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Research Article

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Development and Validation of Stability Indicting Assay Method for the Simultaneous Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form by UPLC

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ABSTRACT

A stability indicating RP-UPLC method was developed and validated for simultaneous estimation Ofloxacin and Ornidazole in tablet dosage form. The separation was achieved under optimized chromatographic condition on a Waters® C_{18} Acquity UPLC BEH (100 mm × 2.1 mm, 1.7µm) column with mobile phase consist of Water: Acetonitrile: Triethylamine in the ratio of 85: 15: 0.1 v/v. An isocratic elution at a flow rate of 0.2 ml/min at ambient oven temperature was carried out with PDA detection at 300 nm. The retention time for Ofloxacin and Ornidazole was 3.9 min and 6.4 min respectively. The degradation was observed under acidic, alkali, oxidative, photolytic and thermal conditions. The linearity was found to be in the concentration range of 50-150µg/ml for Ofloxacin and 125-375µg/ml for Ornidazole. The % recoveries at 50% were found to be 100.46% & 100.22% for Ofloxacin & Ornidazole respectively. The % recoveries at 100% were found to be 99.70% & 99.83% for Ofloxacin & Ornidazole respectively. The % recoveries at 150% were found to be 99.67% & 100.30% for Ofloxacin & Ornidazole respectively. The method was validated as per ICH guideline and the values were found to be within the limits. So, the proposed method was found to be simple, linear, accurate, precise, stability indicating, robust and specific.

Keywords: Ofloxacin, Ornidazole, Linearity, Forced Degradation, Method validation.

INTRODUCTION

Ofloxacin, $C_{18}H_{20}FN_3O_4$ that is (RS)-9-fluoro-3-methyl-10-(-4-methylpiperazine-1-yl)-7-oxo-2, 3-dihydro-7H pyrido[1,2,3,-de]-1 ,4-benzoxazine6-carboxylic acid is used as antibacterial drug. Ornidazole, $C_7H_{10}ClN_3O_3$ that is 1-(-3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole, is used as an antiprotozoal drug. [1-11] Ofloxacin is official in Indian pharmacopeia [7], United States Pharmacopeia [8], British Pharmacopoeia [9] and Japanese Pharmacopoeia. [10] Ornidazole is official in Indian Pharmacopeia, [7] to establish a shelf-life of formulation degradation studies required to decide the storage condition and for that purpose forced degradation study is required to mimic the long term stability condition. [12-15]

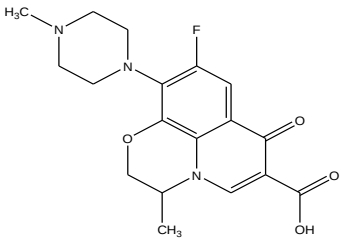

Fig. 1: Structure of Ofloxacin

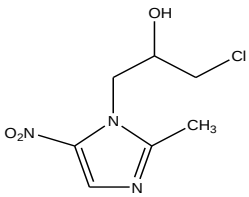

Fig. 2: Structure of Ornidazole

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Stability indicating assay method is one which accurately and precisely measures the non-degraded drug in presence of impurities/ degradation products. [15] Forced degradation study gives the idea of degradation behaviour of drug as well as degradation pathways. [15] UPLC offers advantages like improved resolution, greater sensitivity, less solvent consumption and speed of analysis. [17-36]

The aim of present work is to develop simple, rapid, selective, precise stability indicating UPLC method with PDA detection for Ofloxacin and Ornidazole. A study is facilitated with all the adventitious features of UPLC analysis.

MATERIALS AND METHODS

Instrument: Waters Acquity UPLC with PDA detector equipped with Empower 2 software was used to perform the analysis of the tablet formulation.

Materials: Standard gift sample of Ofloxacin and Ornidazole were provided by Glenmark Pharmaceuticals, Mumbai (India). Tablet formulation (OFLOSTAR-OZ) was purchased from market, manufactured by Cadila Pharmaceuticals.

Method Development

Preparation of Mobile Phase

For each liter of mobile phase, 850 ml water was mixed with 150 ml Acetonitrile and 1ml Triethylamine added. Mixed well and PH was adjusted to 2.3 with diluted ortho-phosphoric acid solution. Mixed well and sonicated for 10 minutes.

Chromatographic Condition

The optimised Chromatographic Condition as given in Table 1.

Preparation of Standard Solution

Accurately weighed 100 mg of OFL and 250 mg of ORN were transferred into 100 ml volumetric flask, to which 50 ml of Methanol was added and sonicated for 15 min, dissolved completely and diluted up to the mark with Methanol to give a stock solution containing 1000 µg/ml of OFL & 2500µg/ml of ORN. Working solution was prepared by taking 1 ml of above stock solution into 10 ml volumetric flask and diluting it up to the mark with Methanol to get the final working solution containing 100µg/ml of OFL & 250µg/ml of ORN. This solution was used further for all the trials related to the optimization of chromatographic conditions.

Sample Preparation

The contents of twenty tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet (containing 200 mg of OFL and 500 mg of ORN) was transferred in to 100 ml of volumetric flask containing few ml of methanol and sonicated for 20 min to dissolve the drug as completely as possible and diluted up to the mark with methanol. The flask was shaken and the solution was filtered through 0.45µ PVDF filter. 1 ml from this solution was taken in 20 ml volumetric flask and diluted with methanol to get the solution containing 100µg /ml and 250µg/ml of OFL and ORN respectively. The solution was analyzed by proposed method and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve.

Forced Degradation Study

Standards of OFL, ORN and formulation were subjected to forced degradation in acidic medium in presence of 0.1 N HCl at 80°C for 1 hour. Standards of OFL, ORN and formulation were subjected to forced degradation in basic medium in presence of 0.05 N NaOH at 50°C for 10 min. Standards of OFL, ORN and Formulation were subjected to forced degradation in 3% v/v solution of hydrogen peroxide (oxidizing medium) at room temperature for 24 hours. Thermal degradation study of standards of OFL, ORN and formulation was carried out in a dry stability chamber at 105°C for 24 hours by exposing formulation in tablet form. Photo degradation study of standards of OFL, ORN and formulation was carried out in a photo stability chamber by exposing to UV light in a Petri dish for 1 ICH cycle.

Table 1: Optimised Chromatographic Condition

Column	Acquity UPLC BEH C_{18}(100 mm×2.1 mm, 1.7µm)
Mobile phase	Water: ACN: TEA (85: 15: 0.1)
pH	2.3 by OPA
Flow rate	0.2 ml/min
Injection volume	1µl
Column temperature	30°C
Detection	300 nm by PDA Detector
Diluent	Methanol

Table 2: Result of System Suitability Test

Parameters	Observation		Specification
	OFL	ORN	
Repeatability	0.31	0.32	RSD≤ 1%
Resolution (R_S)	10		R_S > 2
Tailing Factor (T)	1.2	1.2	T ≤ 2
Theoretical Plates (N)	5177	9277	≥2000

Table 3: Result of Forced Degradation Study

Type of Degradation	Degradation Condition	% Degradation	
		OFL	ORN
Acid Degradation	0.1 N HCl at 80°C for 1 h	34.46%	11.62%
Alkali Degradation	0.05 N NaOH at 50°C for 10 min	28.96%	38.31%
Peroxide Degradation	3%H_2O_2 at RT for 24 h	40.91%	0.54%
Thermal Degradation	105°C for 24 h	34.66%	20.41%
Photo Degradation	1.2 million lux h	0.19%	17.93%

Table 4: Result for Linearity of OFL and ORN

	S. No.	Concentration (µg/ml)	Peak Area	R^2 (>0.999)
OFL	1	50	2356508	0.9994
	2	80	3650532	
	3	100	4518419	
	4	120	5585685	
	5	150	6877057	
ORN	1	125	1992729	0.9994
	2	250	3095576	
	3	300	3830952	
	4	350	4739715	
	5	375	5839629	

Method Validation

The method was validated for linearity, accuracy, precision, repeatability and specificity. Accuracy was assessed by measuring recovery at three different levels, 50, 100 and 150% of the amount expected from analysis of the formulation, in accordance with ICH guidelines.

Table 5: Result of Accuracy of OFL and ORN

Drug	Level	Amt of Std taken (mg)	Amt of Std Recovered (mg)	Mean % Recovery
OFL	50	49.99	49.87	99.75
	100	99.69	99.38	99.70
	150	149.95	149.46	99.67
ORN	50	125.02	124.99	99.98
	100	249.98	249.57	99.83
	150	375.01	374.65	99.91

RESULTS AND DISCUSSION

RP-UPLC method was developed and validated for simultaneous estimation of Ofloxacin and Ornidazole in tablet dosage form. All system suitability parameters were passed in acceptable range. % Degradations of both drugs in different conditions was achieved as per ICH guidelines. Linearity of the developed method was near to 1, range was found 50–150µg/ml for Ofloxacin and 125–375µg/ml for Ornidazole. %RSD was found to be less than 2 for repeatability, intraday precision and intermediate precision. %Recoveries were found to be 99.67–99.75% and 99.83–100.98% for Ofloxacin and Ornidazole respectively. These results indicate that the developed method is accurate, precise, specific, robust and simple and less time consuming. It can be used in the routine quality control of marketed dosage form.

Table 6: Robustness study of OFL and ORN

Parameters	Condition	Retention Time		Tailing Factor		Plate count		Resolution
		OFL	ORN	OFL	ORN	OFL	ORN	
Flow rate	0.1 ml/min	4.128	6.957	1.2	1.1	5171	9136	10
	0.2 ml/min	3.950	6.406	1.1	1.1	5180	9244	10
	0.3 ml/min	3.260	5.957	1.1	1.1	5190	93337	10
Wavelength	298 nm	3.936	6.399	1.1	1.1	5136	9255	10
	300 nm	3.936	6.402	1.1	1.1	5179	9248	10
	302 nm	3.937	6.396	1.1	1.1	5134	9254	10
Mobile Phase ratio	83:17	3.573	4.774	1.2	1.2	4287	7544	3.2
	85:15	3.948	6.399	1.1	1.1	5136	9297	10
	87:13	5.574	5.900	1.2	1.2	8544	10326	2
pH	2.2	3.941	6.402	1.1	1.1	5180	9136	10
	2.3	3.936	6.399	1.1	1.1	5187	9197	10
	2.4	3.956	6.399	1.1	1.1	5189	9244	10

Table 7: Summary of Validation Parameters

S. No	Parameters	Acceptance Criteria	Result	
			OFL	ORN
1	Specificity	No Interference	No Interference	No Interference
2	Precision	% RSD (<2%)	0.31%	0.32%
3	Linearity	R^2 (>0.999)	0.9994	0.9994
4	Accuracy (% Recoveries)			
	50%	98-102%	99.75%	99.98%
	100%		99.70%	99.83%
	150%		99.67%	99.91%
5	Robustness			
	Flow Rate	% RSD (<2%)	0.99	0.66
	Wavelength		1.54	1.32
	Mobile Phase Ratio		0.52	0.58
	pH Change		0.48	0.17

Table 8: Analysis of Marketed Formulation of OFL and ORN by Proposed Method (n = 6)

Sample No.	Label Claim (mg/tablet)		Amount Found (mg/tablet)		% Assay	
	OFL	ORN	OFL	ORN	OFL	ORN
1	200	500	197.71	498.84	98.86	99.77
2	200	500	203.30	506.00	101.65	101.20
3	200	500	197.93	479.44	98.97	95.89
4	200	500	200.18	506.11	100.09	101.22
5	200	500	203.98	511.63	101.99	102.33
6	200	500	200.32	504.50	100.16	100.90
Mean			200.57	501.09	**100.29**	**100.22**
S.D.			0.91	3.56	0.46	0.71
% RSD					0.46	0.71

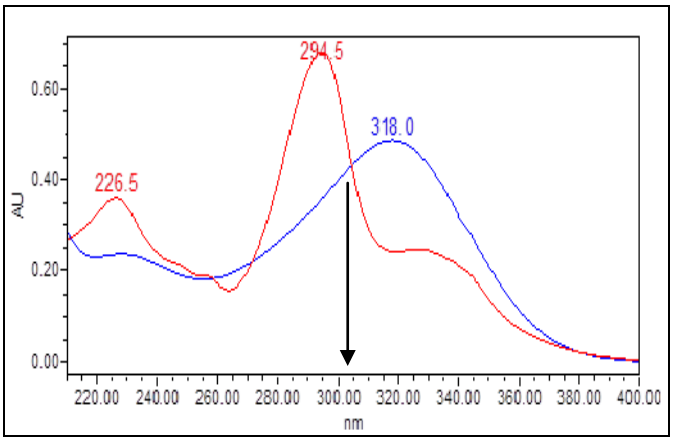

Fig. 3: Overlay PDA Spectrum of OFL and ORN Showing Selection of Detection Wavelength

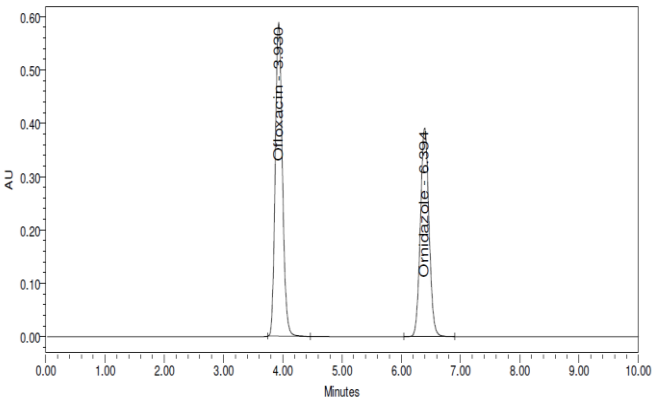

Fig. 4: Chromatogram of Standard OFL and ORN

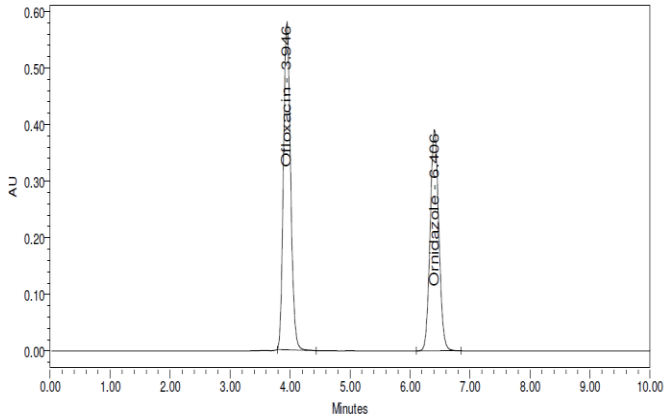

Fig. 5: Chromatogram of Tablet

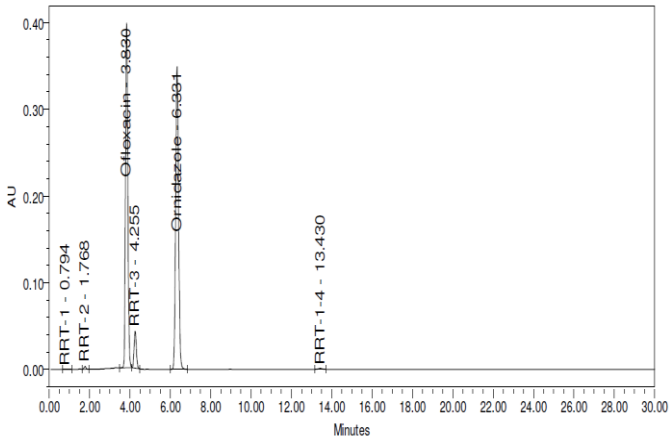

Fig. 6: Chromatogram of Tablet in Acidic condition

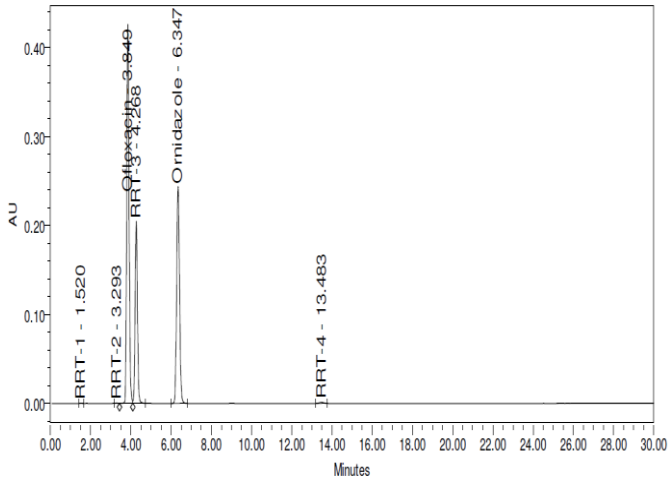

Fig. 7: Chromatogram of Tablet in Alkaline condition

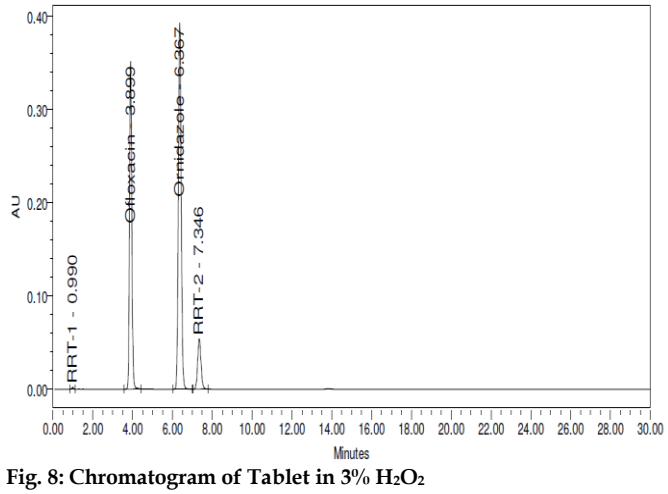

Fig. 8: Chromatogram of Tablet in 3% H_2O_2

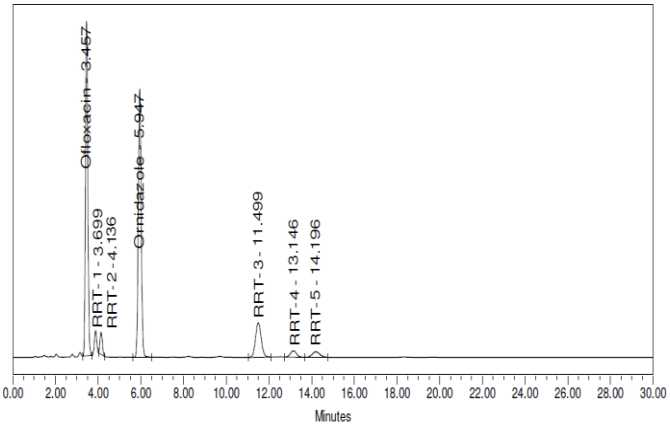

Fig. 9: Chromatogram of Tablet in Thermal condition

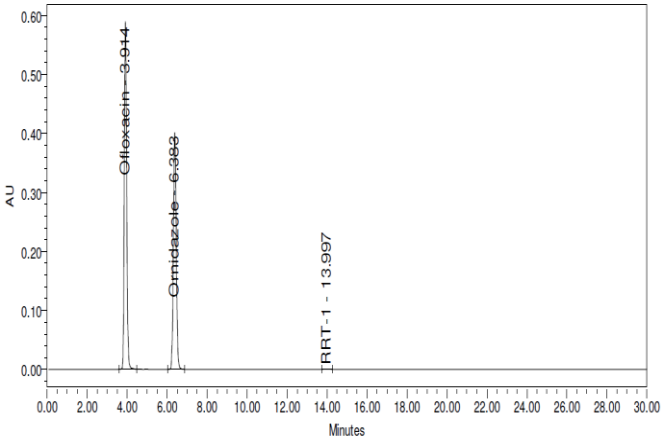

Fig. 10: Chromatogram of Tablet in photolytic condition

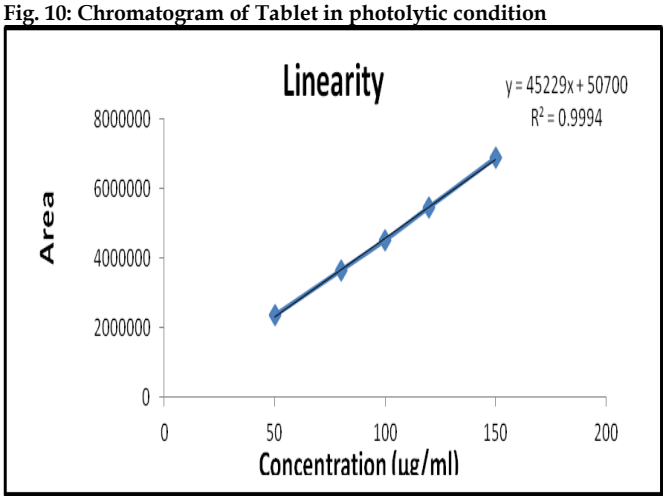

Fig. 11: Linearity Curve of OFL

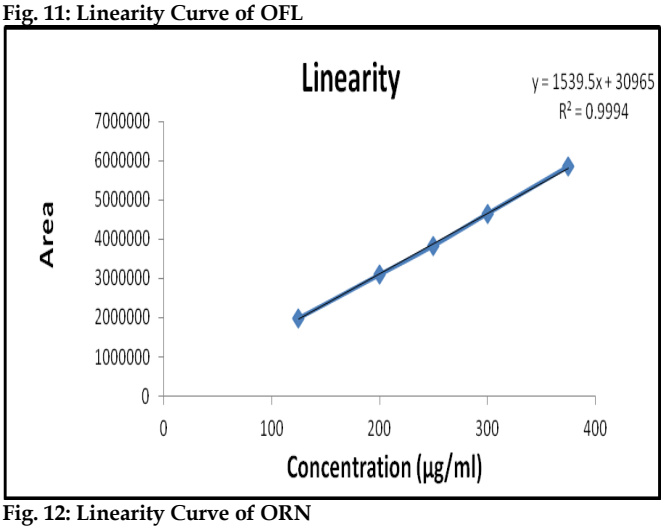

Fig. 12: Linearity Curve of ORN

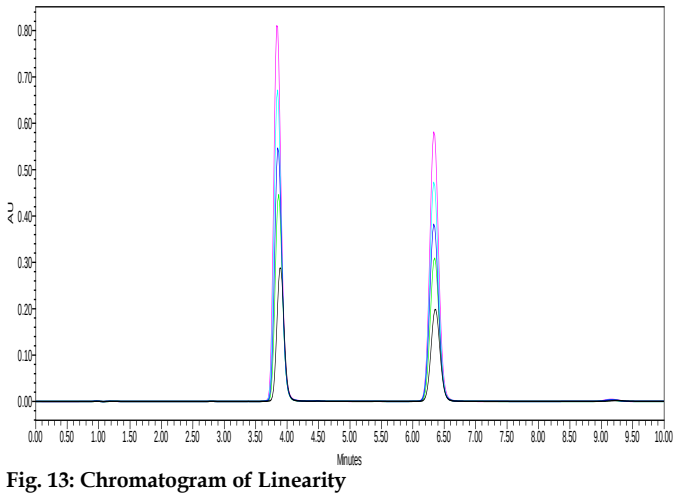

Fig. 13: Chromatogram of Linearity

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