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Development and validation of stability indicating assay method for simultaneous determination of Metronidazole and Ofloxacin in pharmaceutical dosage form by using RP-HPLC

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ABSTRACT

A simple, accurate, precise sensitive, repeatable and stability indicating RP-HPLC method was developed for simultaneous determination of Metronidazole (MET) and Ofloxacin (OFL) in combine dosage form. The method was developed by using reverse phase Hypersil BDS C_{18}, 150x4.6, 5µ as stationary phase with phosphate buffer and acetonitrile : (90:10 %v/v) as a mobile phase, pH was adjusted to 5.0 ± 0.1 by ortho-phosphoric acid at a flow rate of 1.0 ml/min and column temperature maintained at 30ºC. Quantification of eluted compound was achieved with UV-Vis detector, Thermostat column compartment connected with Waters (alliance) Empower software at 289 nm. The combination drug product was exposed to water, acid, base hydrolytic, and oxidative stress conditions and the stressed samples were analyzed by proposed method. Metronidazole and Ofloxacin followed linearity in the concentration range of 25-150 µgml^{-1} and 12-75 µgml^{-1} with r^2=0.9993 (n=6) and r^2=0.9997 respectively. Limit of detection (LOD) and limit of quantification (LOQ) values for Metronidazole was 0.3795 and 1.15 µgml^{-1} and for Ofloxacin was 0.818 and 2.477 µgml^{-1} respectively. Chromatographic peak purities are 1.0and 1.0 for MET and OFL respectively. This demonstrated the specificity of assay method for their estimation in presence of degradation products. The validation study is carried out as per International Conference on Harmonization (ICH) guidelines. This method was also successfully applied for determination of Metronidazole and Ofloxacin in pharmaceutical formulation.
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KEYWORDS

Metronidazole;
Ofloxacin;
Stability Indicating Assay Method;
RP-HPLC.

INTRODUCTION

Metronidazole (Figure 1) chemically is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol[1]. Metronidazole is a nitro imidazole antibiotic drug used against anaerobic organisms, amoebozoa infections and antiprotozoal. It is frequently used for mild-to-moderate Clostridium difficile infection[2]. Metronidazole is also used to treat bacterial vaginosis, pelvic inflammatory disease, pseudo membranous colitis, aspiration pneumonia, rosacea, fungating wounds, intra-abdominal infections, lung abscess, gingivitis, amoebiasis, giardiasis, trichomoniasis and infections caused by susceptible anaerobic organisms such as Bacteroides fragilis spp,

Fusobacterium spp, Clostridium spp, Peptostreptococcus spp and Prevotella spp[3]. It has a molecular formula of $C_6H_9N_3O_3$and a molecular weight of 171.15 g/mol.

Figure 1 : Structure of metronidazole

Ofloxacin (OFL) is a fluoroquinolone derivative with potent activity against a broad spectrum of bacteria. Chemically, it is (±)-9-fluoro-2, 3-dihydro- 3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7Hpyrido-[1,2,3-de]-1,4-benzoxazine -6-carboxylic acid [4-8] in (Figure 2). It is mainly used as an antibacterial for the treatment of urinary tract infection and sexually transmitted diseases.

It has a molecular formula of $C_{18}H_{20}FN_3O_4$ and a molecular weight of 361.367 g/mol.

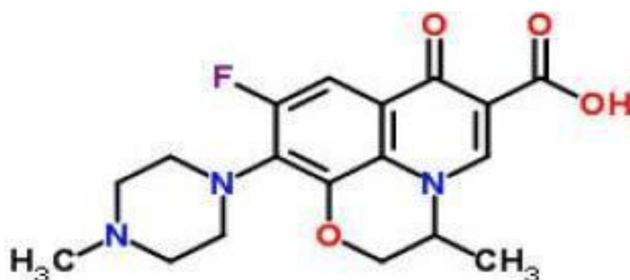


Figure 2 : Structure of ofloxacin

Literature survey reveals that, UV, HPLC, Spectrophotometric methods are reported for Metronidazole alone and combination with other drugs[9 -16]. And various analytical methods have been reported for the estimation of OFL in single and combination form such as spectrophotometric[17,18], conductometric[19], HPLC[20-24], LC/MS/MS[25,26]. There is no reported stability indicating RP-HPLC method for estimation of simultaneous determination of Metronidazole (MET) and Ofloxacin (OFL) in combine dosage forms of both these drugs. The aim of the present work is to develop a simple, accurate, precise sensitive, repeatable and Stability indicating RP-HPLC method was developed for simultaneous determination of Metronidazole (MET) and Ofloxacin (OFL) in combine dosage form.

EXPERIMENTAL

Materials and methods chemicals and reagents

Dipotasssium hydrogen phosphate and potassium hydrogen phosphate were bought from SR Scientifics - Tirupati, India. Acetonitrile (HPLC grade) purchased from SR Scientifics - Tirupati, India. BioLeo Labs pLtd. Hyderabad, Telangana, India was kind enough and supplied the reference standards of MET and OFL for this research work. All the chemicals used throughout the research work were of analytical grade. O-Phosphoric acid was also purchased from SR Scientifics - Tirupati, India. Commercial tablets of METOLOR-AM consist of MET (100 mg) and (50 mg) was purchased from local market manufactured by Bio Leo Labs pLtd. Hyderabad, India

Instrumentation

Waters HPLC 2 2695 series consisting pump, Auto sampler, UV-Vis detector, Thermostat Column compartment connected with Waters(alliance) Empower software.

Chromatographic conditions

The chromatographic separation was performed on Hypersil BDS C18, 150x4.6, 5µ. The column temperature was kept at 30ºC. Separations were performed in isocratic mode using a mobile phase consisting of Mix buffer and acetonitrile in the ration 90:10. (P^H5.0 ±0.1 adjusted by using Ortho-phosphoric acid): acetonitrile in ratio of (90:10 v/v %) with a flow rate of 1.0 ml/minute. The detection wavelength was set at 289 nm.

Analytical methodology

Preparation of reagents and standards Mobile phase

Precisely weighed and dissolved 2.6gms of potassium hydrogen phosphate and 0.6gms of dipotasssium hydrogen phosphate in 1000ml, adjusted p^H 5.0±0.1 with dilute orthophosphoric acid solution. The above prepared buffer and acetonitrile were mixed

in the proportion of 90:10 v/v. The mobile phase was then duly filtered through 0.45 μm nylon membrane vacuum filtration and duly degassed by sonication.

Preparation of metronidazole and ofloxacin stock and standard solutions

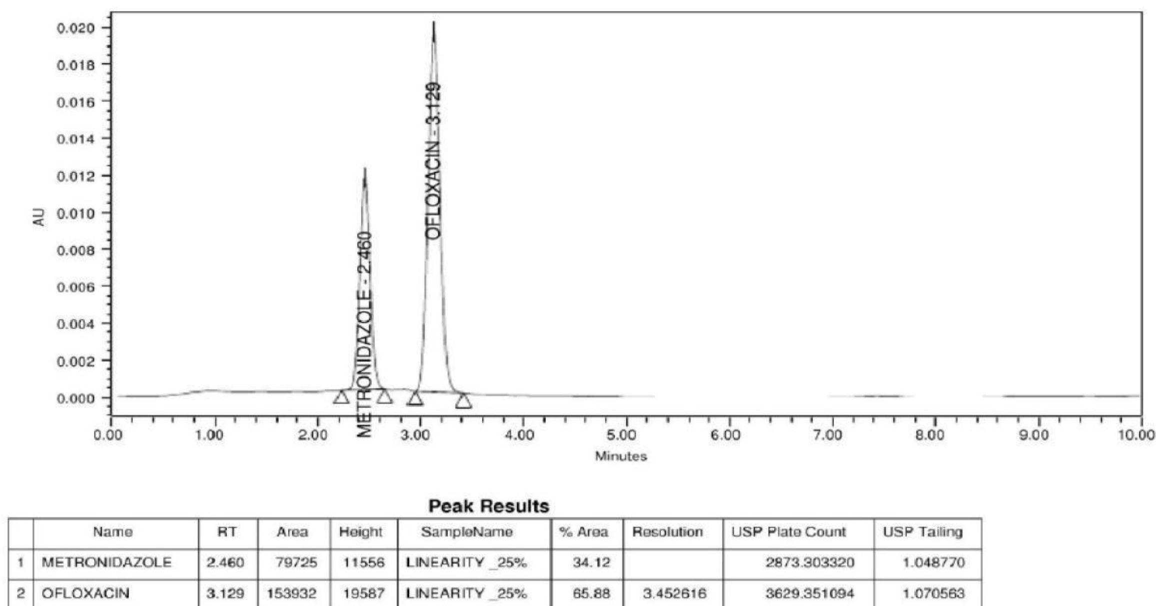
100mg of Metronidazole and 50 mg Ofloxacin were weighed accurately and transferred in to 100 ml volumetric flasks. 30 ml of diluents were added and sonicated to dissolve the compound. This was made up to mark with buffer and acetonitrile in the ratio 90:10 v/v which yields 1000 μgml^{-1} & 500 μgml^{-1} (stock solution A). 10 ml of solutions were pipetted out into 100ml volumetric flasks separately and volumes were made up to mark with diluents which gave 100 μgml^{-1} and 50 μgml^{-1} (stock solution B). The standard solution ranging from 2.5-15 mL were transferred into a series of 100 ml volumetric flasks to provide a final concentration range of Metronidazole 25-150 μgml^{-1} and Ofloxacin 12-75 μgml^{-1}, and the contents of each flask was made up to the mark with diluents.

Preparation of formulation test solutions

Twenty tablets containing Metronidazole and twenty tablets containing Ofloxacin were weighed and finely powered. An accurately weighed portion of the powder equivalent to 100 mg of Metronidazole and 50 mg of Ofloxacin was transferred into 100 ml volumetric flasks. 10 ml of diluents were added and shaken for 20 minutes by manually and further sonicated for 10 minutes. This was diluted up to the mark with diluents. These solutions were centrifuged at 8000 rpm for 10 minutes. The supernatant solution was decanted into another test tube (i.e. 1000 μgml^{-1} and 500 μgml^{-1}) 10 ml of supernatant solution was transferred into another 100 ml volumetric flask and made up to the mark with diluents (100 μgml^{-1} and 50 μgml^{-1}). 2.5-15 mL of solutions were transferred into other 100 ml volumetric flasks separately and made up to the mark with diluents to provide a final concentration range of Metronidazole 25-150 μgml^{-1} and Ofloxacin 12-75 μgml^{-1}. The solutions were filtered through 0.45 μm Nylon membrane filter paper. 20 μL of blank solution, placebo solution, three times of standard solutions were injected, disregarding peaks due to blank and placebo.

Assay procedure

The column was equilibrated for at least 30 minutes with mobile phase flowing through the system with a flow rate of 1.0 ml/min. Detector was set at a wavelength of 289 nm. Twelve sets of the drug solutions were prepared in diluents containing Metronidazole and Ofloxacin at a concentration range of 25 – 150 μg/ml and 12-75 μg/ml. Then 20 μl of each standard and sample solution were injected for Six times separately. The retention time for Metronidazole and Ofloxacin



Peak Results

	Name	RT	Area	Height	SampleName	% Area	Resolution	USP Plate Count	USP Tailing
1	METRONIDAZOLE	2.460	79725	11556	LINEARITY _25%	34.12		2873.303320	1.048770
2	OFLOXACIN	3.129	153932	19587	LINEARITY _25%	65.88	3.452616	3629.351094	1.070563

Figure 3: Chromatograms of metronidazole and ofloxacin

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succinate were found to be 2.460 and 3.129 min (Figure 3). The peak areas of the drug concentrations were calculated.

System suitability solution

Metronidazole and Ofloxacin standard working solution was used as system suitability solution.

Procedure

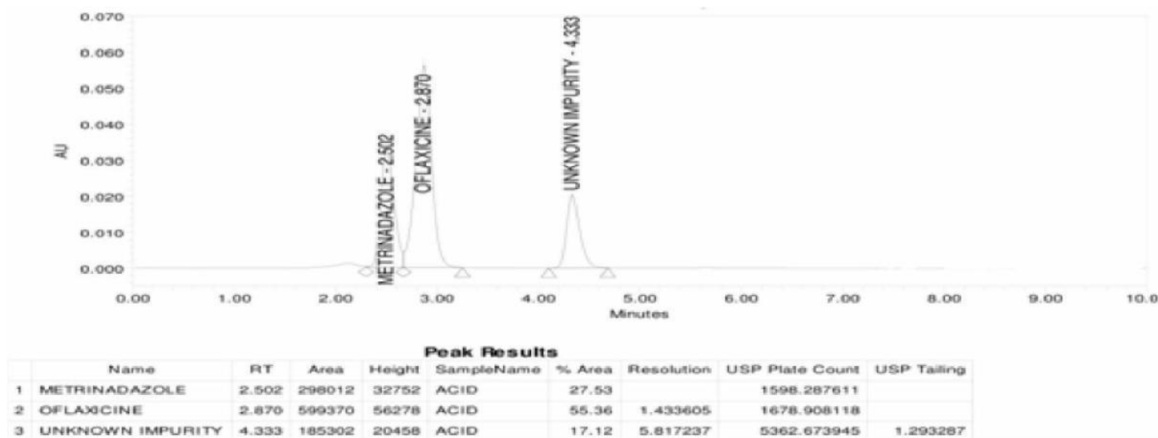
Equal volumes of blank were injected and twelve replicate injections of system suitability solutions in to column (Metronidazole and Ofloxacin standard working solution). The chromatograms were recorded. Disregarded any peaks due to blank in the test solution. % RSD of twelve replicate injections of system was calculated (Metronidazole and Ofloxacin standard working solution). Tailing factor and theoretical plates of the peak in the chromatogram obtained with 12th injection of system suitability solution (Metronidazole and Ofloxacin standard working solution) were checked.

System suitability requirements from SST solution:

a) Tailing factor : NMT 2.0
b) Theoretical Plates : NLT 2000
c) Resolution : NLT 2.0

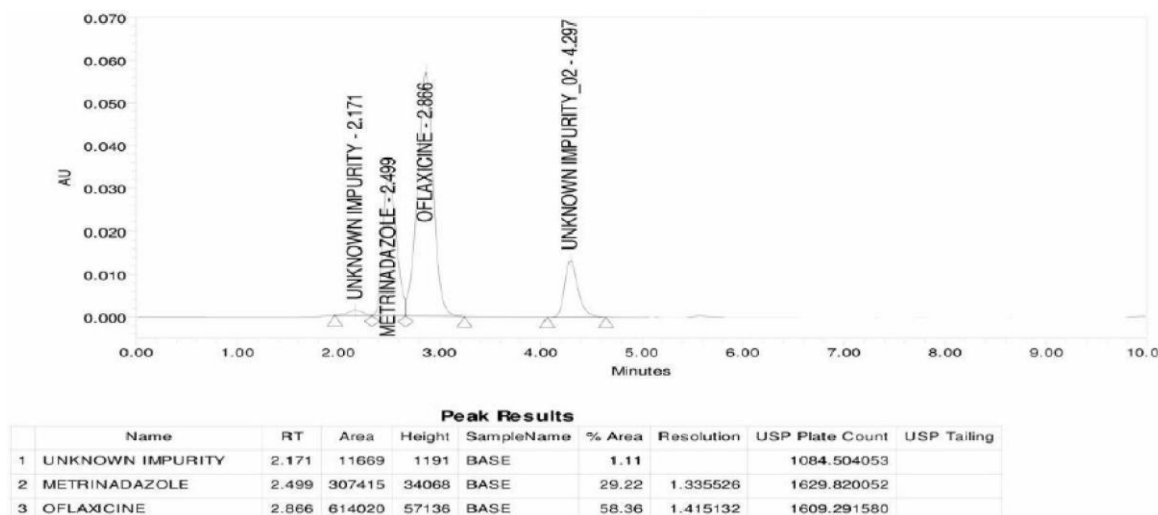
Linearity and construction of calibration curve

Linearity of the peak area response was determined by taking measurements at twelve concentrations of working standard of Metronidazole and Ofloxacin solutions in the range of 25-150 µgml^{-1} and 12-75 µgml^{-1}. 20µL quantity of the solution was injected each time in to the column. The drug elutes were monitored at 289 nm at a column temperature of 30°C and the corresponding chromatograms were recorded. The Linearity of the calibration curve was plotted between the mean peak areas versus respective Concentration in (Figure 8 &9).



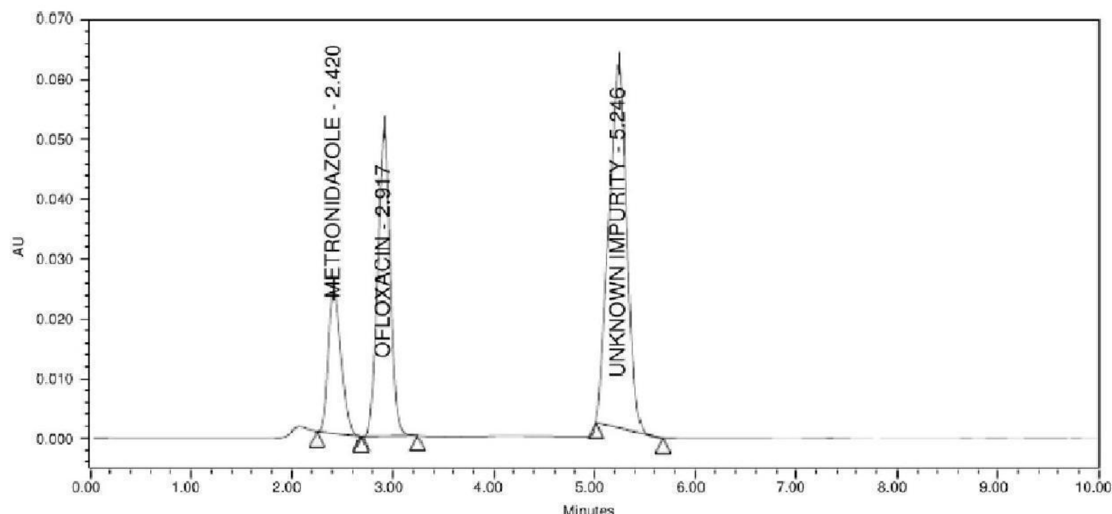
	Name	RT	Area	Height	SampleName	% Area	Resolution	USP Plate Count	USP Tailing
1	METRINADAZOLE	2.502	298012	32752	ACID	27.53		1598.287611	
2	OFLAXICINE	2.870	599370	56278	ACID	55.36	1.433605	1678.908118	
3	UNKNOWN IMPURITY	4.333	185302	20458	ACID	17.12	5.817237	5362.673945	1.293287

Figure 4 : Chromatograms of metronidazole and ofloxacin in acid degradation



	Name	RT	Area	Height	SampleName	% Area	Resolution	USP Plate Count	USP Tailing
1	UNKNOWN IMPURITY	2.171	11669	1191	BASE	1.11		1084.504053	
2	METRINADAZOLE	2.499	307415	34068	BASE	29.22	1.335526	1629.820052	
3	OFLAXICINE	2.866	614020	57136	BASE	58.36	1.415132	1609.291580	

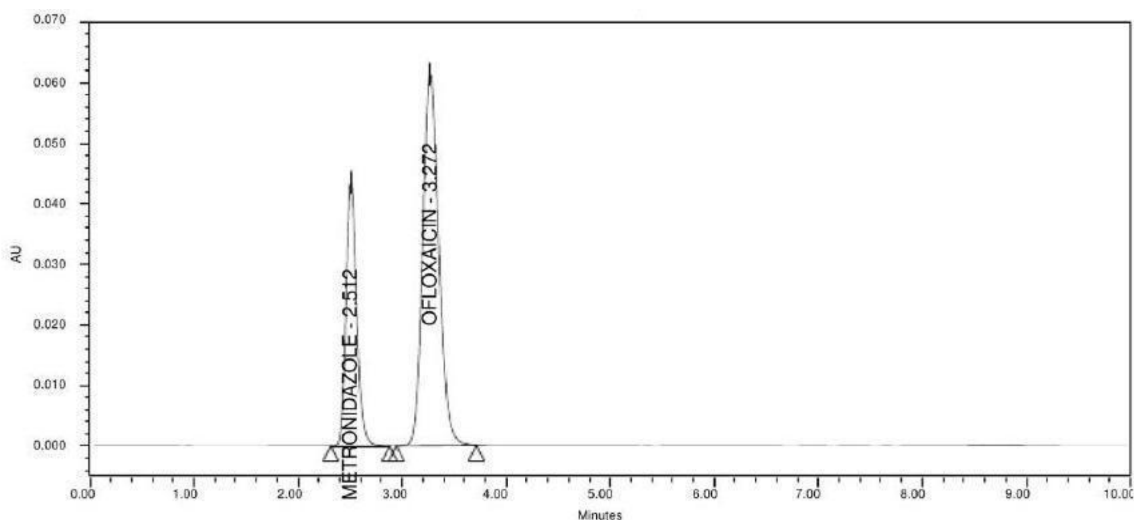
Figure 5 : Chromatograms of metronidazole and ofloxacin in base degradation



Peak Results

	Name	RT	Area	Height	SampleName	% Area	Resolution	USP Plate Count	USP Tailing
1	METRONIDAZOLE	2.420	212208	24141	PEROXIDE	14.99		1629.158485	1.259467
2	OFLOXACIN	2.917	443639	51446	PEROXIDE	31.35	2.179499	2540.958346	0.996040
3	UNKNOWN IMPURITY	5.246	759480	61266	PEROXIDE	53.66	8.307270	3336.319574	0.986506

Figure 6 : Chromatograms of metronidazole and ofloxacin in peroxide degradation



Peak Results

	Name	RT	Area	Height	% Area	Resolution	USP Plate Count	USP Tailing
1	METRONIDAZOLE	2.512	308563	43772	31.93		2959.562341	1.186521
2	OFLOXACIN	3.272	658985	61732	68.07	3.294852	2176.643523	1.163485

Figure 7 : Chromatograms of metronidazole and ofloxacin in water degradation

METHOD VALIDATION

Specificity study

Mobile phase along with placebo were injected to check the interference at the retention time of MET and OFL in the established chromatographic condition and no interference were observed at the designated Retention Time which was established by peak purity of the chromatogram by UV-Vis detector.

Stress studies

Acid, Alkaline, Peroxide and water degradation

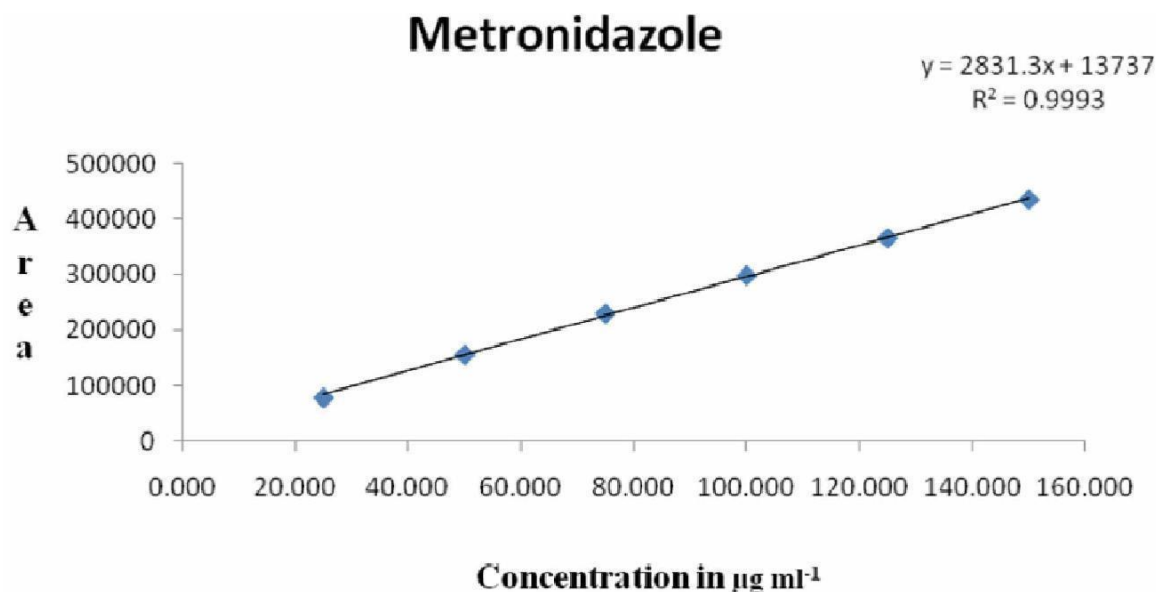


Figure 8 : Linearity chromatogram of metronidazole

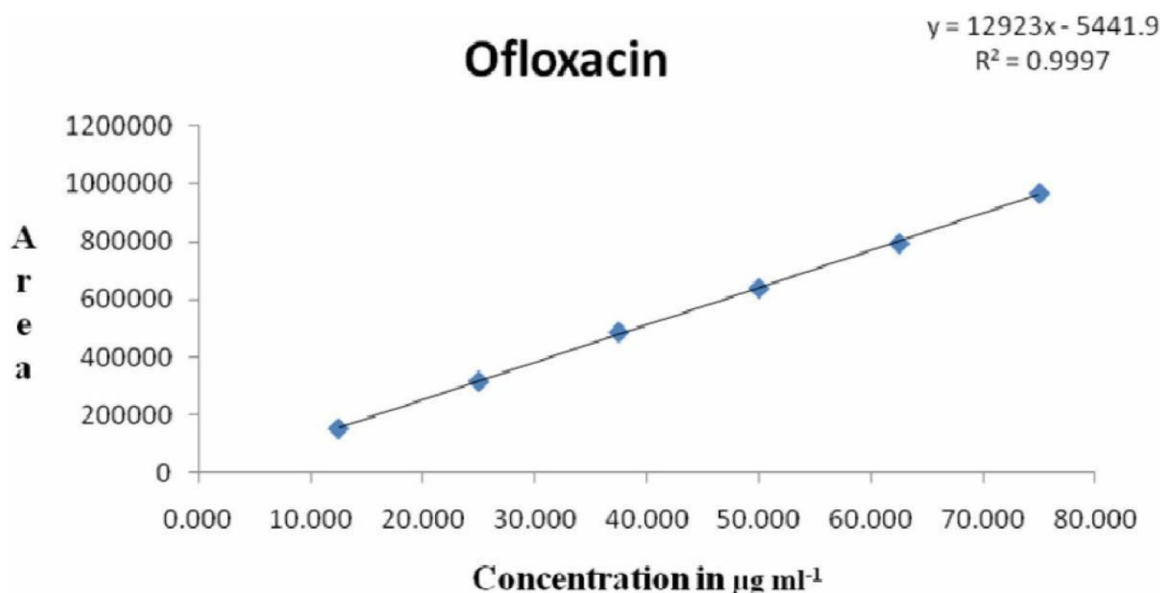


Figure 9 : Linearity chromatogram of ofloxacin

studies were conducted and MET and OFL were subjected to this condition. 1N HCl, 0.5N NaOH, 3% hydrogen peroxide and water at room temperature were used for stress testing studies. The samples are neutralized before injecting into the system for acid and alkaline samples. Oxidative and water samples were injected after proper dilution as such. Placebo and mobile phase were also subjected to same treatment as sample to check for interferences Figures 4, 5, 6 &7.

Precision

ICH describes precision as closeness of individual measure of analytes when the procedure is applied repeatedly to multiple times interday and intraday precision has been established in the method.

Accuracy

It was evaluated at three levels of 50%, 100% and 150% of test concentration by adding known amount of drug to placebo and extracting the sample. Three sets were prepared and analyzed.

Robustness

Varying conditions of wavelength and temperature were carried out as per ICH guidelines to estimate the effects on the method.

TABLE 1 : Performance calculations, detection characteristics precision and accuracy of the proposed method for metron-idazole and ofloxacin

Parameter	HPLC method for Metronidazole	HPLC method for Ofloxacin
Wavelength (nm)	289	289
Retention times (t) min	2.460	3.129
Linearity range (µg ml^{-1})	25-150	12-75
LOD (µg ml^{-1})	0.3795	1.15
LOQ (µg ml^{-1})	0.818	2.477
Regression equation	(y=bc+a)	(y=bc+a)
Slope (b)	2831.3	12923
Intercept (a)	13737	5441.9
Correlation coefficient(r^2)	0.9993	0.9997
Relative Standard deviation (%RSD)	0.22	0.78
Intermediate Precision (%RSD)	0.45	1.35

%RSD of five independent determinations

TABLE 2 : Results of linearity of sample

Metronidazole		Ofloxacin	
Conc(µg ml^{-1})	Area	Conc(µg ml^{-1})	Area
25.000	79725	12.500	153932
50.000	156831	25.000	317079
75.000	230829	37.500	486741
100.000	299097	50.000	639780
125.000	366823	62.500	794350
150.000	435557	75.000	967709

TABLE 3 : System precision and system suitability

S No	Metronidazole		Ofloxacin	
	RT	Area	RT	Area
1	2.483	308049	3.185	651416
2	2.512	309233	3.272	660050
3	2.483	306957	3.185	650199
4	2.512	308118	3.272	658915
5	2.512	307004	3.272	657213
6	2.512	304457	3.272	655581
Avg	2.502	307303	3.243	655562
Std Dev	0.0150	1628.16	0.0449	4001.55
%RSD	0.598	0.530	1.385	0.610

RESULTS AND DISCUSSIONS

Method development and optimization

Actual chromatographic conditions were established after number of preliminary experiments for selecting the proper mobile phase system. Different mobile phase systems were tested, and selection of the proper system depended on its ability to give good separation between the pure drugs and their possible degradation products. Acceptable separation was achieved on Hypersil BDS

TABLE 4 : Method precision

S No	Metronidazole		Ofloxacin	
	RT	Area	RT	Area
1	2.483	307660	3.185	654352
2	2.483	308029	3.185	653344
3	2.512	308754	3.272	663425
4	2.512	309229	3.272	663788
5	2.512	309229	3.272	663788
6	2.512	309229	3.272	664068
Avg	2.502333	308688.3	3.243	660460.8
Std Dev	0.014976	688.9794	0.044927	5136.247
%RSD	0.60	0.22	1.39	0.78

TABLE 5 : Ruggedness of metronidazole day 1 and day 2

S No	Name	RT	Area
1	Injection-1	2.483	307660
2	Injection-2	2.483	308029
3	Injection-3	2.512	308754
4	Injection-4	2.512	309229
5	Injection-5	2.512	309229
6	Injection-6	2.512	309229
7	Injection-7	2.423	309105
8	Injection-8	2.423	308659
9	Injectoion-9	2.423	305087
10	Injection-10	2.424	309097
11	Injection-11	2.426	309231
12	Injection-12	2.421	306056
	AVG	2.462833	308280.4
	STDEV	0.042488	1378.783
	%RSD	1.73	0.45

TABLE6 : Ruggedness of ofloxacin day 1 and day 2

S No	Name	RT	Area
1	Injection-1	3.185	654352
2	Injection-2	3.185	653344
3	Injection-3	3.272	663425
4	Injection-4	3.272	663788
5	Injection-5	3.272	663788
6	Injection-6	3.272	664068
7	Injection-7	2.987	648082
8	Injection-8	2.98	646054
9	Injectoion-9	2.987	646066
10	Injection-10	2.98	639780
11	Injection-11	2.988	643513
12	Injection-12	2.985	656066
	AVG	3.11375	653527.2
	STDEV	0.138375	8827.851
	%RSD	4.44	1.35

C_{18}, 150x4.6, 5µ using a mobile phase composed of Mix buffer and acetonitrile in the ration 90: 10. (pH5.0 ±0.1 adjusted by using Ortho-phosphoric acid): acetonitrile in ratio of (90:10 v/v %) pumped with a flow rate of 1.0 ml/min the column temperature was kept constant at 30ºC. under these chromatographic conditions, the run time sample was 10 min, and the retention times of MET and OFL 2.460 and 3.129 min. The representative chromatogram is shown as Figure 3.

Performance calculations, detection characteristics precision and accuracy of the proposed method for Metronidazole and Ofloxacin were reported in the TABLE 1.

System suitability

System suitability parameters like theoretical plates per meter, tailing factor, percentage relative standard deviation of area and retention time of twelve injections

were carried out and the values are well within the limits as shown in TABLES-3 & 4.

Linearity and sensitivity

A linear calibration plot of MET and OFL was constructed at nine point concentration levels 25-150 μgml^{-1} and 12-75 μgml^{-1} in duplicate. Average peak area of MET and OFL were plotted against respective concentrations and linear regression analysis was performed. Correlation coefficient was found to be r^2=0.9993 (n=6) and r^2=0.9997 respectively. Limit of detection (LOD) and limit of quantification (LOQ) values for Metronidazole was 0.3795 and 1.15 μgml^{-1} and for Ofloxacin was 0.818 and 2.477 μgml^{-1} respectively. The results were shown in Table- 2.

Precision

The precision of the assay method was evaluated for repeatability and intermediate precision. For intra-day precision and inter-day precision, the percentage relative standard deviation of MET and OFL was found to be 0.22% and 0.78% respectively. These values were well within the acceptable limit of 2%, as per USP. Result is given in (TABLES 5& 6).

Accuracy

Known amount of standard was spiked in 50%, 100%, 150% concentration in triplicate to test solution and recovery of drug was calculated. The accuracy of method was established at three concentration levels at 50, 100 and 150 μgml^{-1} of MET and 25, 50and 75 of OFL standards. The recoveries at three different concentrations were found to be within the range of 95. 0 to 105 % as per ICH guidelines. Mean % recovery (mean ± SD) was found to between 99.10 to 100. 91.

The results indicated that the recovery of CLOT in three different concentrations (TABLES 10 & 11).

Robustness

The robustness of assay method was studied by incorporating small but deliberate changes in the analytical method (wavelength and temperature) and also by observing the stability of the drugs for 24 hours at room temperature in the dilution solvent. In all the varied chromatographic conditions, there was no significant change in chromatographic parameters. Result is given in (TABLE 7).

STRESS STUDIES

Acid and alkali degradation studies

100mg of the MET and 50mg of OFL were weighed accurately and transferred to 100ml volumetric flasks containing 50ml 1N HCl and 50ml 0.5N NaOH and kept at room temperature for 24 hours. The samples were filtered and neutralized up to volumes with 1N HCl and 0.5N NaOH. Diluted 10ml of each of above solution to 100ml with diluent. 20 μl^{-1} of each of above solution was injected into chromatographic system and chromatograms were recorded. The results are given in Table- 8.

TABLE 7 : Robustness study of metronidazole and ofloxacin

S.N	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Met. W-289nm	2.485	307843	43641	32.10	---	2886.321421	1.116052
	Ofl. W-289nm	3.189	653520	66986	67.89	3.215413	2481.605214	1.095241
2	Met. W-287nm	2.481	308142	43645	32.12	---	2882.231025	1.114952
	Ofl. W-287nm	3.187	651201	66983	67.88	3.216842	2483.562143	1.095042
3	METTem-35°C	2.483	307065	43637	32.08	---	2879.135997	1.115818
	OFL Tem-35 °C	3.185	650028	66979	67.92	3.217907	2480.628302	1.094242
4	MET Tem-33°C	2.512	307997	43750	31.84	---	2956.501516	1.180324
	OFL Tem-33°C	3.272	659200	61723	68.16	3.296534	2171.930492	1.163936

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TABLE 8: Degradation study of metronidazole and ofloxacin

Stress conditions	Time in hours	Area		Assay of active		Deg%		Peak purity	
		MET	OFL	MET	OFL	MET	OFL	MET	OFL
Acid	24h	298012	599370	96.7	28.26	3.3	71.74	1.0	1.0
Base	24h	307415	614020	99.5	93.63	0.5	6.37	1.0	1.0
Peroxide	12h	212208	443639	68.9	67.65	31.1	32.35	1.0	1.0
Water	6 h	308563	658985	99.6	100.1	0.4	No Deg	1.0	1.0

Oxidation degradation

100mg of the MET and 50mg of OFL working standards were weighed accurately and transferred to 100ml volumetric flasks containing 3 % H_2O_2 and kept at room temperature for 12 hours. It was filtered. Diluted 10ml of solution to 100ml with diluent. 20 μl^{-1} of the was injected in to the chromatographic system and the chromatogram was recorded. The results are given in Table- 8.

Water degradation

Sufficient amount of MET and of OFL powder was transferred into petridish spread evenly for NMT 1mm thickness and kept inside hot air oven at 40^{0C} for 6 hours. Samples were collected at different time intervals and final dilution were done with the mobile phase and loaded into HPLC system. The results are given in TABLE 8.

Applicability of the method

MET and OFL were subjected to the analysis by the proposed method. The label claim percentage was

TABLE 9: Assay results of metronidazole and ofloxacin

Drug	Amount present/tablet	Amount Found /tablet	% of Assay
Metronidazole	100	100.22	100.22
Ofloxacin	50	50.31	100.62

TABLE 10 : Accuracy data (Triplicate values at 50,100 &150 percent levels) of metronidazole

S.No	Spike level	Peak area	Amount Added (μgml^{-1})	Amount Recovered (μgml^{-1})	%Recovery	Avg	%RSD
1	50%	156831	50	50.72	101.44	100.91	0.76
		156529	50	50.63	101.25		
		154654	50	50.02	100.04		
2	100%	309105	100	99.97	99.97	99.49	0.72
		308659	100	99.83	99.83		
		305087	100	98.67	98.67		
3	150%	455557	150	147.33	98.22	99.56	1.17
		464444	150	150.21	100.14		
		465300	150	150.48	100.32		

TABLE 11 : Accuracy data (Triplicate values at 50,100 &150 percent levels) of ofloxacin

S.No	Spike level	Peak area	Amount Added (μgml^{-1})	Amount Recovered (μgml^{-1})	%Recovery	Avg	%RSD
1	50%	328005	25	24.91	99.64	99.10	0.73
		323526	25	24.57	98.28		
		327141	25	24.85	99.38		
2	100%	648082	50	49.22	98.43	98.23	0.18
		646054	50	49.07	98.13		
		646066	50	49.07	98.13		
3	150%	987709	75	75.01	100.01	99.23	0.69
		976735	75	74.18	98.90		
		975449	75	74.08	98.77		

99.99 ±0.07%. This acceptable value indicates the applicable of the method for the routine quality control of MET and OFL without interference from the excipients and degradation products. The results are given in TABLE 8.

CONCLUSION

A simple and precise stability indicating HPLC method has been developed for determination of MET and OFL in tablet dosage form. The %RSD values in precision, recovery studies, robustness and ruggedness studies was found less than 2.0% which indicates that the method is precise, accurate and robust. Limit of detection (LOD) and limit of quantification (LOQ) values for Metronidazole was 0.3795 and 1.15 µg/ml and for Ofloxacin was 0.818 and 2.477 µg/ml respectively. This indicates that the method is sensitive for the determination of lower concentration of the both the drugs. The % assay was found to be well within the acceptable limit. In degradation studies it was found that MET was more sensitive to peroxide degradation, while OFL showed more degradation in acidic conditions. The peaks of the degradants in each condition were well separated from main peaks. Purity plot confirmed that there is no interference of any degradants at the retention time of the main peaks indicates that the developed method is stability indicating. The study showed that the drug is stable for the water degradation condition where as least degraded in base (0.5% & 6.37). Moderately degraded in peroxide (31.1 & 32.5%) condition, highly degraded in the acid (3.3 &71.74) and no degradation in water conditions. The proposed method can be used as an alternative method for the analysis of MET and OFL in its dosage forms.

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