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Simultaneous spectrophotometric estimation of ofloxacin and ornidazole from combined tablet dosage form

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ABSTRACT

Two simple, sensitive, accurate, precise, rapid and economical methods were developed for the simultaneous estimation of Ofloxacin and Ornidazole from combined tablet dosage form. First method is based simultaneous equations and second method is based on Q-analysis (absorbance ratio method). Ofloxacin and Ornidazole show absorbance maxima at 293nm and 275nm in 0.1N HCI respectively. The linearity was obtained in the concentration ranges of 2-14 μg/ml for Ofloxacin and 5-35 μg/ml for Ornidazole. In the first method concentration and subsequently amount of drug determined by using simultaneous equations and in second method concentration and amount of drugs determined by using ratio of absorbance at iso-absorptive point and at λmax of one of the drug. The parameters were used for method validation are linearity; accuracy, precision, robustness, ruggedness, LOD and LOQ. Proposed method was found to be simple, precise, and accurate and can be successfully applied for routine quality control analysis of the two simultaneously in the drug formulation.

KEYWORDS

Ofloxacin;
Ornidazole;
Absorbance ratio;
Iso-absorptive point;
Method validation.

INTRODUCTION

In the topical countries like India, the major problems of health arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. Infections caused by the microorganisms like, fungi, protozoa, are most common. Drugs with antifungal and antiprotozoal activity have been used in the treatment of the same.

Ofloxacin $C_{18}H_{20}FN_3O_4$ that is (*RS*)-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 is used as a antibacterial drugs. (Molecular weight:- 361.368 g/mol)

Ornidazole, $C_7H_{10}ClN_3O_3$ that is 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole, is used as an antiprotozoal drug. (Molecular weight: - 219.625 g/mol)

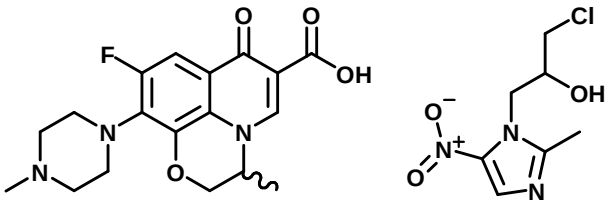
It is highly effective for bacterial and protozoan infections and is available in the tablet form. A literature surveys reveals few Chromatographic methods i.e. HPLC, HPTLC, Derivative and Extractive spectrophotometric methods for the simultaneous determination of Ofloxacin and Ornidazole. Very little attention has been paid to the use of electroanalytical method.

Ofloxacin and Ornidazole in combined dosage form is available in the market, has gained great acceptance

in diarrhooea, bacterial and protozoal infections. In many cases, drugs with two active ingredients are prescribed to the patients to have an added advantage. Many of these antibacterial drugs are found in combination with antifungal and antiprotozoal drugs which are highly effective against fungal and protozoal infections.

In the present study a successful attempt has been made to estimate both these drugs i.e. Ofloxacin and Ornidazole simultaneously in combined pharmaceutical formulation by two simple spectrophotometric methods. First method is based simultaneous equations and second method is based on Q-analysis (absorbance ratio method). The proposed methods have been validated as per ICH guidelines[9,10].

Structure



Ofloxacin Ornidazole

MATERIALS AND METHODS (EXPERIMENTAL)

Instruments

A Jasco model V-530 with Serial No. B086960512, double beam UV-Vis spectrophotometer with spectral width of 2.0 nm, wavelength accuracy 0.5 nm and pair of 10 mm matched quartz cells was to measure the absorbance of resulting solutions.

Materials

Pure standard of Ofloxacin and Ornidazole was obtained from Cipla pharmaceutical Pvt. Ltd. The tablet formulations of the said combination were purchased from a local pharmacy (The label claim contained 200mg of Ofloxacin and 500mg of Ornidazole.) All the solutions were prepared in double distilled water. All the reagents use were of AR grade.

Preparation of standard solutions

10 mg of standard Ofloxacin and 25 mg of standard Ornidazole was accurately weighed and dissolved in 0.1N HCI and made up to a volume of 50 ml in standard flask to give stock solution (200 µg/ml of Ofloxacin and 500 µg/ml of Ornidazole respectively). Further all the standard solutions containing the mixture of Ofloxacin and Ornidazole were prepared by using this stock solution.

Procedure

Method-1: Simultaneous equation method

Working standard solutions were scanned in the entire range of 200-400 nm to determine λmax of both the drugs. The λmax of Ofloxacin and Ornidazole were found at 293 nm and 275 nm respectively. A series of solutions were prepared having concentration ranges 1-40 µg/ml by using 0.1N HCI from working standard solutions. Absorbances of resulting solutions were measured at 293 nm and 275 nm and calibration curves plotted at these wavelengths. The linearity was obtained in the concentration ranges of 2-14 µg/ml for Ofloxacin and 5-35 µg/ml for Ornidazole. The absorptivity coefficients of these two drugs were determined by using calibration curve equation. Two simultaneous equations were formed using these absorptivity coefficients values.

$$A_1 = 60.1\ C_X + 15.0\ C_Y \quad (1)$$

$$A_2 = 35.7\ C_X + 23.4\ C_Y \quad (2)$$

Where, A_1 and A_2 are the absorbances of sample at 293 nm and 275 nm respectively. C_X and C_Y are the concentration (µg/ml) in of Ofloxacin and Ornidazole in sample respectively. From the resulting concentration obtained after solving above equations, then amount of Ofloxacin and Ornidazole present in the given sample was found out.

ANALYTICAL METHOD VALIDATION

System suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recording absorbance at working concentrations for Ofloxacin (4 µg/ml,8 µg/ml, 12 µg/ml) and for Ornidazole (10 µg/ml, 20 µg/ml, and 30 µg/ml) with five replicates and the mean was used for the whole calculations.

Specificity

The specificity of method was confirmed by recording the spectra of both the standard solution and the drug sample solutions. The spectra obtained from the drugs sample solution were found to be identical to those

obtained for standard solution.

The addition of the standard solution to the drug sample solution for recovery analysis did not change the characteristics of spectra . This gives the validity of method for the determination of both drugs from combined pharmaceutical formulation.

Linearity and range

A good linearity was achieved for Ofloxacin and Ornidazole in the concentration ranges of 2-14 μg/ml for Ofloxacin and 5-35 μg/ml Ornidazole. The calibration curves were constructed with concentration (C) against absorbance of both drugs.

Limit of detection and limits of quantitation

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. For LOD and LOQ analysis twenty readings for blank recorded then their standard deviation calculated i.e. for LOD= (SD×3+ Mean absorbance of Blank) and for LOQ= (SD×10 + Mean absorbance of Blank.).

Intra-day and inter-day precision/ruggedness

The intra-day and inter-day precision was used to study the variability of the method. According to USP, ruggedness is the degree of reproducibility of the results obtained under variety of test conditions. It is expressed as percent RSD. It is also called as reproducibility or intermediate precision. It is the analysis of same sample under variety of normal test condition such as different laboratories, different analysts, different instruments, different lots of reagents, different days etc.

It was checked by recording the absorbance as well as spectra of standard solutions of Ofloxacin and Ornidazole i.e. working concentrations for Ofloxacin (4 μg/ml, 8 μg/ml, 12 μg/ml) and for Ornidazole (10 μg/ml, 20 μg/ml, and 30 μg/ml) with five replicates (both at intra-day (five times within 24 hour) and inter-day (two times each. during 3 days intervals) to check the precision.

Assay

For estimation of drugs from commercial formulations, twenty tablets were weighed accurately and finely powdered. 46 mg of powder which is equivalent to (10 mg of Ofloxacin and 25 mg of Ornidazole) was accurately weighed and transferred to 50ml volumetric flask and dissolved in 25ml of 0.1N HCI and sonicated for 10 mins. The solution was filtered through whatman filter paper No.41 and residue was washed thoroughly with given solvent. The filtrate and washings were combined in 50ml volumetric flask and diluted with 0.1N HCI The spectra were obtained and absorbance was measured at 293 nm and 275 nm and finally concentration of both the drugs was calculated using equations 1 and 2. The amount of Ofloxacin and Ornidazole present in the given sample found out. Assay studies were carried out at from 50 % to 125% level.

Accuracy (Recovery)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of standard addition. A fixed volume of standard Ornidazole solution was mixed with different concentrations of preanalyzed sample solutions and mixtures were analyzed by proposed method. The percent recovery was determined at different levels i.e. from 50% to 150% level.

Method-2: Absorbance ratio method (Q-Analysis)

Absorbance ratio method uses the ratio of absorbance at two selected wavelengths one at iso-absorptive point and other being the λmax of one of the components. From the overlay spectra of two drugs, it is evident that Ofloxacin and Ornidazole show iso-absorptive point at 284nm and the second which is the λmax of Ofloxacin (293nm). The quantification of both drugs was carried out at the selected wavelengths. i.e. 284nm and 293nm.

Linearity and range

A series of solutions having concentration ranges 1-40 μg/ml were prepared by using 0.1N HCI Absorbance of resulting solution was measured at 293 nm and 308 nm. Calibration curves were plotted at these wavelengths. The linearity was obtained in the concentration ranges of 2-14 μg/ml for Ofloxacin and 5-35 μg/ml for Ornidazole. The absorptivity coefficients of these two drugs were determined by using calibration curve equation at 293 nm and 284 nm.

Assay

For estimation of drugs from commercial formulations, 46 mg of powder which is equivalent to (10mg of

Ofloxacin and 25mg of Ornidazole) was accurately weighed and transferred to 50ml volumetric flask and dissolved in 25ml of 0.1N HCI and sonicated for 10 mins. The solution was filtered through whatman filter paper No.41 and residue was washed thoroughly with given solvent. The filtrate and washings were combined in 50ml volumetric flask and diluted with 0.1N HCI Absorbances were measured at 293 nm and 308 nm and concentration of both the drugs was calculated using equations 3 and 4. Finally the amount of Ofloxacin and Ornidazole present in the given sample found out. Assay studies were carried out from 50 % to 125% level.

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{ax_1} \quad (3)$$

$$C_y = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{ay_1} \quad (4)$$

C_x= concentration of Ofloxacin, C_y= concentration of Ornidazole, Q_x= Ratio of Absorptivity of Ofloxacin at 293nm and 284nm, Q_y= Ratio of Absorptivity of Ornidazole at 293nm and 284nm, Q_m= Absorbance ratio of mixture (Sample) 293nm and 284nm, ax_1 and ay_1 = Absorptivity of pure Ofloxacin and Ornidazole respectively at iso-absorptive point i.e. 284nm, A_1= Absorbance of mixture at iso-absorptive point i.e. 284nm

Accuracy (Recovery)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of standard addition. A fixed volume of standard Ornidazole solution was mixed with different concentrations of preanalyzed sample solutions and mixtures were analyzed by proposed method. The percent recovery was determined at different levels i.e. from 50% to 150% level.

RESULTS AND DISCUSSION

1) The proposed methods were found to be simple, accurate, sensitive, precise, economical, and rapid.
2) Proposed methods validated as per as ICH guidelines. All the necessary validation parameters were studied. Method validation parameter for the determination of Ofloxacin and Ornidazole is given in (TABLE 1) i.e. system suitability, the mean % RSD was found to be less than 1 for both Ofloxacin and Ornidazole, which very was acceptable. Specificity, Regression analysis (Linearity and Range), LOD and LOQ.
3) Precision was calculated, as repeatability and also inter and intra day precision (% RSD is less than 1) for both drugs. Given results are shown in (TABLE 1)
4) Accuracy was determined for both the methods by calculating the percent recovery at different levels i.e. from 80% to 120% level. The percentage recovery at three different was found to be from 98.00 % to 102.00 % for both the drugs. The results of recovery analysis of Ofloxacin and Ornidazole for both the methods are shown in (TABLE 3).
5) Quantification of drugs from formulation were done out by assay analysis for both the methods. Assay studies were carried out at three different levels i.e. 80%, 100%, 120% level. The percentage assay at three different levels for Ofloxacin and Ornidazole were found to be from 98.00 % to 102.00 %. The results of assay analysis of Ofloxacin and Ornidazole for both the methods are shown in (TABLE 2).

TABLE 1 : Method validation parameter for the determination of ofloxacin and ornidazole.

Parameters	Method 1 (SEM)		Method 2 (ARM)	
	Ofloxacin	Ornidazole	Ofloxacin	Ornidazole
System suitability (n=5) %RSD	0.69%	0.46%	0.24%	0.51%
Linearity range (μg mL^{-1})	2 to 14 μg/ml	5 to 35 μg/ml	2 to 14 μg/ml	5 to 35 μg/ml
Correlation coefficient (R^2)	0.9998	0.9999	0.9998	0.9998
LOD (μg mL^{-1})	0.3 μg/ml	0.8 μg/ml	0.3 μg/ml	0.8 μg/ml
LOQ (μg mL^{-1})	1 μg/ml	2.5 μg/ml	1 μg/ml	2.5 μg/ml
Intraday precision (n=5) %RSD	0.75%	0.65%	0.35%	0.71%
Interday precision (n=5) %RSD	0.60%	0.45%	0.29%	0.54%
Assay	98% to 102%	98% to 102%	98% to 102%	98% to 102%
Recovery	98% to 102%	98% to 102%	98% to 102%	98% to 102%

SEM: - Simultaneous Equation Method; ARM: - Absorbance Ratio Method

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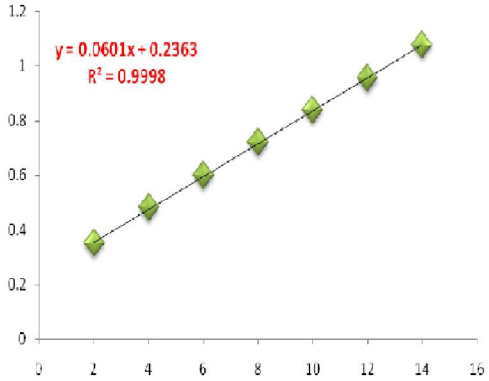
TABLE 2 : Result of assay studies of ofloxacin and ornidazole

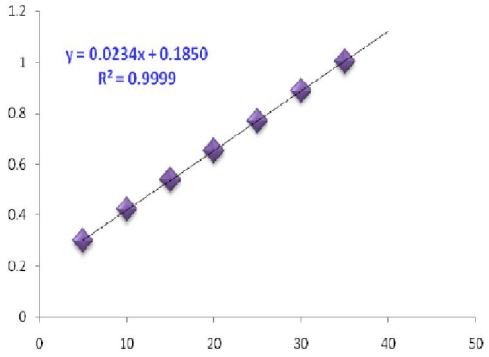
Samples Used
1) OFNOF (Aristo) 2) O2 (Medley)

	Method 1 (SEM)		Method 2 (ARM)	
	Ofloxacin	Ornidazole	Ofloxacin	Ornidazole
Labeled claim (mg)	200mg	500mg	200mg	500mg
Drug found in mg	198.8 mg	499.1 mg	201.2 mg	504.0 mg
% Assay	99.7%	99.85 %	100.3 %	100.6%
SD	0.3	0.4	0.5	0.467
% RSD (n=5)	0.31	0.474	0.51	0.461

SEM: - Simultaneous equation method; ARM: - Absorbance ratio method



Ofloxacin standard (293nm)



Ornidazole standard (275nm)

Figure 1 : Linearity graph for SEM

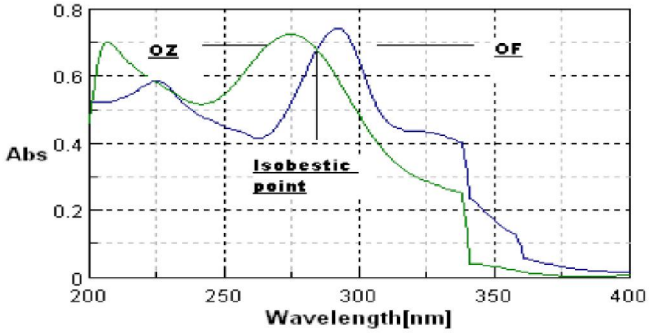


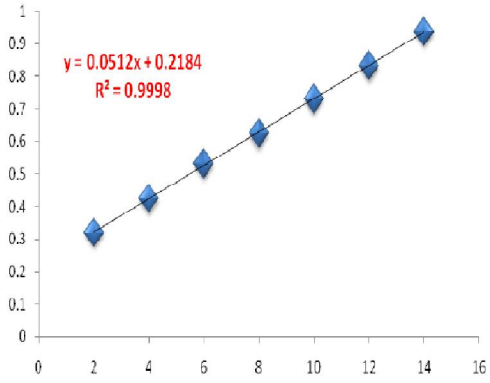
Figure 3 : Overlain spectra of ofloxacin (293nm), ornidazole (275nm) and iso-absorptive point (284nm)

TABLE 3 : Results of recovery studies of ofloxacin and ornidazole

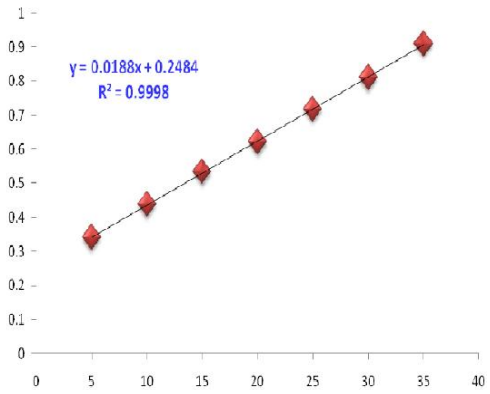
Method Name	Level of % Recovery	% Recovery Found		Standard Deviation (SD)		Relative Standard Deviation % (RSD) (N=5)	
		OF	OZ	OF	OZ	OF	OZ
SEM	0	100.6%	100.2%	0.037	0.032	0.57	0.73
	50 %	99.8%	99.4%	0.041	0.04	0.35	0.51
	100 %	101.01%	101%	0.051	0.045	00.39	0.50
	150%	100.2%	99.7%	0.01	0.07	0.69	0.79
ARM	0	99.6%	100.0%	0.02	0.056	0.33	0.62
	50 %	99.5%	99.9%	0.025	0.015	0.53	0.22
	100 %	100.3%	100.5%	0.032	0.05	0.52	0.55
	150%	101.7%	100.7%	0.033	0.076	0.44	0.69

SEM:- Simultaneous equation method; ARM:- Absorbance ratio method; OF:- Ofloxacin; OZ:- Ornidazole

Iso-absorptive point



Ofloxacin standard (284nm)



Ornidazole standard (284nm)

Figure 2 : Linearity graph for ARM

ABBREVIATION

OF Ofloxacin
OZ Ornidazole

CONCLUSION: - APPLICATION TO ANALYSIS OF PHARMACEUTICAL FORMULATION

All these factors lead to the conclusion that, both the methods described in this paper for simultaneous estimation of Ofloxacin and Ornidazole are found to be simple, accurate, precise, accurate, economical, and rapid, therefore presented methods can be recommended for routine quality control analysis of Ofloxacin and Ornidazole in their combined dosage forms.

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