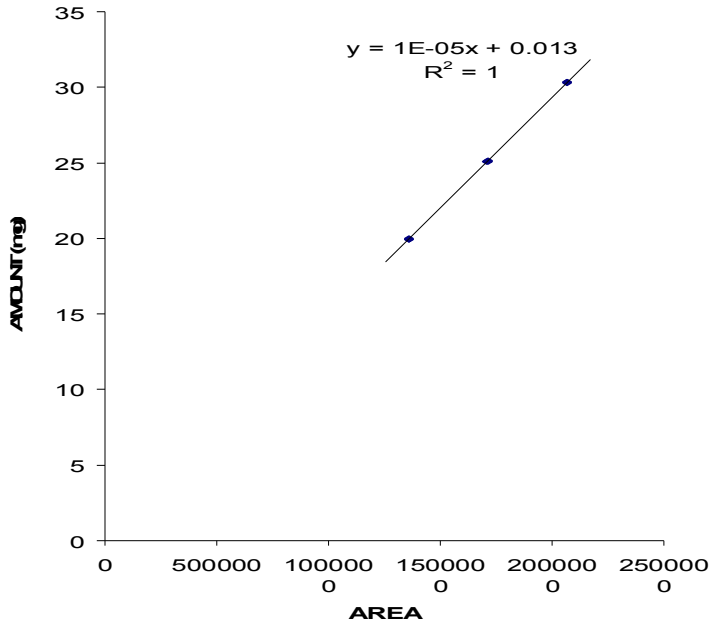

Figure5. Linearity curve of Ornidazole

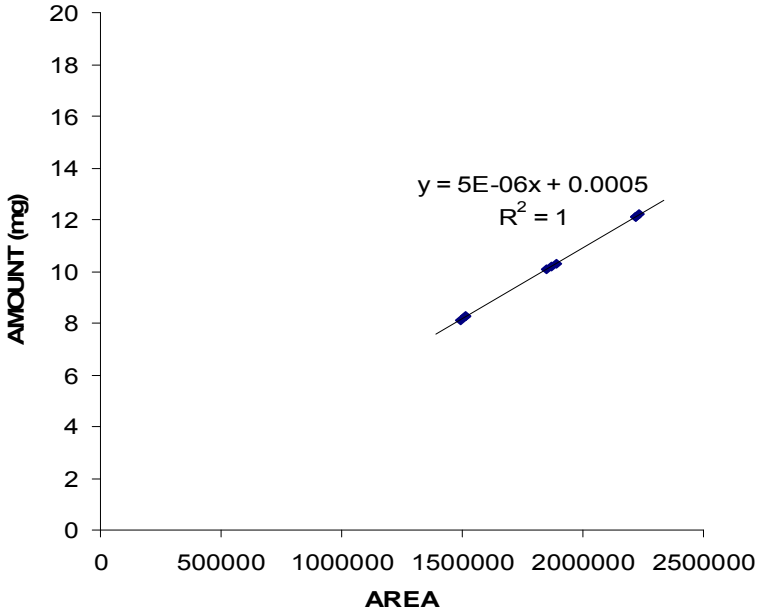

Figure6. Linearity curve for ofloxacin

ACKNOWLEDGEMENTS

Authors are thankful to Mr. Goutam Dey, Managing Director, for facilities and Mr. R. Dey, Jt. Managing Director, Mr. S. Dey, Whole time Director, and Mr. S. K. Dasgupta, Director of Personnel & I.R, for their encouragement during this investigation.

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