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Simultaneous Determination of Ofloxacin and Ornidazole in Tablets by Spectrophotometry and Reverse Phase HPLC

CN Nalini¹, S Ramachandran², K Kavitha³, Harikrishna⁴

¹Dept of Pharmaceutical Chemistry, CL Baid Metha College of Pharmacy, Thoraipakkam, Chennai 600096, (INDIA)

²Dept Pharmaceutical Chemistry¹, Mohamed Sathak A J College of Pharmacy, Shollinganallur, Chennai 600119, (INDIA)

³Anna University of Technology, Tiruchi.

⁴C L Baid Metha College of Pharmacy, Thoraipakkam, Chennai 600096,

ABSTRACT

The analytical work comprise of simple, precise, rapid, sensitive and accurate methods for the simultaneous estimation of Ofloxacin and Ornidazole in combined dosage form. The methods used for the estimation were- Simultaneous equation method, Multicomponent mode of analysis, reverse phase HPLC. From the above analysis it was noted that the three methods of analysis were simple, specific, accurate and precise and can be employed in the routine analytical work. HPLC is the most reliable method since the RSD values are low when compares to the other two methods. But for economical reasons spectrophotometric methods can be employed for regular quantitative works.

Keywords: Ofloxacin, Ornidazole, Reverse Phase HPLC, Simultaneous equation method, Multicomponent mode of analysis.

**Corresponding author*

INTRODUCTION

Ofloxacin [1] is a fluorinated 4-quinilone which is effective against several types of bacteria that tend to be resistant to other commonly used antibiotics. It has an in vitro activity against a broad spectrum of gram positive and gram negative and anaerobic bacteria. It exerts its bacterial effect on susceptible microorganisms by entering the bacterial cell and inhibiting a chemical called DNA gyrase which is involved in the production of genetic material DNA. It is indicated for the treatment of genitourinary infections, respiratory infections, gastro intestinal infections.

Ornidazole [1] is a 5 nitro imidazole which is active against protozoa and anaerobic bacteria. It acts by damage of DNA strands or inhibition of their synthesis. It is indicated for the treatment of several intestinal amoebiasis, hepatic amoebiasis.

The combination of Ofloxacin and Ornidazole in a fixed dose combination is introduced in India which is having better therapeutics profile and less or negligible side effects. Its composition is Each film coated tablet contains Ofloxacin 200mg and Ornidazole 500mg. Both the drug has the category of antibacterial. Ofloxacin and Ornidazole [2, 3] are soluble in water.

Simultaneous analysis may be done when a sample contains two or more components. The components are estimated simultaneously in a single analysis by the following methods- Simultaneous equation method, absorbance ratio method, derivative spectrophotometry, difference spectrophotometry, chemical derivatisation, multicomponent mode of analysis. In simultaneously equation method sample containing two absorbing drugs (X and Y) each of which absorbing at the λ_{\max} of the other may be determined. In this method two equations are constructed 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 two equations are

$$A_1 = ax_1C_1 + ay_1C_2$$

$$A_2 = ax_2C_1 + ay_2C_2$$

Where ax_1 and ax_2 = absorbtivity values of compound X at λ_1 and λ_2 respectively

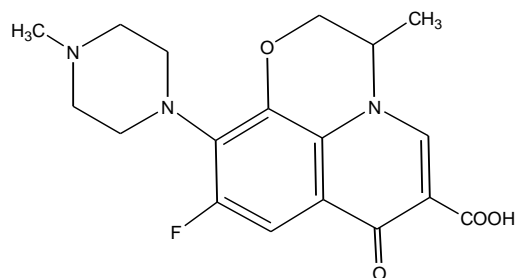
ay_1 and ay_2 = absorbtivity values of compound Y at λ_1 and λ_2 respectively

C_1 and C_2 = concentration of components X and Y in diluted sample

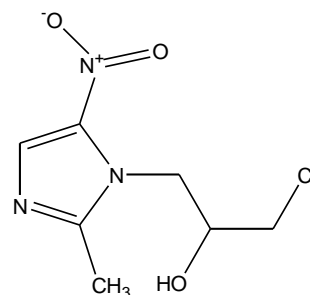
A_1 and A_2 = absorbance of diluted sample at λ_1 and λ_2 respectively.

In multicomponent mode of analysis [4], five mixed standard and sample solutions were prepared and scanned using sampling points of the drugs between 200-400nm in the multicomponent mode of spectrophotometer. The spectral data obtained from these scans are used to determine the concentration of the drugs present in the given formulations. High performance liquid chromatography [5] is the fastest growing analytical technique for the analysis of drugs. It was first developed by Kirkland and Huber. Chromatographic separation in

HPLC [6] is the result of specific interactions between sample molecules with both the stationary and mobile phases. HPLC is a suitable technique for the analysis of compounds with a wide range of polarities, high molecular weights and those that are thermally unstable or have a tendency to ionise in solution. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage form and biological fluids.



Ofloxacin



Ornidazole

MATERIALS AND METHODS

Experimental Section

In the study two different brands of combination tablets containing Ofloxacin and Ornidazole were subjected to the following methods of analysis for quantitative estimation using reference standards (Ofloxacin and Ornidazole)

1. Spectrophotometry
 - a. Simultaneous equation method
 - b. Multicomponent mode of analysis
2. Reverse Phase High Performance Liquid Chromatography

The validation of the assay procedure was carried out using the following parameters such as specificity, accuracy, precision, repeatability, limit of detection, limit of quantitation, linearity, range [7, 8, 9].

Spectrophotometry

Materials

Instrumentation Single pan balance Shimadzu Libror AEG 220

UV Visible spectrophotometer- Shimadzu UV 1601, matched with quartz cells corresponding to 1cm path length.

Reagents Used- 0.1N Hydrochloric acid

Reference standard- Ofloxacin and Ornidazole

Tablets used-

- i. Zenflex OZ (200mg of Ofloxacin and 500mg of Ornidazole)
B.No. 020090, Mfd Jun 2002, Exp May 2004
Manufacturer Mankind Pharma, Meerut
- ii. O2 (200mg of Ofloxacin and 500mg of Ornidazole)
B.No. 1F, Mfd Jun 2002, Exp May 2004
Manufacturer Medley Pharmaceuticals, Mumbai.

Establishment of various parameters

1. Absorption maximum
Concentration - 1000 μ g/ml of individual concentration of Ofloxacin and Ornidazole
Reagent 0.1N Hydrochloric acid
Wavelength used 200-400nm
 λ_{max} for Ofloxacin- 294nm and λ_{max} for Ornidazole – 277nm
2. Beers law concentration range
The stocks solution used individually were 2-30 μ g/ml for Ofloxacin and 5-75 μ g/ml for Ornidazole
Reagent 0.1N Hydrochloric acid
Wavelength used 200-400nm
From the graphs it was found that Ofloxacin and Ornidazole obeys beer law. The regression analysis was carried out for the calibration graphs to find out correlation coefficients, y intercept and slope of the regression line which estimates the degree of linearity

Simultaneous Equation Method

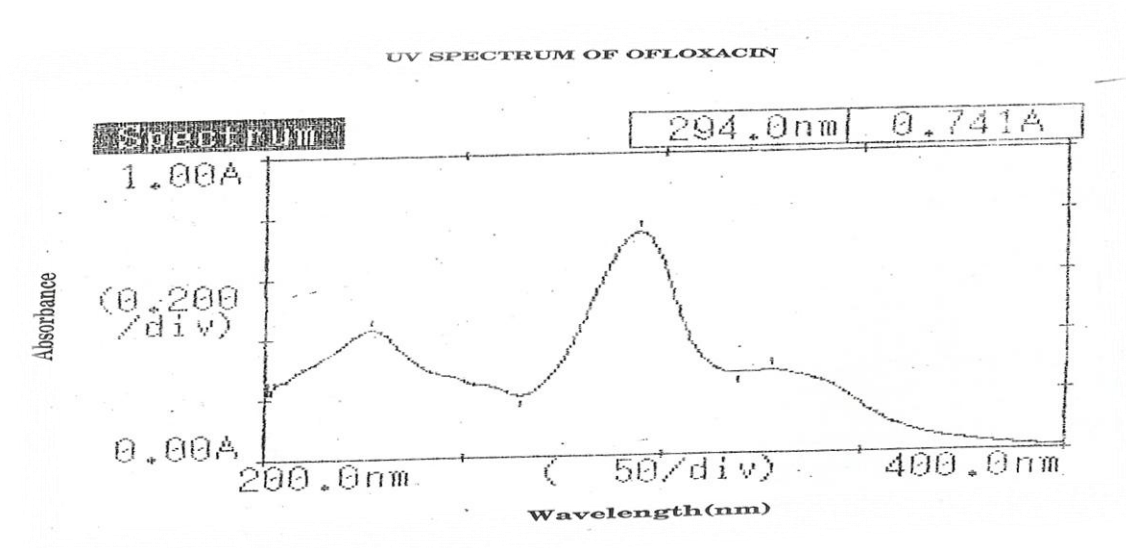


Figure 1: uv spectrum of Ofloxacin- λ_{max} -294nm

The absorption maximum λ_{max} were observed at 294 and 277nm for Ofloxacin and Ornidazole respectively. The data regarding the absorption maxima are depicted in Figure 1 and Figure 2. Ofloxacin showed linearity in the concentration of 2-20 μ g/ml and Ornidazole showed linearity in the concentration of 5-50 μ g/ml. The data regarding the calibration curves are given graphically in figure 3 and figure 4. Absorptivity values for Ofloxacin and Ornidazole were calculated.

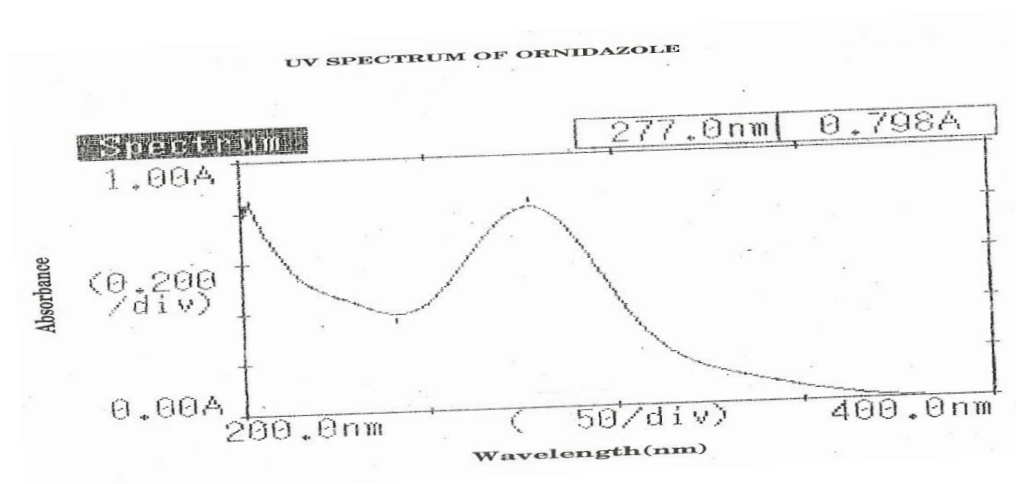


Figure 2: UV SPECTRUM OF ORNIDAZOLE- λ_{max} 277nm

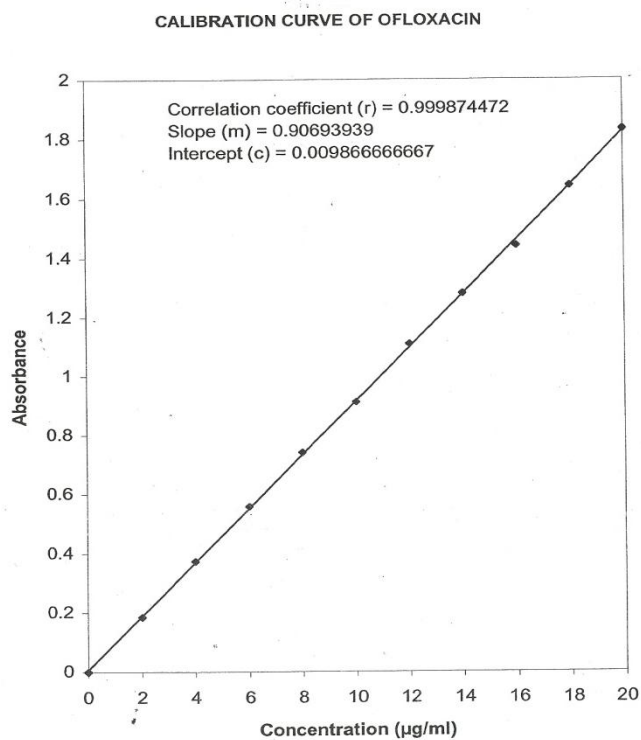


Figure 3: CALIBRATION CURVE OF OFLOXACIN

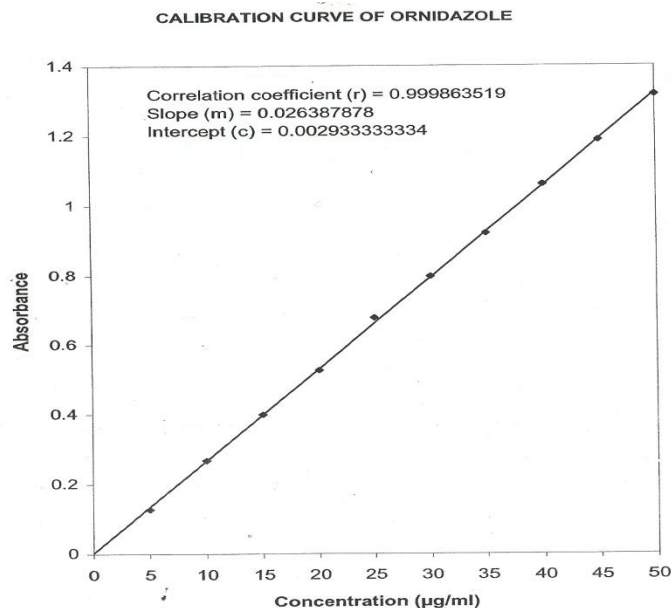


Figure 4: CALIBRATION CURVE OF ORNIDAZOLE

Table 1 QUANTITATIVE ESTIMATION AND THE STATISTICAL DATA- SIMULTANEOUS EQUATION METHOD

Tablet sample	Label claim (mg/tablet)	Amount Present(mg/t ablet)	% label claim (%w/w)	%deviation	Standard deviation (SD)	Relative Standard Deviation (RSD)	Standard error of mean (SE)
Tablet A							
OFX	200	199.01	99.51	-0.49	0.1989	0.1999	0.0812
OZ	500	504.70	100.94	+0.94	0.4718	0.4674	0.1926
Tablet B							
OFX	200	197.00	98.50	-1.5	0.1951	0.1981	0.0796
OZ	500	500.24	100.05	+0.05	0.4518	0.4516	0.1844

Each value is a mean of six readings. OFX- Ofloxacin, OZ- Ornidazole Tablet A- Zenflox-oz Tablet B- OZ

Table 2--RECOVERY STUDIES -SIMULTANEOUS EQUATION METHOD

Sample Component	Tablet A					Tablet B			
	Amt Present in the pre-analysed sample (µg/ml)	Amt std added (µg/ml)	Amt found in the analysed sample (µg/ml)	Amt recovered (µg/ml)	% recovery	Amt Present in the pre-analysed sample (µg/ml)	Amt found in the analysed sample µg/ml	Amt recovered (µg/ml)	% recovery
OFX	5.99	2	8.02	2.03	100.20	5.92	7.95	2.03	100.20
		4	10.02	4.03	99.46		9.96	4.04	99.70
		6	12.03	6.04	99.37		11.96	6.04	99.37
OZ	15.21	5	20.28	5.07	99.8	15.03	20.13	5.10	100.39
		10	25.32	10.11	99.51		25.20	10.17	100.09
		15	30.42	15.21	99.80		30.36	15.33	100.59

Table 3-REPEATABILITY- SIMULTANEOUS EQUATION METHOD

Tablet Sample	Concentration ($\mu\text{g/ml}$)		Label claim(mg/tablet)		Amount found (mg/tablet)		Percentage label claim(%w/w)		Percentage deviation	
	OFX	OZ	OFX	OZ	OFX	OZ	OFX	OZ	OFX	OZ
Tablet A	6	15	200	500	198.92	504.21	99.46	100.84	-0.54	+0.84
Tablet B	6	15			197.14	501.06	98.57	100.21	-1.43	+0.21
Tablet A	8	20	200	500	200.24	504.53	100.1	100.91	+0.12	+0.91
Tablet B	8	20			197.70	504.44	98.85	100.89	-1.15	+0.89
Tablet A	10	25	200	500	199.62	504.05	99.81	100.81	-0.19	+0.81
Tablet B	10	25			198.15	507.09	99.08	101.42	-0.92	+1.42

Each value is a mean of three readings

The quantitative estimation was carried out on two different brands of tablets formulations by taking a concentration of $6\mu\text{g/ml}$ of Ofloxacin and $15\mu\text{g/ml}$ of Ornidazole. The data regarding the quantitative estimation and statistics are given in table 1. Both the brands of tablet formulation show percentage purity values ranging from 98.5 – 99.51%w/w for Ofloxacin and 100.05-100.95%w/w for Ornidazole. The percentage deviation values were found to lie between -1.5 to -0.49 for Ofloxacin and +0.05 to +0.94 for Ornidazole. The relative standard deviation value were below 2% indicating the precision of the methodology and low standard error values show the accuracy of the method.

The validation of the proposed simultaneous equation method was further confirmed by recovery studies. The recovery data is given in table 2. The percentage recovery values vary from 99.37 to 100.20% for Ofloxacin and 99.51% to 100.59% for Ornidazole. This serves as a good index of accuracy and reproducibility of the study. The repeatability of the method was confirmed by repeating the assay procedure with three different concentrations of three replicates each. The data is presented in table 3. The results obtained in repeatability test expresses the precision of the given method.

Multicomponent Mode of Analysis

Table 4-QUANTITATIVE ESTIMATION AND STATISTICAL DATA- MULTICOMPONENT METHOD

Tablet sample	Label claim (mg/tablet)	Amt Present (mg/tab)	Percentage label claim (%w/w)	Percentage deviation	Standard deviation (SD)	Relative Standard Deviation (RSD)	Standard error of mean (SE)
Tablet A OFX OZ	200	199.99	99.99	-0.01	0.1933	0.1933	0.0789
	500	502.46	100.49	+0.49	0.1837	0.1828	0.0750
Tablet B OFX OZ	200	201.95	100.98	+0.98	0.2009	0.1990	0.0820
	500	507.22	101.44	+1.44	0.1987	0.1959	0.0811

Each value is a mean of six readings

Five mixed standard solutions Ofloxacin (2,4,6,8,10 $\mu\text{g/ml}$) and Ornidazole (5,10,15,20,25 $\mu\text{g/ml}$) were prepared and scanned over the range of 200-400nm in the multicomponent mode of spectrophotometer using the two sampling points 294nm and 277nm for Ofloxacin and Ornidazole respectively. The quantitative estimation was carried out on two different brands of tablets formulations by taking a concentration of 6 $\mu\text{g/ml}$ of Ofloxacin and 15 $\mu\text{g/ml}$ of Ornidazole respectively. The data regarding the quantitative estimation and the statistics are given in table 4. Both the brands of tablet formulation show percentage purity values ranging from 99.99 – 100.98%w/w for Ofloxacin and 100.49-101.44%w/w for Ornidazole. The percentage deviation values were found to lie between -0.01 to +0.98 for Ofloxacin and +0.49 to +1.44 for Ornidazole. The percentage purity values close to 100%w/w and low percentage deviation values show the accuracy of this method. The relative standard deviation value were below 2% indicating the precision of the methodology and low standard error values show the accuracy of the method.

Table 5-RECOVERY STUDIES- MULTICOMPONENT METHOD

Tablet-A						Tablet-B			
Tablet component	Amt Present in the pre-analysed sample ($\mu\text{g/ml}$)	Amt std added ($\mu\text{g/ml}$)	Amt found in the analysed sample ($\mu\text{g/ml}$)	Amt recovered ($\mu\text{g/ml}$)	% recovery	Amt Present in the pre-analysed sample ($\mu\text{g/ml}$)	Amt found in the analysed sample ($\mu\text{g/ml}$)	Amt recovered ($\mu\text{g/ml}$)	% recovery
OFX	6.01	2	8.02	2.01	100.30	6.07	8.11	2.04	101.19
		4	10.02	4.01	100.05		10.13	4.06	100.69
		6	12.03	6.02	100.13		12.17	6.10	100.86
OZ	15.10	5	20.12	5.07	100.10	15.25	20.27	5.02	99.90
		10	25.17	10.11	100.40		25.33	10.08	100.30
		15	30.22	15.21	100.50		30.27	15.02	99.64

Table 6-REPEATABILITY- MULTICOMPONENT METHOD

Tablet Sample	Concentration ($\mu\text{g/ml}$)		Label claim(mg/tablet)		Amount found (mg/tablet)		Percentage label claim(%w/w)		Percentage deviation	
	OFX	OZ	OFX	OZ	OFX	OZ	OFX	OZ	OFX	OZ
Tablet A	6	15	200	500	200.30	503.18	100.15	100.64	+0.15	+0.64
Tablet B	6	15			200.27	508.00	101.14	101.6	+1.14	+1.60
Tablet A	8	20	200	500	201.19	504.92	100.60	100.98	+0.60	+0.96
Tablet B	8	20			202.39	508.37	101.20	101.67	+1.20	+1.67
Tablet A	10	25	200	500	201.48	502.85	100.74	100.57	+0.74	+0.57
Tablet B	10	25			201.87	507.07	100.04	101.42	+0.94	+1.41

Each value is a mean of three readings

The validation of the proposed multicomponent method was further confirmed by recovery studies. The recovery data is given in table 5. The percentage recovery values vary from 100.05 to 100.86% for Ofloxacin and 99.64% to 100.50% for Ornidazole. This serves as a good index of accuracy and reproducibility of the study. The repeatability of the method was

confirmed by repeating the assay procedure with three different concentrations of three replicates each. The data is presented in table 6. The results obtained in repeatability test expresses the precision of the given method.

Reverse Phase High Performance Liquid Chromatography

Materials and method

Instrumentation : Single pan balance , Shimadzu, Libror, AEG 220
Control Dynamics pH meter
Shimadzu HPLC SPD10A VP equipped with UV Visible detector
Hypersil ODS C18 column 250x4.6mm 5 μ particle size
Rheodyne injector, Hamilton syringe with 20 μ l lopp

Reagents and chemicals

Double Distilled water
Acetonitrile HPLC grade(Ranbaxy fine chemicals Ltd. New Delhi)
Triethylamine, analytical grade (Sisco research Laboratories Pvt.Ltd, Mumbai)
O-Phosphoric acid , analytical grade (Qualigens fine Chemicals, Mumbai)

Reference standards- Ofloxacin and Ornidazole

Tablets used-

- i. Zenflex OZ (200mg of Ofloxacin and 500mg of Ornidazole)
Manufacturer Mankind Pharma, Meerut
- ii. O2 (200mg of Ofloxacin and 500mg of Ornidazole)
Manufacturer Medley Pharmaceuticals, Mumbai.

Operational Conditions

Mode of operation- Isocratic
Temperature – Ambient
Flow rate – 1ml/min
UV detection – 284nm (isobestic point)

The Mobile Phase was prepared by adding 1ml of triethylamine to 1000ml of double distilled water. This solution and acetonitrile were mixed in to the ratio of 75:25 and the pH was adjusted to 3 ± 0.1 with o-phosphoric acid. An accurately weighed quantity of 40mg of Ofloxacin and 125 mg of Ornidazole were dissolved in mobile phase to obtain a mixed standard stock solution having a concentration of 1000 μ g/ml of Ofloxacin and 2500 μ g/ml of Ornidazole respectively.

The isobestic point measured in the UV region for Ofloxacin and Ornidazole was found to be 285nm and taken as the suitable wavelength for UV Visible detector in HPLC .The individual peaks of Ofloxacin and Ornidazole were identified by knowing their retention times which were found to be around 5.06min and 7.50 minutes. Linearity was evaluated by visual inspection of plot of peak areas as a function of analyte concentration for both Ofloxacin and Ornidazole. The chromatograms and linearity for both the drugs are given graphically in figures

5,6,7,8 respectively. From the linearity studies the specified range was determined for both the drugs and given as 10-50 μ g/ml for Ofloxacin and 25-125 μ g/ml for Ornidazole respectively.

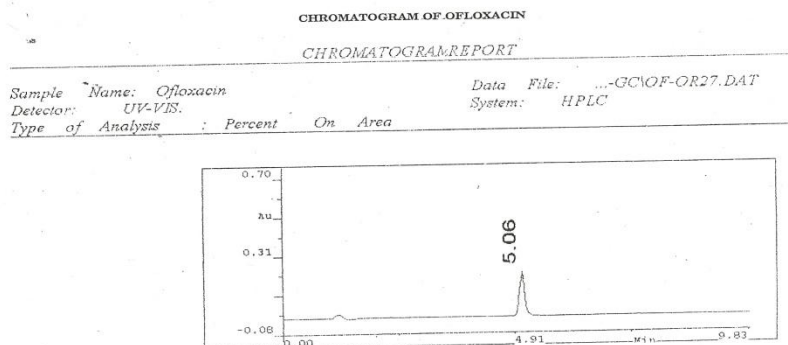


Figure 5- Chromatogram of OFLOXACIN

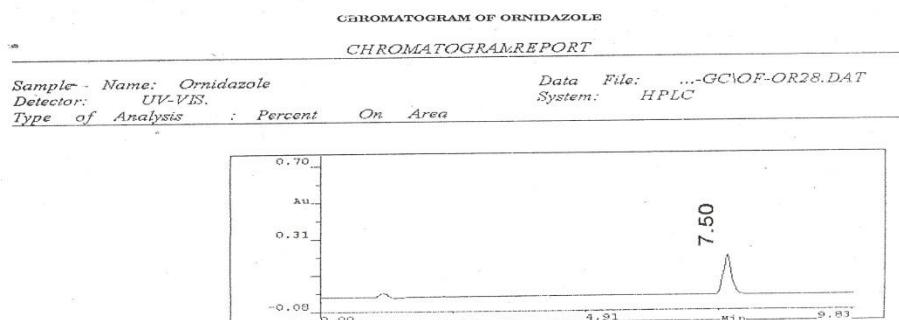


Figure 6- Chromatogram of ORNIDAZOLE

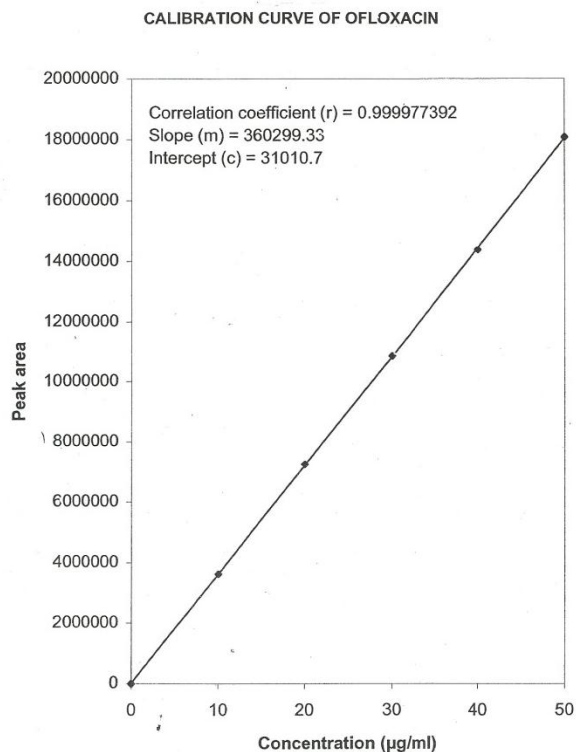


Figure 7 : CALIBRATION CURVE OF OFLOXACIN

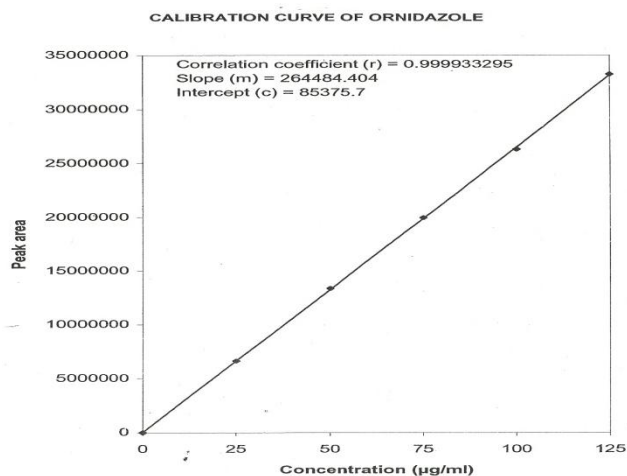


Figure 8: CALIBRATION CURVE OF ORNIDAZOLE

The simultaneous estimation of Ofloxacin and Ornidazole in tablets was carried out by RP-HPLC using water acetonitrile and triethylamine in the ration of 75:25:0.1 adjusted to the pH of 3.5 ± 0.1 with o-phosphoric acid as the mobile phase and C18 column as the stationary phase. The flow rate of the mobile phase was set at 1ml.min and the detection was carried out at 254nm.

Table 7-SYSTEM SUITABILITY PARAMETERS – RP HPLC

Parameter	Ofloxacin	Ornidazole
Resolution	--	7.97
Tailing Factor	1.17	1.21
Number of theoretical plates	8389	9881

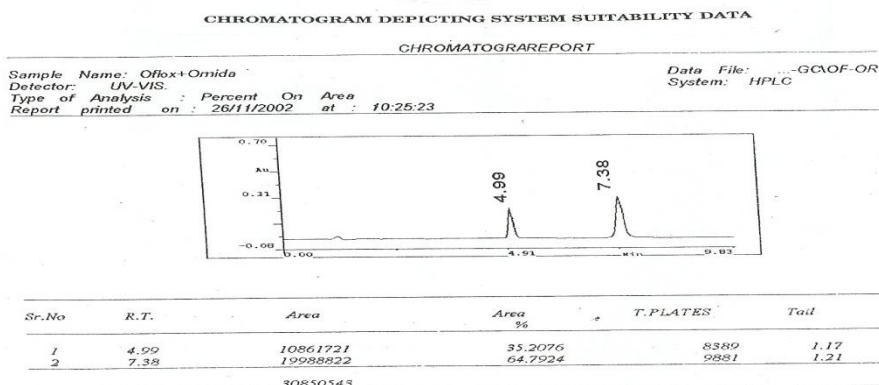


Figure 9 : System Suitability

System suitability parameters such as resolution, tailing factor and number of theoretical plates were calculated and the results are presented in table 7 and its graphical representation in figure 9. The resolution value of more than 1 indicates satisfactory results in quantitative work and the high resolution value obtained indicates the complete separation of the drugs. The symmetry factor or the tailing factor values for Ofloxacin and Ornidazole were found to be 1.17 and 1.21 indicating the symmetrical nature of the peaks. The number of theoretical peaks were high indicating the efficient performance of the column. The retention time of both the drugs were also well within the specified limits of 10 mts.

Table 8-LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION– RP HPLC

Drug	Limit of detection ($\mu\text{g/ml}$)	Limit of quantification ($\mu\text{g/ml}$)
Ofloxacin	0.19	0.58
Ornidazole	0.13	0.40

The limit of detection and limit of quantification of both the drugs were calculated and found to be 0.19 and 0.58 $\mu\text{g/ml}$ for Ofloxacin and 0.13 and 0.40 $\mu\text{g/ml}$ for Ornidazole which are represented in table 8. The retention times of Ofloxacin and Ornidazole in the mixed standard solution having a concentration of 30 $\mu\text{g/ml}$ of Ofloxacin and 75 $\mu\text{g/ml}$ of Ornidazole were found to be around 4.99 and 7.38 min respectively.

The quantitative estimation was carried out on tablet formulations of two different manufacturers by taking the same concentration as the mixed standard solution. The data regarding quantitative estimation and statistical analysis is depicted in table 9. Both the two different brands of tablet show percentage purity values ranging from 99.79 -100.45% w/w for

Ofloxacin and 99.55-100.25%w/w for Ornidazole. The percentage deviation was found to lie between -0.21 to +0.45 for Ofloxacin and -0.45 -+0.25 for Ornidazole. The percentage purity values close to 100% w/w and low percentage deviation values show the accuracy of the method.. The RSD values obtained were below 2% indicating the precision of the applied methodology. The low standard error values also indicate the accuracy of the proposed method.

Table 9-QUANTITATIVE ESTIMATION AND STATISTICAL PARAMETERS- RP HPLC

Tablet sample	Label claim (mg/tab)	Peak area	Amt found (mg/tablet)	% label claim (%w/w)	% deviation	Standard deviation (SD)	Relative Standard Deviation (RSD)	Standard error of mean (SE)
Tablet A								
OFX	200	10596914	199.58	99.80	-0.20	0.0940	0.0942	0.0384
OZ	500	19463293	497.77	99.56	-0.44	0.0886	0.0890	0.0362
Tablet B								
OFX	200	10729954	200.90	100.46	+0.46	0.2009	0.1146	0.0420
OZ	500	19715955	501.27	100.25	+0.25	0.1987	0.1077	0.0441

Each value is a mean of six readings

Table 10-RECOVERY STUDIES- RP HPLC

Tablet A						Tablet B			
Tablet content	Amt Present in the pre-analysed sample (µg/ml)	Amt std added (µg/ml)	Amt found in the analysed sample (µg/ml)	Amt recovered (µg/ml)	% recovery	Amt Present in the pre-analysed sample (µg/ml)	Amt found in the analysed sample (µg/ml)	Amt recovered (µg/ml)	% recovery
OFX	29.94	5	34.97	5.03	100.40	30.14	35.18	5.04	100.60
		10	39.91	9.97	99.50		40.27	10.13	101.10
		15	45.16	15.22	101.28		45.16	15.02	99.93
OZ	74.67	12.5	87.23	12.56	100.32	75.19	87.70	12.51	99.92
		25.0	99.31	24.64	98.40		100.28	25.09	100.20
		37.5	112.65	37.98	101.12		112.41	37.22	99.09

Table 11-REPEATABILITY-- RP HPLC

Tablet Sample	Concentration (µg/ml)		Label claim (mg/tablet)		Amt found (mg/tablet)		% label claim(%w/w)		% deviation	
	OFX	OZ	OFX	OZ	OFX	OZ	OFX	OZ	OFX	OZ
Tablet A	20	50	200	500	198.51	496.75	99.26	99.35	-0.74	-0.65
Tablet B	20	50			200.11	498.93	100.06	99.79	+0.06	-0.21
Tablet A	30	75	200	500	199.5	497.57	99.75	99.51	-0.25	-0.49
Tablet B	30	75			200.71	500.83	100.36	100.17	+0.36	+0.17
Tablet A	40	100	200	500	199.20	496.85	99.60	99.37	-0.40	-0.63
Tablet B	40	100			201.43	502.37	100.72	100.47	+0.72	+0.47

Each value is a mean of three readings

The validation of the proposed reverse phase HPLC method was further verified by recovery studies. The data regarding recovery studies is presented in table 10. The percentage recovery range was found to be within 99.50-101.26% for Ofloxacin and 98.40-101.12% for Ornidazole. This serves as a good index of the accuracy and reproducibility of the method. The repeatability of the method was confirmed by repeating the procedure with three different concentrations of three replicates each. The data is presented in table 11. The results obtained in repeatability test express the precision of the proposed method. All the parameters including flow rate, detection wavelength and sensitivity were maintained constant throughout the procedure.

SUMMARY AND CONCLUSION

Present analytical work comprises of simple, precise, rapid, sensitive and accurate methods for the simultaneous estimation of Ofloxacin and Ornidazole in combined dosage form. The methods used for the estimation were- simultaneous equation method, multicomponent mode of analysis, reverse phase HPLC.

In Simultaneous equation method, the percentage purity values of the two brands vary from 98.5-99.51%w/w for Ofloxacin and 100.05 to 100.94%w/w of Ornidazole respectively. The quantitative results obtained are subjected to the statistical validation. The values of RSD are less than 2% indicating the accuracy and precision of the method. The percentage recoveries vary from 99.37 to 100.20% for Ofloxacin and 99.51 to 100.59% for Ornidazole.

In the multicomponent mode of analysis, the quantitative estimation was carried out on two different brands of tablets. The percentage purity values for Ofloxacin and Ornidazole vary from 93.99-100.98%w/w and 100.49-101.44%w/w respectively. The quantitative results obtained are subjected to the statistical validation. The values of RSD are less than 2% indicating the accuracy and precision of the method. The percentage recoveries vary from 100.05- 100.86% and 99.64-100.50% of Ofloxacin and Ornidazole respectively.

Simultaneous estimation of Ofloxacin and ornidazole was done by reverse phase HPLC. The mobile phase used consists of water –acetonitrile-triethylamine (75; 25:0.1, v/v/v) adjusted to pH 3±0.1 with o-phosphoric acid. A C₁₈ column containing octadecyl silane (ODS) chemically bonded to porous silica particles (250x4.6mm, 5μ particle size) is used as the stationary phase. The detection is carried out using UV Visible detector set at 284nm (isosbestic point). The solutions are chromatographed at a constant flow rate of 1ml/min. The retention times of Ofloxacin and ornidazole in the mixed standard solution are around 4.99min and 7.38min. The linearity range of Ofloxacin and ornidazole are 10-50μg/ml and 25-125μg/ml.

The percentage purity values vary from 99.79 – 100.45 %w/w and 99.55- 100.25% w/w for Ofloxacin and Ornidazole respectively. The quantitative results obtained are subjected to the statistical validation. The values of RSD are less than 2% indicating accuracy and precision of

the method. The percentage recoveries vary from 99.50-101.26% for Ofloxacin and 98.40 - 101.12% for Ornidazole respectively.

Table 12 - COMPARISON OF THE THREE METHODS

Method	Tablet sample	Label claim(mg/tablet)	^o Amount found(mg/tablet)	Percentage Label claim(%w/w)	Relative Deviation (RSD)	Percentage recovery
Simultaneous equation method	Tablet A					
	OFX	200	199.01	99.51	0.1999	99.68
	OZ	500	504.70	100.94	0.4674	99.70
	Tablet B					
	OFX	200	197.00	98.50	0.1981	99.76
	OZ	500	500.24	100.05	0.4516	100.34
Multicomponent mode of analysis	Tablet A					
	OFX	200	199.99	99.99	0.1933	100.16
	OZ	500	502.46	100.49	0.1828	100.33
	Tablet B					
	OFX	200	201.95	100.98	0.1990	100.91
	OZ	500	507.22	101.44	0.1959	99.95
Reverse Phase HPLC	Tablet A					
	OFX	200	199.58	99.80	0.0942	100.39
	OZ	500	497.77	99.56	0.0890	99.95
	Tablet B					
	OFX	200	200.90	100.46	0.1146	100.54
	OZ	500	501.27	100.25	0.1077	99.74

^oEach value is a mean of six readings

The results obtained by the three methods are recapitulated in table 12. From the table it is clear that all the above three methods of analysis are simple, specific, accurate and precise and can be employed in the routine analytical work. HPLC is the most reliable method since the RSD values are low when compared to the other two methods. But for economical reasons spectrophotometric methods can be employed for regular quantitative works.

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