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Method Development and Validation for Simultaneous Estimation of Ofloxacin and Ornidazole In Bulk and Pharmaceutical Dosage forms

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ABSTRACT

A simple, rapid, sensitive reverse-phase high-performance liquid chromatography method was developed and validated for simultaneous estimation of ofloxacin and ornidazole, at single wavelength of 343nm.chromotographic separation was performed on an enable aligent zorabax(thermo) column(250nmx4.6mm ID particle size 5 um) and a mobile phase consisting of acetonitrile and buffer (600:4300v/v) at a flow rate of 1.0ml/min. the calibration curve was linear($r^2 \geq 0.0999$) over the concentration range. 400-1200µg/mL of ofloxacin and 1000-3000µg/mL of ornidazole. the limit of detection 0.00246µg/ml for ofloxacin 0.00508µg/ml for ornidazole and no interference was found by the excipients in the synthetic mixture. The proposed methods were validated for international conference on harmonization guideline for linearity, accuracy, precision, and robustness for estimation of ofloxacin and ornidazole in bulk and synthetic mixture, and The results were found to be satisfactory

Keywords: Ofloxacin, Ornidazole, RP-HPLC Validation

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INTRODUCTION

Ofloxacin (Figure 1)[1] 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 track infection and sexually transmitted diseases. Ornidazole (Figure 2)[2], 1-chloro-3-(2-methyl-5-nitroimidazole-1-yl)propan- 2-ol, is a derivative of nitroimidazole, with antiprotozoal and antibacterial properties. It is used in the prevention and treatment of infections due to anaerobic germs. The drug is specifically useful in abdominal and gynecological surgery. To date, only two methods were reported for simultaneous estimation of ofloxacin and ornidazole by HPLC[3] and HPTLC[4]. Hence, it was felt necessary to develop a simple, inexpensive, sensitive liquid chromatography method for the determination of ofloxacin and ornidazole in combined dosage forms.

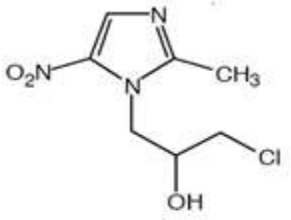


Figure 1: Structure of Ofloxacin

Figure 2: Structure of Ornidazole

MATERIALS AND METHOD

Reagents & Materials:

Milli-Q water, methanol (HPLC Grade), orthophosphoric acid (GR Grade),potassium dihydrogen phosphate monohydrate (GR Grade) was obtained from Qualigens Ltd., Mumbai. All other chemical of analytical grade were procured from local sources. All dilutions were performed in standard class-A, volumetric glassware.

Instrumentation:

In the present chapter the analysis was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector. An Reverse phase HPLC column Aligent, Zorbax(150 mmx4.6 mm I.D; particle size 5 µm) was used and the output signal was monitored and integrated using waters Empower 2 software.

Mobile Phase Preparation:.

Prepare a filtered and degassed mixture of buffer and Acetonirile in the ratio of40:60v/v respectively

Diluent Preparation:

Mobile phase was used as a diluent in the present assay.

Standard Preparation:

About 100mg ofloxacin and ornidazole were accurately weighed and transferred into taken in 100ml volumetric flask and dissolved with the diluent and was sonicated for 5 mins. Later the volume of the flask was adjusted to the mark with the same diluent to give stock solution of concentration 1.0mg/ml for ofloxacin and ornidazole respectively. From this calibration standard solutions were prepared in the concentration range of 400 - 1200µg/ml for ofloxacin and 1000 - 3000µg/ml for ornidazole respectively.

Sample Preparation:

Ten tablets [Oftrum; ofloxacin, 200mg + ornidazole, 500mg)] were procured from local pharmacy, weighed and was grounded to finely powdered in a pestle and mortar. Tablets powder equivalent to 100mg of ofloxacin and ornidazole was transferred to 100ml volumetric flask containing 50 ml of diluent. This solution were sonicated for 15min., diluted to the mark with diluent and then filtered through 0.45µm membrane filter(1.0mg/ml) respectively. From this aliquots of the sample solution were transferred to 50ml volumetric flasks and diluted with diluent to obtain 400 -1200µg/ml of ofloxacin and 1000 - 3000µg/ml of ornidazole.

Chromatographic Conditions:.

In the present assay an Aligent, Zorbax (Make: 150 mmx4.6 mm I.D; particle size 5µm) column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of1.0ml/min. The sample injection volume was 10µL. The photodiode array detector was set to a wavelength of 343nm for the detection with an chromatographic runtime of 6 minutes.

RESULTS AND DISCUSSION

Development and optimization of the HPLC method:

Several tests were performed in order to get satisfactory separation-resolution of ofloxacin and ornidazole in mobile phases with various ratios of organic phase and water by using C_{18} column. Acetonitrile with buffer in the ratio (40:60v/v) was preferred because it resulted in a greater response to ofloxacin and ornidazole. The retention time of ofloxacin and ornidazole on analytical column was evaluated at 2.703 and 5.113 min at a flow rate of 1.0 $mL.min^{-1}$. The injection volume was 10µl. The retention time of standard and sample for were satisfactory with good resolution. Under these conditions, the analyte peak was well-defined and free from tailing was obtained.

METHOD VALIDATION

System Suitability:

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate, resolution and % RSD of peak area were determined for same. Acceptance criteria for system suitability, Asymmetry not more than 2.0, theoretical plate not less than 2000 for ofloxacin and 5000 for ornidazole and the % RSD of peak area not more then 2.0, were full fill during all validation parameter

Blank and **Placebo Interference:**

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the column as described in the above chromatographic conditions and the blank and placebo chromatograms were recorded. The chromatogram of blank solution (Figure 3) showed no peaks at the retention time of ofloxacin and ornidazole peak, revealing that the diluent solution used in sample preparation do not interfere in estimation of ofloxacin and ornidazole in tablets. Similarly the chromatogram of placebo solution (Figure 4) showed no peaks at the retention time of ofloxacin and ornidazole peak revealings that the placebo used in sample preparation did not interfere in estimation of ofloxacin and ornidazole in their formulations.

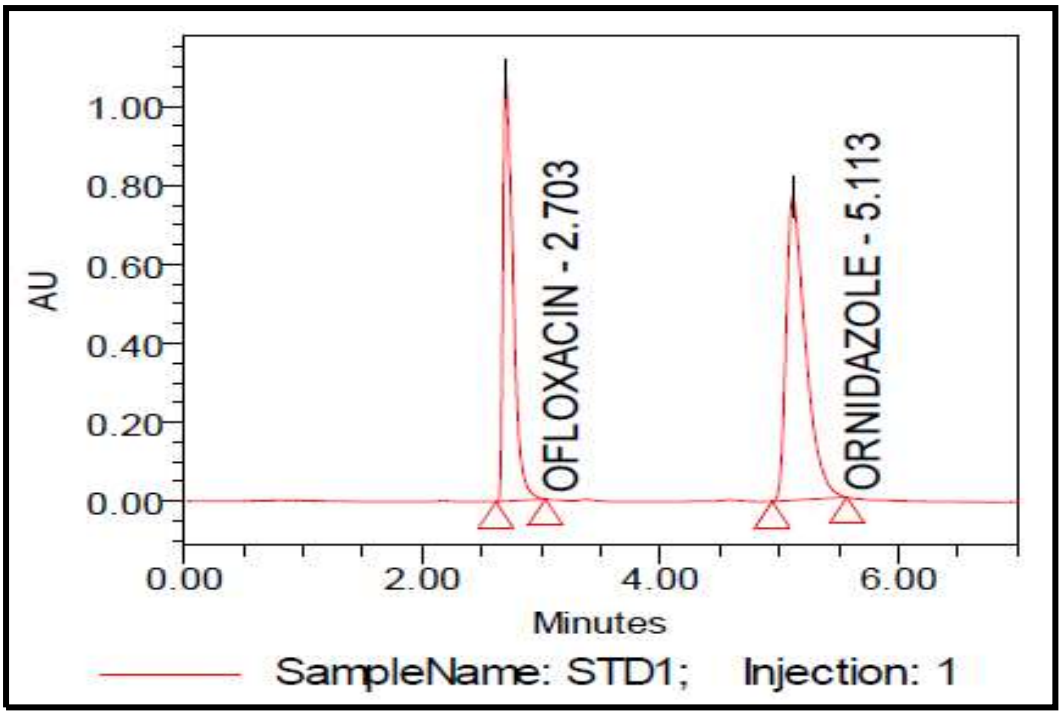


Figure 3: Typical HPLC Chromatogram Ofloxacin and Ornidazole

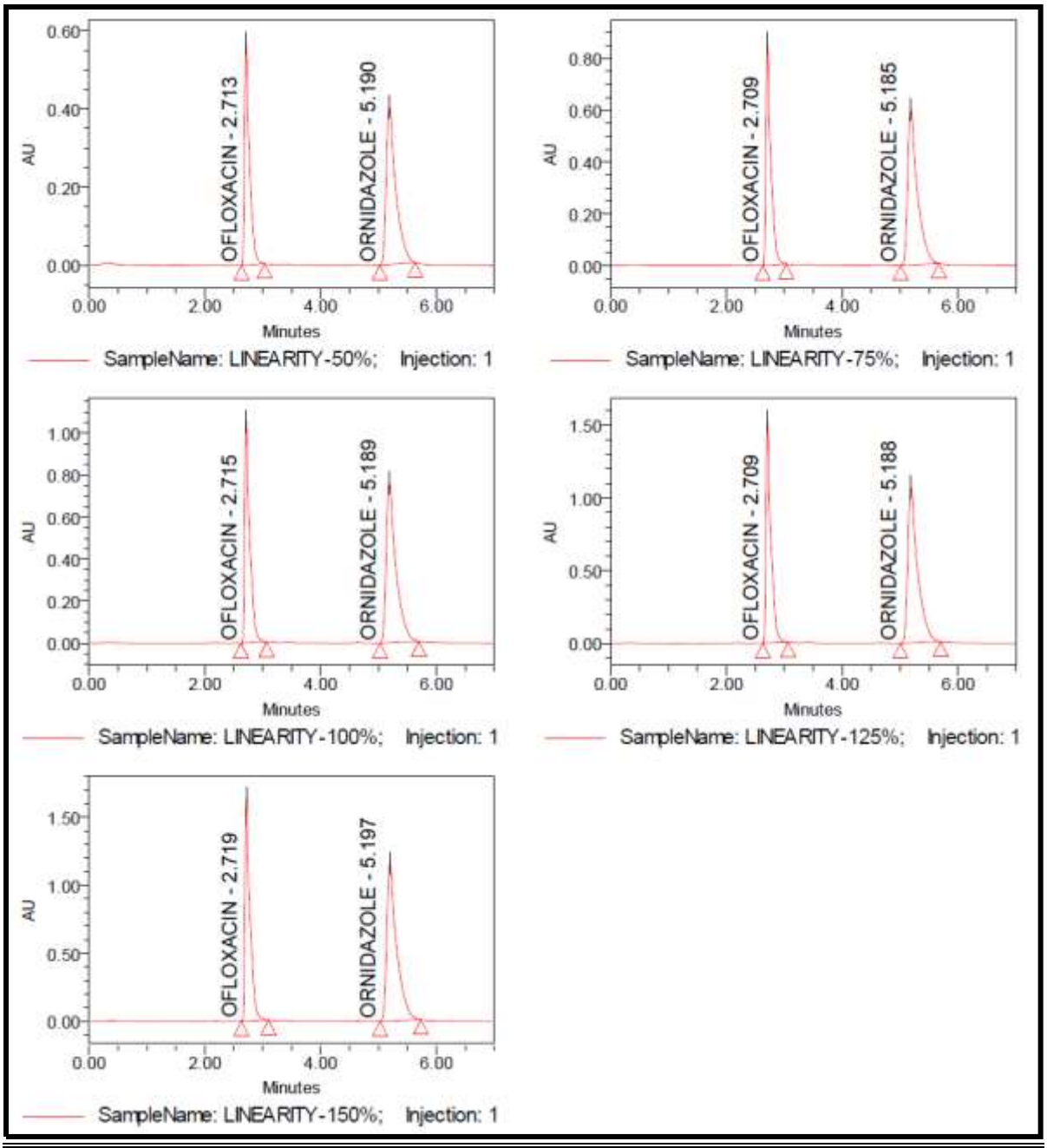


Figure: 4: Linearity Chromatograms of Ofloxacin and Ornidazole

Linearity:

A standard curve was obtained in the concentration range of 400 -1200µg/ml for ofloxacin and 1000-3000µg/ml for ornidazole. The linearity of this method was evaluated by linear regression analysis. The slope, intercept and correlation coefficient [r^2] of standard curve were plotted and calculated and are given in Figure 5 & Table 1 for ofloxacin Figure 6 & Table: 2 and for ornidazole respectively demonstrating the linearity of the proposed RP-HPLC method. The LOD value for ofloxacin and ornidazole were found to be 0.00246µg/mL and 0.00508µg/mL,

respectively and are reported in Table:1 and 2 respectively.

Table: 1 & Figure: 5 Linearity Studies & Curve of Ofloxacin By The Proposed Method

Linearity study for ofloxacin		
% Level (approx.)	**Conc. µg/ml**	**Area**
50	400	3185370
75	600	4775271
100	800	6361263
125	1000	7957587
150	1200	9540107
Slope		7945.5
RSQ(r2)		1.000
LOD		0.00246

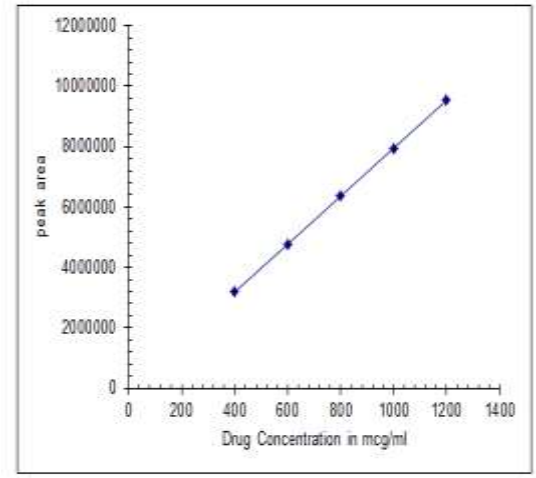
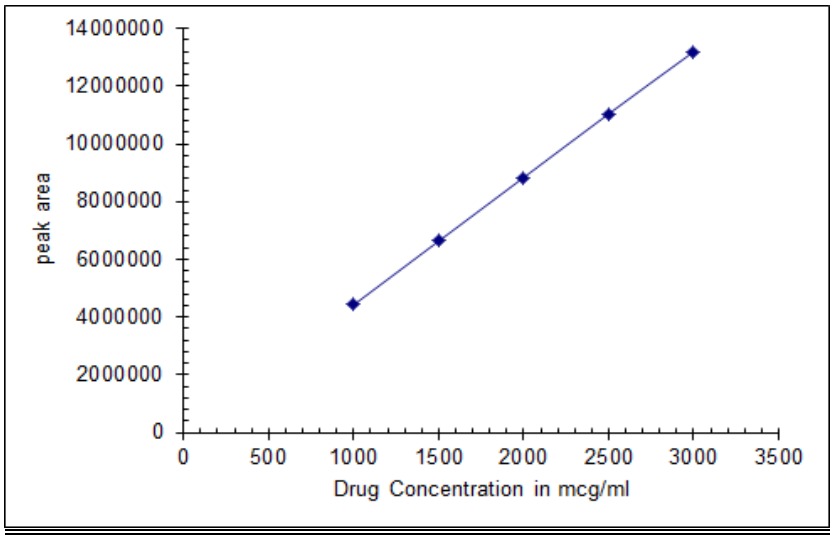


Table: 2 & Figure: 6: **Linearity Studies & Curve Of Ornidazole By The Proposed Method**

Linearity study for ornidazole		
% Level (approx.)	**Conc. µg/ml**	**Area**
50	1000	4427315
75	1500	6637991
100	2000	8842182
125	2500	11048909
150	3000	13200688
Slope		4391.53
RSQ(r2)		0.9999
LOD (µg/ml)		0.00508



Precision:

In the present study, precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. The method precision study for six sample preparations in marketed samples showed (Figure 7 a RSD of 0.0273% and the 95% confidence interval with the assay range of 99.6-100 for ofloxacin. Similarly the method precision study for six sample preparations in marketed samples showed a % RSD of 0.023% and the 95% confidence interval with the assay range of 99.9-100% for ornidazole respectively.

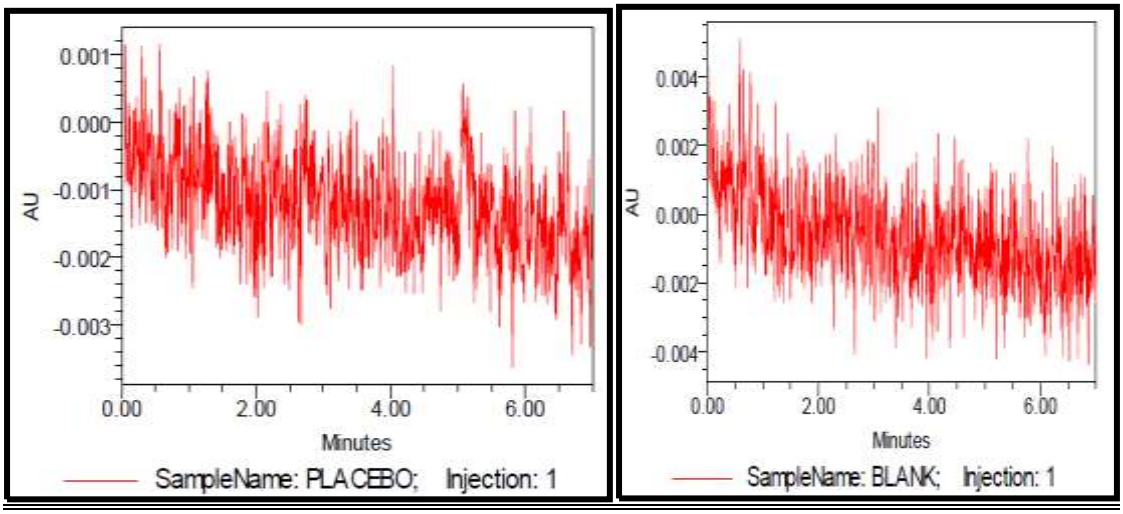


Figure: 7.A & B - Typical HPLC Chromatogram Showing the No Interference Of Blank And Placebo For Ofloxacin And Ornidazole

Accuracy:

The accuracy of the present RP-HPLC method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on

composite blend collected from formulations of ofloxacin and ornidazole, and were analyzed as per the proposed method. The percentage recoveries were found in the range of 100% with an overall %RSD of 0.487 and 0.34 for ofloxacin for ornidazole respectively. From the data reported in Table 3, that the developed RP- HPLC method was found to be accurate for ofloxacin and ornidazole assay.

Table- 3: Method Precision (Inter and Intraday) Studies for Ofloxacin And Ornidazole By The Proposed Method

Method precision by proposed method		
For ofloxacin **Method precision (inter & intra day**		**For ornidazole** **Method precision (inter & intra day**
Set-1	9547551	13242234
Set-2	9544603	13297494
Set-3	9543210	13208445
Set-4	9541744	13256820
Set-5	9540425	13222174
Set-6	9541080	13245464
Over All Avg.	9543102	1345439
Over All Std Dev.	2651.638	30852.36
Over All %RSD	0.027	0.232

Robustness:

The robustness study of the developed RP-HPLC assay method for ofloxacin and ornidazole were established in slight variance conditions. Basing on the results obtained the assay value of the test solution was not affected and it was in accordance with that of actual even with slight variations in chromatographic parameters. The system suitability parameters were found be satisfactory revealing the robustness of the proposed RP-HPLC method. (Figure 8)

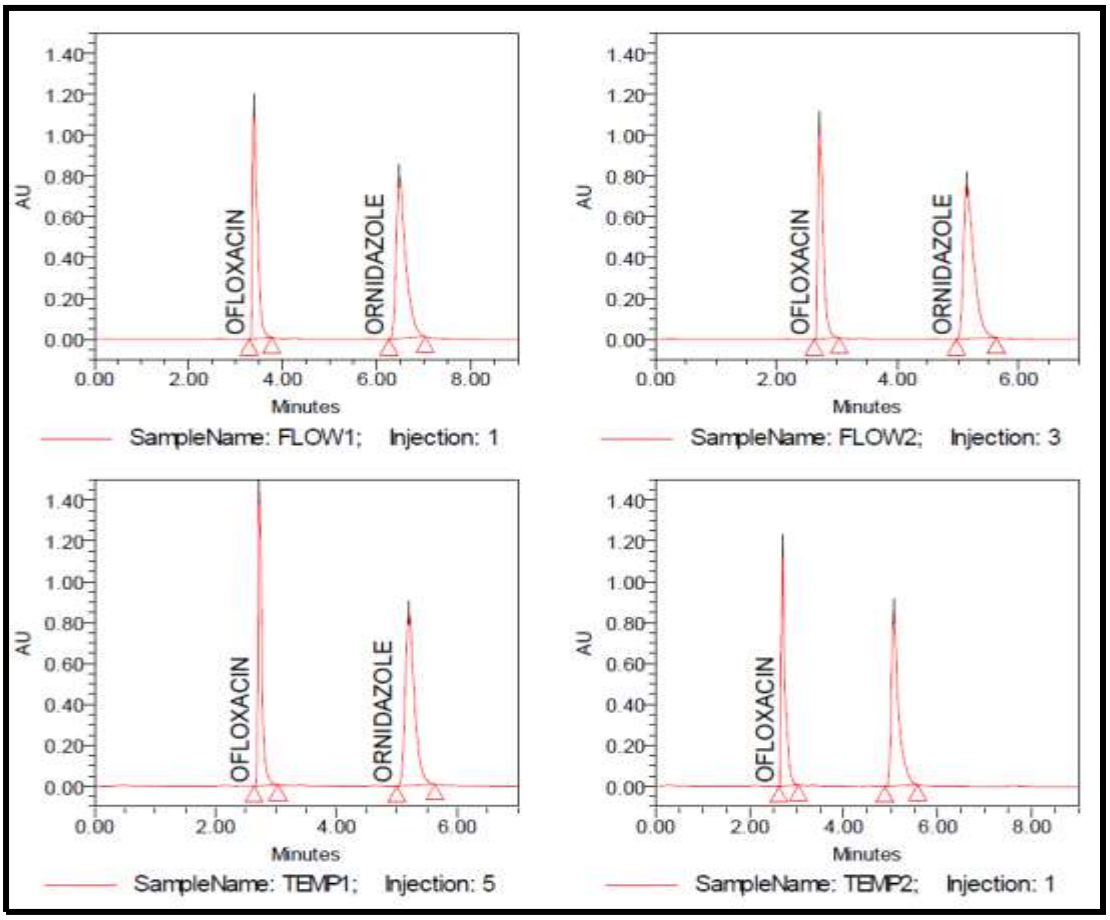


Figure 8: Robustness Studies Chromatograms of Ofloxacin and Ornidazole

Assay in formulations:

The analysis of marketed tablets for ofloxacin and ornidazole was carried out using optimized HPLC conditions. The % assay of the drug content of tablets obtained by the proposed method for ofloxacin and ornidazole was found to be 99.94 and 99.96, respectively. This showed that the estimation of dosage forms with the proposed RP-HPLC method was accurate and was within the acceptance level of 95% to 100% and the results are given in Table.4.

Table: 4 Recovery Studies for Ofloxacin By The Proposed Method

Spiked Level	Sample Weight	Sample Are	µg/ml added	µg/ml found	% Recovery	% Mean
50%	458.43	3187048	396.404	397.13	100	100
50%	458.43	3184170	396.404	396.77	100	
50%	458.43	3186941	396.404	397.12	100	
50%	458.43	3185914	396.404	396.99	100	
50%	458.43	3181026	396.404	396.38	100	
50%	458.43	3188995	396.404	397.37	100	
100%	916.85	6369529	792.800	793.69	100	100
100%	916.85	6360919	792.800	792.62	100	

100%	916.85	6368448	792.800	793.56	100	
150%	1375.30	9547551	1189.222	1189.70	100	100
150%	1375.30	9544603	1189.222	1189.33	100	
150%	1375.30	9543210	1189.222	1189.16	100	
150%	1375.30	9541744	1189.222	1188.98	100	
150%	1375.30	9540425	1189.222	1188.81	100	
150%	1375.30	9541080	1189.222	1188.89	100	

Table: 5 Recovery Studies for Ornidazole by the Proposed Method

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	458.43	4421464	997.011	997.01	100	100
50%	458.43	4426497	997.011	998.15	100	
50%	458.43	4423298	997.011	997.43	100	
50%	458.43	4424958	997.011	997.80	100	
50%	458.43	4428429	997.011	998.58	100	
50%	458.43	4421806	997.011	997.09	100	
100%	916.85	8842719.00	1994.000	1993.98	100	100
100%	916.85	8841444.00	1994.000	1993.69	100	
100%	916.85	8844142.00	1994.000	1994.30	100	
150%	1375.30	13242234	2991.054	2996.04	100	100
150%	1375.30	13297494	2991.054	2991.50	100	
150%	1375.30	13208445	2991.054	2991.42	100	
150%	1375.30	13256820	2991.054	2999.33	100	
150%	1375.30	13222174	2991.054	2981.52	100	
150%	1375.30	13245464	2991.011	2991.01	100	

Table.6: Analysis of Marketed Tablets

Drug	Label claim	Quantity found*	%Assay
Ofloxacin	200mg	199.89	99.94
Ornidazole	500mg	499.98	99.96

CONCLUSION

A new high performance liquid chromatographic method has been developed and validated for the simultaneous determination of ofloxacin and ornidazole in pure and tablet formulations. It was observed from the validation results that the developed RP-HPLC method is more sensitive, accurate, precise, repeatable with wide range of linear range as compared to the reported method[4].The run time of the present assay is relatively short i.e. less than 10 min, which enable rapid quantitation of samples in the routine analysis of tablet formulation.. Basing on the above aspects it is concluded that the developed RP-HPLC method can be used for the simultaneous determination of ofloxacin and ornidazole in tablet formulations.

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