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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF OFLOXACIN AND ORNIDAZOLE IN COMBINED DOSAGE FORM USING SIMULTANEOUS EQUATION METHOD

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ABSTRACT

A simple, precise UV spectrophotometric method has been developed for determination of ofloxacin and ornidazole in its tablet dosage form by using distilled water as a solvent. The absorption maximum (λ max) was observed at 287 nm and 318 nm respectively. The method obeys beer lamberts linearity in the range of 2-12 ug/ml for ofloxacin and 5-30 ug/ml for ornidazole with a value of correlation coefficient (R^2) 0.999 for both the drugs at their respective wavelength. Assay was performed on two different brands of marketed formulation. Precision studies were carried out as per ICH guidelines Q2 $R_1$and were found to be within the limits NMT 2 % concluding that the method is precise. The proposed method can be used for routine purity testing in bulk and combined dosage form.

KEYWORDS: Ofloxacin, Ornidazole, OFX, ORN, simultaneous equation method, UV spectrophotometry.

INTRODUCTION

Ofloxacin is a second generation fluoroquinolone being a broader-spectrum analog of norfloxacin, useful in treatment of various bacterial infections like pneumonia, UTIs etc. Chemically it is 7-fluoro-2-methyl-6(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo (7.3.1.0) (5,13) trideca-5(13),6.8,11 tetraene-11 carboxylic acid. It exerts its bacterial effect on susceptible microorganisms by entering the bacterial cell and inhibits DNA gyrase, a type

2 topoisomerase and topoisomerase 4 an enzyme that seperates replicated DNA, thereby inhibiting bacterial cell division.

Ornidazole belongs to nitroimidazole class of drugs namely tissue amoebicides. Chemically it is 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol. It causes damage to the DNA strands or inhibits their synthesis. It is used in treatment of intestinal amoebiasis, hepatic amoebiasis.

The combination is accepted and prescribed worldwide for broader spectrum of antibacterial activity. Ofloxacin and ornidazole are official in U.S.P and I.P. Literature review reveals that ofloxacin is estimated by spectrophotometry, H.P.L.C and spectroflourimetry. Both these drugs are available in combined tablet dosage form (200 mg and 500 mg) as an antiamoebic.
Simultaneous equation method can be performed when a sample contains two absorbing drugs (X and Y) each of which absorb at their absorption maxima. In this method two equations are formed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance of X and Y.

The equations are: $A_1=ax_1Cx+ay_1Cy$
$A_2=ax_2Cx+ay_2C_y$
Where ax_1 and ax_2 =absorbtivity values of compound X at λ_1
and λ_2 respectively
ay_1 and ay_2=absorbtivity values of compound Y at λ_1 and λ_2
respectively
Cx and Cy =concentration of components X and Y in diluted
Sample
A_1 and A_2 =absorbance of diluted sample at λ_1 and λ_2

Respectively

The main objective of this study was to develop simple precise newer analytical method for simultaneous estimation of ofloxacin and ornidazole in bulk and combined tablet dosage form which is more economical and validate the same as per ICH guidelines Q2 R1.

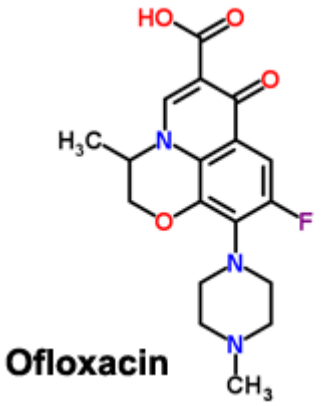

Fig 1(a): Chemical structure of ofloxacin.

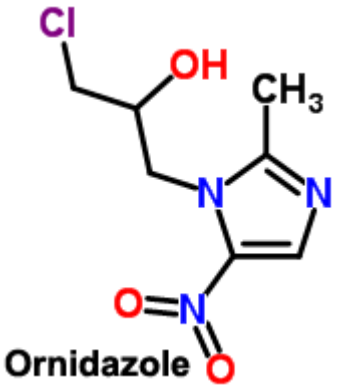

Fig 1(b): Chemical structure of ornidazole

MATERIALS AND METHODS

Instrumentation and apparatus

UV method was performed on Shimadzu UV-2700 UV-VIS spectrophotometer with UV probe ver 2.51 software and all weighing was done on Wensar Electronic Weighing Balance, MAB 220, ISO 9001:2001 at room temperature.

Reagents and Chemicals

Analytically pure ofloxacin and ornidazole were kindly provided by Vaishali Pharma Pvt Ltd Mumbai, India and Cadila Healthcare Limited Kundaim Goa respectively as gift samples. The tablet formulation of ofloxacin 200 mg and ornidazole 500 mg was procured from local market.

METHOD DEVELOPMENT

Preparation of standard stock solution

Standard stock solutions of OFX and ORN were prepared by dissolving 100 mg in 100 ml of volumetric flask of both the drugs separately with distilled water to give 1000 ug/ml concentration. From the above standard stock solution, 10 ml each of solution was transferred in 100 ml volumetric flask separately to give 100 ug/ml concentrations as working standard of OFX and ORN respectively.

Selection of Analytical Wavelength

Solutions of 10 ug/ml were prepared from working standard solution and were analyzed in the UV range of 200-400 nm against distilled water as a blank. The absorption maxima of OFX and ORN were found to be 287 nm and 318 nm respectively.

Preparation of Sample Solution from Standard Stock Solution

The sample solutions were prepared from standard stock solution by aliquots dilution of working standard solution.

Calibration Curve

The drug solutions of OFX were prepared from 2-12 ug/ml and ORN was prepared from 5-30 ug/ml from their respective working standard solutions each having the concentration of 100 ug/ml. The volume was made up to the mark in 10 ml volumetric flask with distilled water. Absorbance for each drug was measured at 287 nm and 318 nm respectively. Calibration curve was plotted against absorbance v/s concentration. Absorptivities were calculated by taking a mean of three determinations for each concentration of OFX and ORN.

Methodology

Standard drug solutions of OFX and ORN were prepared from their respective working standard solutions each having a concentration of 10 ug/ml. The solutions were scanned in the UV range of 200-400 nm against distilled water as blank runs. The overlain spectra of both the drugs were recorded and OFX showed an absorbance peak at 287 nm whereas ORN showed an absorbance peak at 318 nm.

Due to difference in absorbance maxima, and no interference with each other, both the concentration of drugs can be determined through simultaneous equation method.

The absorbance and absorptivity values at particular wavelength were calculated and substituted in the following equation to get concentration of each drug.

$Cx=(A_2ay_1-A_1ay_2)/(ay_1ax_2-ay_2ax_1)$

$Cy=(A_1ax_2-A_2ax_1)/(ay_1ax_2-ay_2ax_1)$

A1 A2 = absorptivities of mixture at λ_1 and λ_2 respectively

ax1= absorptivity of OFX at 287 nm

ax2= absorptivity of ORN at 318 nm

ay1= absorptivity of OFX at 287 nm

ay2= absorptivity of ORN at 318 nm

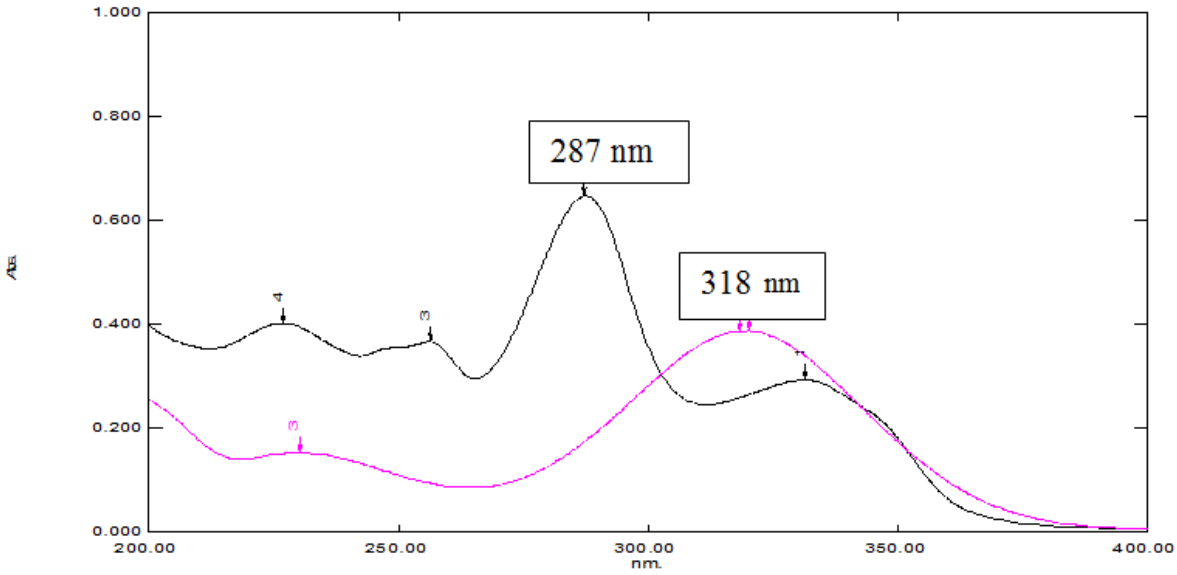

Fig. 3: Overlain spectra of OFX and ORN in distilled water.

Validation Procedure

The main objective of validation of analytical procedure is to determine whether the developed method is intended for use. The various parameters that were validated for this method are linearity, range, accuracy and precision according to ICH guideline Q2 R1.

Linearity and Range

The linearity range was prepared from working standard solutions from 2-12 ug/ml and 5-30 ug/ml for OFX and ORN respectively. Calibration curve was plotted against concentration v/s absorbance to obtain regression equation. The correlation coefficient for OFX and ORN was found to be 0.999 at each other's wavelength respectively.

Table 1: Data of linearity range of OFX in distilled water.

Concentration (ug/ml)	Absorbance at 287 nm	Absorbance at 318 nm
2	0.089	0.035
4	0.183	0.070
6	0.282	0.113
8	0.378	0.155
10	0.460	0.184
12	0.561	0.218

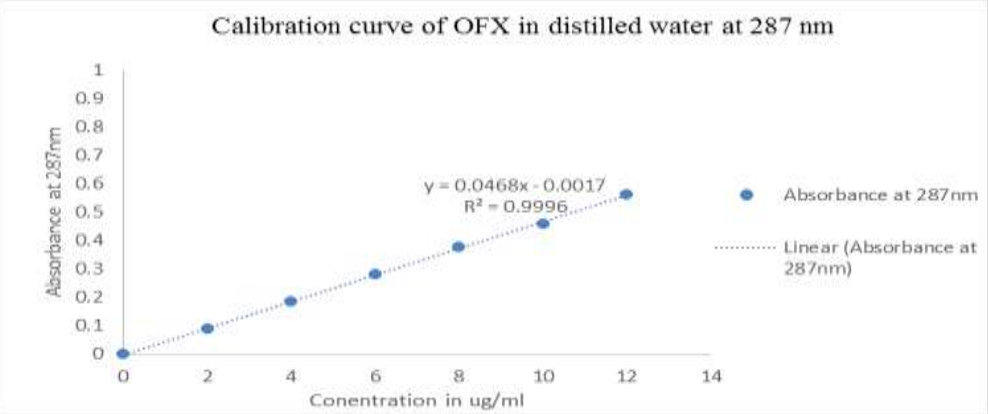

Fig.4: Calibration curve of OFX at 287 nm in distilled water.

Table 2: Data of linearity range of ORN in distilled water.

Concentration(ug/ml)	Absorbance at 287 nm	Absorbance at 318 nm
5	0.077	0.166
10	0.144	0.313
15	0.219	0.476
20	0.291	0.630
25	0.356	0.773
30	0.412	0.907

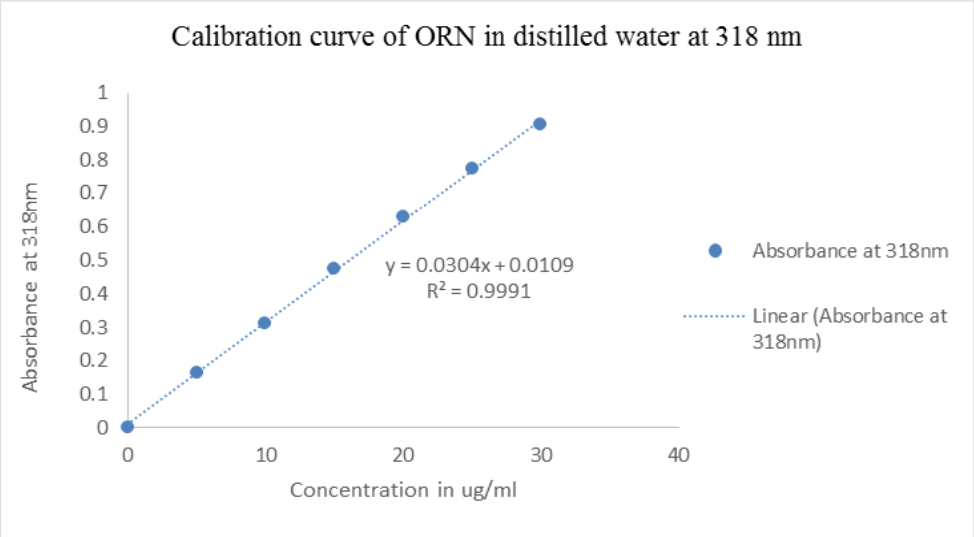

Fig. 5: Calibration curve of ORN at 318 nm in distilled water.

Accuracy (Recovery): Recovery studies were carried out to evaluate accuracy of the method. Accuracy of the method was carried out on two different brands of OFX 200 mg and ORN 500 mg tablets at three different spiked levels (80%, 100%, and 120%). At each level three determination (n=3) were obtained. The amount recovery and the values of percent recovery were calculated as shown in table 3 below.

Table 3: Data of accuracy study of OFX (Brand name-OFLOX-OZ tablets).

Amount of sample in tablet	Level of addition	Amount of pure drug spiked (ug/ml)	Total concentration (ug/ml)	Abs at 287 nm	Abs at 318 nm	Total content found (ug/ml)	Amount of standard drug recovered(ug/ml)	Mean recovery %(n=3)
8.57ug/ml	80	6.8	15.3	0.834	0.570	15.126	6.556	95.5
		6.8		0.834	0.570	15.126	6.556	
		6.8		0.829	0.574	14.945	6.375	
	100	8.5	17	0.919	0.613	16.852	8.282	95.1
		8.5		0.910	0.599	16.784	8.034	
		8.5		0.902	0.603	16.524	7.954	
	120	10.2	18.7	1.012	0.656	18.789	10.219	98.7
		10.2		0.999	0.648	18.542	9.972	
		10.2		1.003	0.653	18.587	10.017	

Table 4: Data of accuracy study of ORN (Brand name-OFLOX-OZ tablets).

Amount of sample in tablet	Level of addition	Amount of pure drug spiked (ug/ml)	Total concentration (ug/ml)	Abs (287 nm) A1	Abs (318 nm) A2	Total content	Amount of standard drug recovered (ug/ml)	Mean recovery% (n=3)
	80	16.00	36.00	0.837	1.218	34.468	14.468	97.4
				0.819	1.218	34.745	14.468	
				0.919	1.334	37.838	17.838	
20.00ug/ml	100	20.00	40.00	0.899	1.370	39.422	19.422	103.0
				0.926	1.411	40.600	20.600	
				0.973	1.462	41.821	21.821	
	120	24.00	44.00	0.985	1.499	43.113	23.113	103.1
				1.007	1.565	45.340	25.34	
				1.014	1.581	45.840	25.824	

Table 5: Data of accuracy study of OFX (Brand name-OFLOREN-OZ tablets).

Amount of sample in tablet tablet of sam	Level of addition	Amount of pure drug spiked (ug/ml)	Total concentration (ug/ml)	Abs (287 nm) A1	Abs (318 nm) A2	Total content	Amount of standard drug recovered (ug/ml)	Mean recovery % (n=3)
7.889 ug/ml	80	6.3	14.1	0.790	0.568	13.986	6.097	95.7
				0.795	0.573	14.058	6.169	
				0.777	0.561	13.727	5.839	
	100	7.8	15.6	0.859	0.593	15.508	7.619	99.6
				0.870	0.598	15.738	7.849	
				0.872	0.603	15.730	7.841	
	120	9.4	17.2	0.964	0.641	17.701	9.812	102.6
				0.951	0.632	17.467	9.578	
				0.950	0.632	17.440	9.551	

Table 6: Data of accuracy study of ORN (Brand name-OFLOREN-OZ tablets).

Amount of sample in tablet (ug/ml)	Level of addition	Amount of pure drug spiked (ug/ml)	Total concentration (ug/ml)	Abs (287 nm) A1	Abs (318 nm) A2	Total content (ug/ml)	Amount of standard drug recovered (ug/ml)	Mean recovery %(n=3)
20.751 ug/ml	80	16.6	37.3	0.897	1.338	38.209	17.458	102.8
				0.886	1.327	37.951	17.2	
				0.873	1.306	37.334	16.583	
	100	20.7	41.4	0.953	1.463	42.206	21.455	100.6
				0.935	1.438	41.511	20.75	
				0.923	1.421	41.035	20.284	
	120	24.9	45.6	0.969	1.538	44.874	24.123	96.6
				0.972	1.541	44.945	24.194	
				0.964	1.530	44.640	23.889	

Method Precision

a) Repeatibility

Repeatibility study was carried out in the same manner on OFLOX-OZ and OFLOREN-OZ tablets. Aliquots of 2 ml each of working sample solution were transferred in six 10 ml volumetric flask and were diluted up to the mark with distilled water (8 ug/ml of OFX and 20 ug/ml of ORN). The absorbance's were recorded at 287 nm and 318 nm against blank sample. The data of repeatability study is shown in table 7

Table 7: Data of repeatability study (OFLOX-OZ tablets).

Sr. No	Absorbance (287 nm)	Absorbance (318 nm)	Concentration (ug/ml) OFX	Concentration (ug/ml) ORN
1	0.689	0.803	8.45	20.61
2	0.681	0.793	8.36	20.35
3	0.689	0.799	8.44	20.49
4	0.680	0.791	8.35	20.28
5	0.689	0.803	8.45	20.61
6	0.706	0.822	8.67	21.09
		Mean % content	98.59	102.85
		SD	0.115	0.287
		% RSD	1.36	1.40

Table 8: Data of repeatability study (OFLOREN-OZ tablets).

Sr. No	Absorbance (287 nm)	Absorbance (318 nm)	Concentration (ug/ml) OFX	Concentration (ug/ml) ORN
1	0.665	0.799	7.86	20.82
2	0.670	0.805	7.92	20.98
3	0.666	0.802	7.85	21.02
4	0.675	0.809	8.00	21.06
5	0.679	0.802	8.19	20.79
6	0.670	0.805	7.92	20.98
		Mean % content	100.50	100.91
		SD	0.126	0.110
		% RSD	1.59	0.53

b) Method Precision

The intermediate precision study was carried out in the same manner on OFLOX-OZ and OFLOREN-OZ tablets. The intra-day and inter-day precision was carried by analyzing the corresponding responses 6 times on the same day and on two different days (8 ug/ml of OFX and 20 ug/ml of |ORN).

Aliquotes of 2 ml each of working sample solution was withdrawn and transferred to six 10 ml volumetric flask which were diluted up to the mark with distilled water. The solutions

were scanned at their respective wavelength against blank sample. The results were reported in terms of percent relative standard deviation (% RSD) in table 9.

Table 9: Data showing Intermediate Precision (OFLOX-OZ tablets).

Sample	Abs		Abs		Concentration (ug/ml)				Inter-day	
	Day1		Day2		Day1		Day 2			
	287 nm	318 nm	287 nm	318 nm	OFX	ORN	OFX	ORN	OFX	ORN
1	0.689	0.803	0.660	0.789	8.45	20.61	7.85	20.51		
2	0.681	0.793	0.644	0.773	8.36	20.35	7.62	20.14		
3	0.689	0.799	0.649	0.775	8.44	20.49	7.73	20.14		
4	0.680	0.791	0.668	0.796	8.35	20.28	7.98	20.66		
5	0.689	0.803	0.646	0.776	8.45	20.61	7.64	20.22		
6	0.706	0.822	0.652	0.781	8.67	21.09	7.74	20.32		
Mean % content					98.59	102.85	90.54	101.65	94.56	102.25
% RSD					1.36	1.40	1.75	1.04	1.03	1.22

Table 10: Data showing Intermediate Precision (OFLOREN-OZ tablets).

Sample	Abs		Abs		Concentration (ug/ml)				Inter-day	
	Day1		Day2		Day1		Day 2			
	287 nm	318 nm	287 nm	318 nm	OFX	ORN	OFX	ORN	OFX	ORN
1	0.665	0.799	0.654	0.788	7.86	20.82	7.70	20.57		
2	0.670	0.805	0.654	0.793	7.92	20.98	7.64	20.76		
3	0.666	0.802	0.648	0.779	7.85	21.02	7.65	20.31		
4	0.675	0.809	0.658	0.789	8.00	21.06	7.80	20.54		
5	0.679	0.802	0.665	0.803	8.19	20.79	7.81	20.98		
6	0.670	0.805	0.668	0.803	7.92	20.98	7.89	20.93		
Mean % content					100.50	100.91	98.22	99.66	99.36	100.28
% RSD					1.59	0.53	1.29	1.24	1.44	0.88

Sample Solution Stability

Sample solution containing 8 ug/ml and 20 ug/ml of OFX and ORN respectively was prepared freshly and stored for 3 hours at room temperature. The solutions were scanned at 287 nm and 318 nm and the results (% RSD) obtained were compared with the results of the freshly prepared solution. The results are tabulated in table no 11

Table 11: Stability profile of sample solution (OFLOX-OZ tablets).

Time in hours	Absorbance at 287 nm	Absorbance at 318 nm
Freshly prepared	0.675	0.786
1	0.662	0.770
2	0.660	0.768
3	0.647	0.754
Mean	0.661	0.769
% RSD	1.73	1.7

Table 12: Stability profile of sample solution (OFLOREN-OZ tablets).

Time in hours	Absorbance at 278 nm	Absorbance at 318 nm
Freshly prepared	0.612	0.752
After 1hr	0.625	0.765
After 2hrs	0.624	0.766
After 3hrs	0.606	0.755
Mean	0.616	0.759
% RSD	1.51	0.93

Assay of Formulation

Assay was performed in same manner on two different brands of OFX 200 mg and ORN 500 mg tablets i:e OFLOX-OZ and OFLOREN-OZ .

Procedure: Five tablets were weighed to get the average weight of each tablet. A sample of powdered tablets equivalent to100 mg of ORN was transferred in 100 ml of volumetric flask. About 50 ml of water was added to the flask and was kept for sonication for 30 minutes and volume was made up to the mark with same solvent to give 1000 ug/ml stock solution.

The contents were then filtered through Whatmann filter paper (No.45). From this 10 ml was withdrawn and transferred to 100 ml volumetric flask and was made it up to the mark to give working standard solution of 100 ug/ml.

From this working standard solution, 2 ml was transferred in a series of six 10 ml volumetric flask. The same solvent was added up to the mark to give solutions containing 8 ug/ml and 20 ug/ml of OFX and ORN respectively.

The solutions were scanned at 287 nm and 318 nm and absorbances A_1 and A_2 were noted down. These values along with absorptivity values were substituted in the equations to give concentration of each component denoted as Cx and Cy for OFX and ORN respectively.

Table 13: Assay of Formulation.

Brand name	OFLOX-OZ tablets		OFLOREN-OZ tablets	
	OFX	ORN	OFX	ORN
Label claim (mg)	200mg	500mg	200mg	500mg
Drug found (mg)	214.2mg	500mg	197.2mg	518mg
% RSD	1.36	1.40	1.59	0.53
Assay	107.1	100.0	98.6	103.6

RESULT TABLE

Table 14: Results of validation parameters of OFX and ORN in distilled water.

Parameters	OFX	ORN	OFX	ORN
Range (ug/ml)	2-12	5-30	2-12	5-30
Linearity (ug/ml)	2-12	5-30	2-12	5-30
Regression coefficient(R^2)	0.999	0.999	0.999	0.999
Assay (n=6)	107.1	100.0	98.6	103.6
Recovery (n=3)	95-99	98-103	95-102	96-102
Intra-day (repeatability)	1.36	1.40	1.59	0.53
Inter-day (intermediate)	1.03	1.22	1.44	0.88

RESULTS AND DISCUSSION

The UV study was carried out to determine OFX and ORN by simultaneous equation method in combined dosage form. The objective of this method was to develop a simple precise and reproducible method to analyse both the drugs for routine use.

The method was developed using distilled water as a solvent. It involved formation of simultaneous equations using 287 nm and 318 nm as the wavelengths. (Absorption maxima of both the drugs).The absorptivity values (A 1% 1cm) were calculated at both the wavelengths (287 nm and 318 nm).

The concentration of each component was determined by using following simultaneous equations: Two equations were constructed based upon the fact that at λ_1 and λ_2 the absorbances of the mixture is the sum of individual absorbances of X and Y.

$Cx = (A_2ay_1\text{-}A_1ay_2)/(ay_1ax_2\text{-}ay_2ax_1)$

$Cy = (A_1ax_2\text{-}A_2ax_1)/(ay_1ax_2\text{-}ay_2ax_1)$

Cx is the concentration of OFX and Cy is the concentration of ORN.

The linearity range that was observed for OFX was 2-12ug/ml and ORN was 5-25ug/ml. The regression coefficient was 0.999 for both OFX and ORN at 287nm and 318nm respectively.

The precision studies showed insignificant variations in results, which demonstrated that the method was reproducible with

%RSD (Repeatability study): 1.36% and 1.40% for OFX and ORN respectively.

% RSD(Intermediate precision):1.03% and 1.22% for OFX and ORN respectively.

CONCLUSION

UV Spectrophotometric method was developed for the simultaneous estimation of OFX and ORN.

An attempt was made to develop a simple, accurate, precise and economical simultaneous equation method for quantitative determination of OFX and ORN in pharmaceutical tablet dosage form.

The optimized method was validated according to ICH guidelines Q2 R1.

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