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STABILITY IDICATING RP-UHPLC METHOD FOR SIMULTANEOUS DETERMINATION OF OFLOXACIN AND ORNIDAZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, stable, economic, accurate, precise and robust reverse phase ultra high pressure liquid chromatography (RP-UHPLC) method was developed and validated for simultaneous estimation of Ofloxacin and Ornidazole in pharmaceutical dosage form. The UHPLC separation was achieved on Agilent C18 column (150 mm x 4.6 mm id, 5 µ particle size) with isocratic condition at ambient temperature using mobile phase as a buffer (2.72 g of potassium dihydrogen phosphate in 1000ml of water and adjust the pH to 2.4 with Orthophosphoric acid): Acetonitrile in the ratio (80:20 v/v). The analysis was performed at flow rate 2 ml/min. Separation was achieved with UV detection at 294 nm. Retention time of Ofloxacin and Ornidazole were found to be 3.068 ± 0.20 minute and 4.955 ± 0.20 minute respectively. The linearity was studied in the concentration range 40-200 µg/ml and 100-500 µg/ml for Ofloxacin and Ornidazole respectively. The assay was validated for the parameters like system suitability, linearity, accuracy, precision, robustness, LOD & LOQ.

KEYWORD: RP-UHPLC; Ofloxacin; Ornidazole; Method Validation.

INTRODUCTION

Ofloxacin is one of a new generation of fluorinated quinolones structurally related to nalidixic acid. It is an orally administered broad spectrum antibacterial drug active against most Gram-negative bacteria, many Gram-positive bacteria and some anaerobes. Chemically, Ofloxacin is a fluorinated carboxyquinolone, is the racemate,(±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylicacid and Ornidazole is Chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)

propan-2- ol. Ornidazole is useful for some protozoan infections and mainly used in poultry industry. Ciprofloxacin is the only other quinolone with superior in vitro antibacterial activity. However, the pharmacokinetic profile of ofloxacin is superior to that of ciprofloxacin, with more rapid absorption and a peak serum concentration several times higher. Moreover, ofloxacin achieves high concentrations in most tissues and body fluids. Ofloxacin is official in Indian Pharmacopeia[1] United States Pharmacopeia[2] and British Pharmacopoeia.[3] But there is no official method available for the analysis of Ornidazole. The combination of Ofloxacin and Ornidazole is widely used in treatment of various G.I. tract infections. Literature search reveals that some analytical methods like spectrophotometric[4,5,6], HPLC[7,8] and HPTLC[9] methods are available for the estimation of Ofloxacin and Ornidazole in combine dosage form. There is no reported method for estimation of Ofloxacin and Ornidazole in their combined dosage form by RP-UHPLC. Ultra High pressure liquid chromatography (UHPLC) is more advanced and sophisticated method of separation. This prompted the present work. The method was developed and validated as per ICH[10,11 & 12] and USP[13] guidelines. The aim of the present work is to develop a simple yet quick, accurate and precise and economic RP-UHPLC method for estimation of Ofloxacin and Ornidazole in their marketed formulation which is more efficient method than the available RP-HPLC method.

The developed RP-UHPLC method utilized economical solvent system having advantages like better retention time, very sharp peak, symmetric peak shapes and resolution.

MATERIALS AND METHOD

Materials

Working standards of Ofloxacin (potency = 99.35%) and Ornidazole (potency = 99.50%) was obtained as a gift samples from Glenmark pharmaceutical Ltd. (Mumbai, India). HPLC grade Acetonitrile, Ortho phosphoric acid, Potassium di hydrogen phosphate were procured from Merck Ltd. Mumbai India. Water was purified with Milli-Q Millipore system. All the solvents and solutions used in the analysis were filtered through a 0.45µ membrane filter paper. The commercial fixed dose combination product Zenotas –OZ (Marketed by Intas Pharmaceuticals) containing 200 mg Ofloxacin and 500 mg Ornidazole was procured from the local market.

METHOD

The UHPLC system used for analysis consisted of Dionex Ultimate 3000+, autosampler, UV detector with chromeleon software for data acquisition and processing. The chromatographic separation was performed on agilent C18 column (150 mm x 4.6 mm id, 5 µ particle size) with isocratic condition maintained at ambient temperature. The analysis was performed at flow rate 2 ml/min. Quantification was achieved with UV detection at 294 nm. Retention time of Ofloxacin and Ornidazole were found to be 3.068 ± 0.20 minute and 4.955 ± 0.20 minute respectively. Chromatographic conditions are summarized in below Table.

HPLC system	Dionex Ultimate 3000+
Software	Chromelion
Detector	UV Detector
Wavelength	294 nm
Pump	Isocratic Pump
Stationary phase	Agilent(150 mm x 4.6 mm id, 5 µ particle size)
Mobile phase	(2.72 g of potassium dihydrogen phosphate in 1000ml of water and adjust the pH to 2.4 with Orthophosphoric acid):ACN (80:20 v/v)
Flow rate	2 ml/min
Injection volume	10 µl
Diluent	Methanol
Column temperature	25^0c

Standard solution preparation

To prepare a stock solution for assay, weight accurately equivalent to 10 mg of Ofloxacin working standard and transferred into 50 ml volumetric flask and 25mg of Ornidazole working standard and transferred to the same volumetric flask, to this 50 ml Methanol was added to dissolve the substance by sonication for 5 minutes and the volume was made upto the mark by Methanol The final concentration thus achieved was 200mcg for Ofloxacin and 500mcg for Ornidazole.

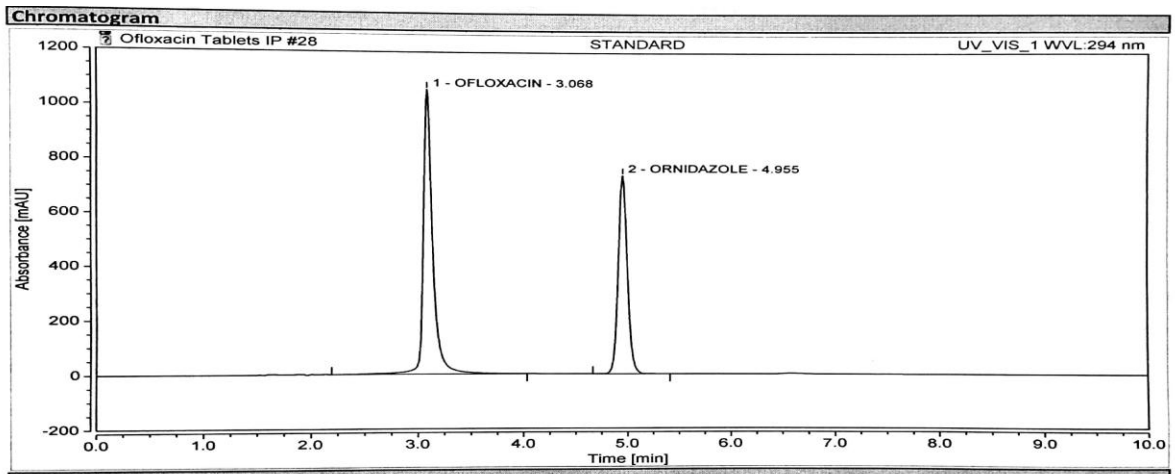
Preparation of tablet dosage form

To prepare a stock solution for assay, 20 tablets (Zenotas –OZ) were weight and mixed well. The average weight was determined and they were finally powdered. An aliquot of powder equivalent to 200mg of Ofloxacin was transferred into 100 ml volumetric flask, to this 40 ml Methanol was added to dissolve the substance by sonication for 10 minutes and diluted to 100ml with methanol. Further 5ml of the above solution was transferred to 100ml of volumetric flask and the volume was made upto the mark.

Method Validation

The developed method was validated according to International Conference on Harmonisation (ICH) (Q2) B guidelines for validation of analytical procedures. As per the ICH guidelines the method validation parameters checked were linearity, accuracy, precision, assay, LOD, LOQ and robustness.[14]

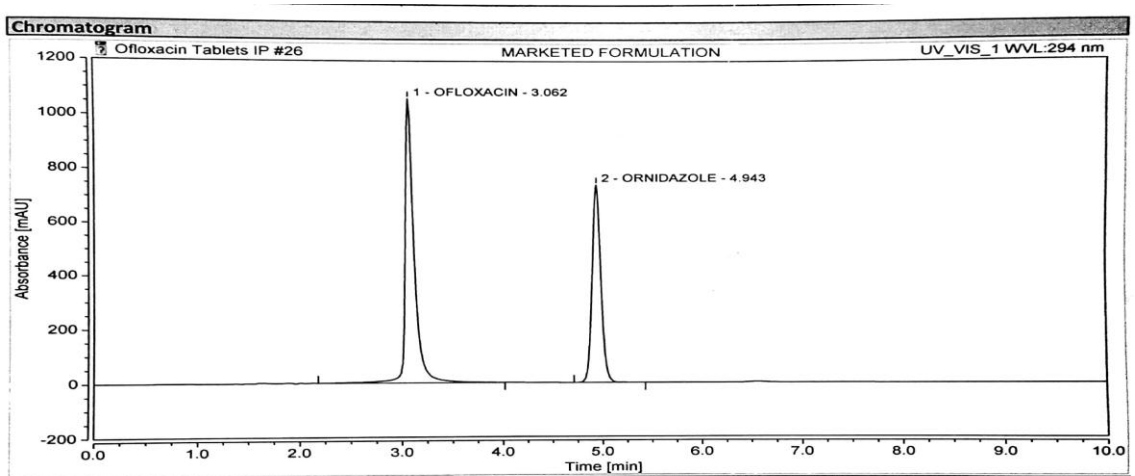
Typical chromatogram of Ofloxacin and Ornidazole are



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	OFLOXACIN	3.068	109.342	1059.689	59.04	58.90	n.a.
2	ORNIDAZOLE	4.955	75.862	739.407	40.96	41.10	n.a.
Total:			**185.204**	**1799.096**	**100.00**	**100.00**	

Fig: Chromatogram Of Standard Ofloxacin & Ornidazole.



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	OFLOXACIN	3.062	109.653	1058.011	59.11	58.87	n.a.
2	ORNIDAZOLE	4.943	75.869	739.183	40.89	41.13	n.a.
Total:			**185.521**	**1797.195**	**100.00**	**100.00**	

Fig: Chromatogram Of Marketed Formulation.

RESULTS AND DISCUSSION

System suitability

System suitability test of the chromatographic system was performed before each validation run. Six replicate injections of standard preparation was injected and tailing factor, theoretical plates, resolution and % RSD of peak area were determine for the same. Acceptance criteria for system suitability parameters that are tailing factor should not be more than 2, theoretical plates should be not less than 2000, resolution should be not less than 2 and % RSD of peak area should not be more than 2.0%. System suitability test parameters for Ofloxacin and Ornidazole for the developed method are reported in table 1.

Table 1: System suitability parameters of Ofloxacin and Ornidazole.

Parameters	Oloxacin ± %RSD (n=6)	Ornidazole ± %RSD (n=6)
Retention Time(min)	3.068 ± 0.120	4.955 ± 0.101
Tailing Factor	1.279±0.503	1.279±0.503
Theoritical Plates	7501 ± 0.235	15521± 0.134

Linearity (Calibration Curve)

For constructing calibration curve, series of five dilutions in the concentration range 40-200 (40, 80, 120, 160 and 200,) µg/ml for Ofloxacin and 100-500 (100, 200, 300, 400 and 500) µg/ml for Ornidazole was taken. Calibration curve were constructed by plotting peak area vs. concentration of Ofloxacin and Ornidazole and regression equation calculated from straight line equation. Linearity curves for Ofloxacin and Ornidazole shown in figure no. 3 and 4 respectively. The method showed good linear response in the concentration range 80-200 µg/ml for ofloxacin (r2 = 0.9998) & 100-500 µg/ml for ornidazole (r2 = 0.9999)

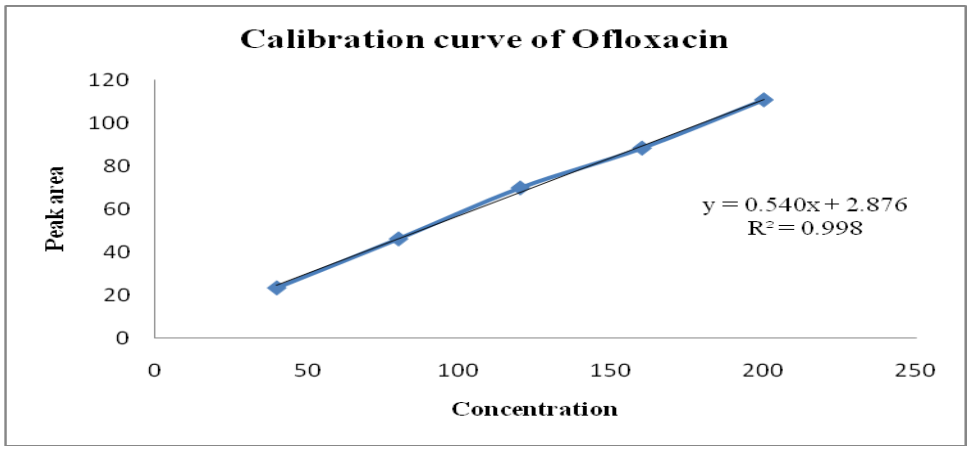


Fig: Calibration Curve of Ofloxacin.

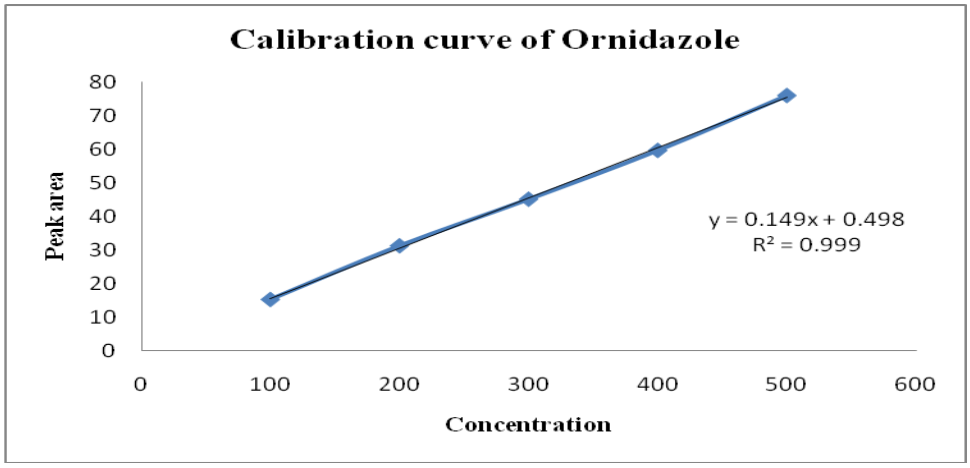


Fig: Calibration Curve of Ornidazole.

Table 2: Regression parameters of calibration curve.

Parameters	Ofloxacin	Ornidazole
Linear range (µg/ml)	40-200	100-500
Regression equation	y = 0.540x + 2.876	y = 0.149x + 0.498
Correlation Coefficient (r^2)	0.9998	0.9999

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of Ofloxacin and Ornidazole by the standard addition method. The accuracy of the analytical method was assessed by determination of recovery for three concentrations (corresponding to 50,100 and150% of test solution concentration). For each concentration, three sets were prepared. The mean recovery and % RSD of recoveries of Ofloxacin and Ornidazole were reported The results of recovery of Ofloxacin and Ornidazole with the %RSD are given in below table.

Table 3: Accuracy study data of Ofloxacin.

Accuracy Level	Set No.	Amount Added (µg/ml)	Amount Found (µg/ml)	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
50%	1	100	99.50	99.5	99.7	0.58	0.20
	2	100	99.15	99.1			
	3	100	100.52	100.5			
100%	1	200	198.56	99.3	99.4	0.14	0.35
	2	200	199.20	99.6			
	3	200	198.59	99.3			
150%	1	300	300.15	100.05	99.97	0.12	0.29
	2	300	300.25	100.08			
	3	300	299.56	99.8			

Table 4: Accuracy study data of Ornidazole.

Accuracy Level	Set No.	Amount Added (µg/ml)	Amount Found(µg/ml)	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
50%	1	250	249.60	99.8	100.08	0.19	0.60
	2	250	250.50	100.20			
	3	250	250.60	100.24			
100%	1	500	499.60	99.8	99.88	0.11	0.54
	2	500	499.10	99.8			
	3	500	500.20	100.04			
150%	1	750	749.60	99.9	100.02	0.08	0.40
	2	750	750.55	100.07			
	3	750	750.80	100.10			

Precision

The precision of analytical method express the degree of agreement among individual test when the procedure is applied repeatedly to multiple sampling of homogenous samples. Precision are considered at three levels that is system precision, method precision (repeatability) and intermediate precision (reproducibility).

System precision

The system precision of the instrument was checked by repeatedly injecting (n =6) standard solutions of the Ofloxacin and Ornidazole under the same chromatographic condition and calculate the % RSD of peak area which should not be more than 2%.

Method precision (Repeatability)

The method precision of the analytical method was determined by analyzing six sets of sample preparation against the same standard. Assay of all six sample preparation was determined and mean of assay, standard deviation and %RSD for the same was calculated.

Intermediate Precision (Reproducibility)

Intermediate precision of the analytical method was determined by performing method precision on another day by another analyst using different instrument under same experimental conditions. Assay of all replicate sample preparation was determined and mean assay, standard deviation and %RSD for the same was calculated. The method was found to be precise and % RSD was found to be less than 2% was shown in below tables.

Table 5: Data of system precision study.

Sl no.	Area of ofloxacin	Area of ornidazole
1	109.653	75.869
2	109.526	75.879
3	109.660	75.201
4	109.879	75.698
5	109.646	75.485
6	109.458	75.468
Mean	109.637	75.60
Standard Deviation	0.131	0.241
% RSD	0.80	0.70

Table 6: Data of method precision study.

Sl no.	Wt of Sample In mg	Avg Area of Ofloxacin	Avg Area of Ornidazole	% Assay Of Ofoxacin	% Assay Of Ornidazole
1	105.70	109.657	75.869	99.50	99.10
2	106.20	109.512	76.000	100.65	100.50
3	105.90	109.346	75.862	99.10	99.90
4	104.60	109.505	76.854	99.85	101.50
5	108.50	109.565	75.897	99.30	99.10
6	106.60	109.390	76.254	99.10	100.80
mean				99.54	100.15
Standard Deviation				0.541	0.878
% RSD				0.20	0.30

Table 7: Data of intermediate precision study.

Sl no.	Wt of Sample In mg	Avg Area of Ofloxacin	Avg Area of Ornidazole	% Assay Of Ofloxacin	% Assay Of Ornidazole
1	106.50	108.667	75.870	99.20	99.50
2	106.20	109.590	76.250	100.60	100.20
3	105.50	109.385	75.750	99.60	99.20
4	105.90	109.560	76.850	99.75	101.02
5	106.50	108.565	75.808	99.10	99.60
6	106.60	108.390	76.256	99.01	100.50
mean				99.54	100
Standard Deviation				0.541	0.629
% RSD				0.18	0.25

Table 8: Results of assay.

Drugs	Label Claimed (mg/Tab)	Amount of Drug Estimated (mg/tab)	% Amount found
Ofloxacin	200	196.25	98.1
Ornidazole	500	504.06	100.8

Robustness

The robustness of the method was established by introducing small changes in various

parameters like, pH of mobile phase, flow rate, wavelength, column temperature and mobile phase composition. The result obtained from assay of test solution was not affected by varying the conditions and in accordance with true value. The robustness of the method was evaluate by calculating % assay of test solution which is not more than ± 2.0% from mean value of method precision and system suitability parameters meets the requirements. Robustness was evaluated by varying different parameters. The results of these variations are given in table 9.

Table 9: Robustness study of Ofloxacin and Ornidazole.

Parameters	Variation	Ofloxacin		Ornidazole	
		Retention Time (min)	Assay (%)	Retention Time (min)	Assay (%)
Flow rate (ml/min)	1.5	4.102	100.50	5.160	100.20
	2.0	3.062	98.90	4.943	100.80
	2.5	2.750	101.60	4.250	101.20
pH	2.4	3.105	100.20	4.865	100.10
	2.6	3.052	99.10	4.945	100.60
	2.8	2.990	100.90	4.290	100.15
Column temperature (°c)	24	4.105	101.60	4.865	100.55
	25	3.052	100.50	4.945	100.60
	26	2.790	99.60	4.290	100.90
wavelength	293	4.104	100.60	4.869	100.85
	294	3.051	101.25	4.942	100.75
	295	2.795	100.50	4.295	100.10

Limit of Detection & Limit of Quantification

Limit of Detection (LOD) is the lowest concentration of analyte in the sample that could be detected under the stated experimental condition and Limit of Quantification (LOQ) is the lowest concentration of the active ingredients in a sample that could be determined with accepted precision and accuracy. According to ICH recommendation, the approach based on the standard deviation (SD) of the response and slope (M) was used for determining the detection and quantification limits. LOD can be calculated according to formula LOD = 3.3 (SD/M) and LOQ = 10(SD/M). The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 & LOQ as the amount for which the signal to noise ratio was 10:1. LOD and LOQ results of ATC and TEL are given in table 10.

Table 10: LOD and LOQ study of ATC & TEL.

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Ofloxacin	0.390	0.460
Ornidazole	0.980	1.160

CONCLUSION

The developed method was found suitable for the simultaneous estimation of Ofloxacin and Ornidazole in tablet dosage form. The chromatographic conditions were optimized by changing the parameters like mobile phase composition, pH of mobile phase, column temperature, wavelength etc. A good sharp peak symmetry, resolution between Ofloxacin and Ornidazole was obtained with mobile phase Buffer (2.72 g of potassium dihydrogen phosphate in 1000ml of water and adjust the pH to 2.4 with Orthophosphoric acid): ACN(80:20 v/v) at a flow rate 2 ml/min. The wavelength of detection selected was 294nm. The retention time of Ofloxacin and Ornidazole was about 3.068 ± 0.20 minute and 4.955 ± 0.20 minute respectively. A validated RP-UHPLC method has been developed for the determination of Ofloxacin and Ornidazole in tablet dosage form. The developed method is simple, rapid, linear, accurate, precise, cost effective and specific. Results from the validation experiments showed that the method is reliable and accurate therefore it can be successfully applied for the routine quality control analysis of the fixed dose comibination of Ofloxacin and Ornidazole.

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