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Spectrophotometric method for simultaneous estimation of ofloxacin and ornidazole in tablet dosage form

S.S.Dengale[1], R.K.Godge[1], K.B.Rasal[1], Y.Madhusudan Rao[2], G.S.Asane[1,*], J.K.Bhatt[1]

[1]Department of Pharmaceutics, Pravara Rural College of Pharmacy, Loni-413736, (M.S.), (INDIA)

[2]Centre for Biopharmaceutics and Pharmacokinetics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal- 506 009, (A.P.), (INDIA)

E-mail : asane2009@rediffmail.com

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ABSTRACT

Ofloxacin (OFX) is broad-spectrum fluoroquinolone antibacterial and ornidazole (ORZ) is an antiprotozoal drug. A simple, precise, rapid and selective spectrophotometric method has been developed for the simultaneous determination of ofloxacin and ornidazole from tablet dosage form. The method involves solving of simultaneous equation based on measurement of absorptivity at two wavelengths 288nm and 319nm for OFX and ORZ respectively. Linearity range for OFX and ORZ were 5-25µg/ml and 10-50µg/ml respectively.

KEYWORDS

Ofloxacin;
Ornidazole;
Simultaneous equations;
Absorbance ratio;
Isobestic point.

INTRODUCTION

Ofloxacin is a synthetic broad spectrum antibacterial agent. Chemically ofloxacin[1] a fluorinated carboxyquinolone, is a racemate, (±)- 9-fluro-2, 3-dihydro-3-methyl-10- (4-methyl- 1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine- 6-carboxylic acid. It is official in BP[2], USP[3], and EP[4]. The assay procedure mentioned in these pharmacopoeias uses nonaqueous titration for estimation of ofloxacin. Literature surveys reveals Spectrophotometric method[5,6] atomic absorption spectrometry[5], spectroflurometry[5], HPLC[7] and microbiological method[8] for its determination. Ornidazole is a 5-nitroimidazole derivative used as anti-infective agent. It is not official in any Pharmacopoeia. Literature survey reveals that ornidazole is estimated by voltametry[9] and HPLC[10] methods for its determination in dosage forms and biological fluids. Ofloxacin and ornidazole in combined tablet dosage form is available in the market, has gained increasing acceptance in diarrhea, bacterial and protozoa infections. Spectrophotometric[11,12] and HPTLC[13] method have been established for their simultaneous estimation in tablet dosage form. This paper presents a simple, accurate and reproducible spectrophotometric methods for simultaneous determination of ofloxacin and ornidazole in tablet dosage form using phosphate buffer pH 6.8.

MATERIALS AND METHODS

Instruments

The instruments used in the experiment were Shimadzu UV-150 Double-beam spectrophotometer (Shimadzu Corporation, Japan) with matched quartz cell corresponding to 1cm path length and spectral bandwidth of 2nm and ultrasonicator.

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Materials

Working standard of Ofloxacin was procured from Ranbaxy laboratories Ltd, Dewas, and Ornidazole from Glenmark Pharmaceuticals Ltd, Mumbai. Combined Ofloxacin-Ornidazole tablets (ZORNO®, FDC Ltd.) were purchased from local market.

Solvents

The solvents used for the experiment were phosphate buffer pH 6.8 (analytical grade)

Stock solutions

Accurately weighed OFX (10mg) and ORZ (10mg) was dissolved in phosphate buffer pH 6.8 and diluted to get a stock solution of 100µg/ml.

Preparation of calibration curve

Solutions were prepared with phosphate buffer pH 6.8 to get concentrations of 5-25µg/ml of OFX and 10-50 µg/ml of ORZ. The Beer's law limit for OFX and ORZ was 5-25µg/ml and 10-50µg/ml at the wavelengths 288nm and 319nm respectively. Absorbance of these solutions was measured at 288nm and 319nm. The coefficient of correlation was found to be 0.9939 for OFX and 0.9948 for ORZ.

Method I: Simultaneous estimation method

The absorptivity for the two drugs is presented in the TABLE 1. The method employs solving of simultaneous equation using Cramer's rule and matrices. A set of two simultaneous equations was formed, using the absorptivity coefficient values as given below.

$$A_1 = 97 \times C_1 + 23.2 \times C_2 \quad (1)$$

$$A_2 = 37.5 \times C_1 + 57.5 \times C_2 \quad (2)$$

Where, C_1 and C_2 are the concentrations of OFX and ORZ in gm/liter respectively in sample solution. A_1 and A_2 are absorbance of sample solution measured at 288nm and 319nm respectively. 97 and 23.2 are absorptivities at 288nm while 37.5 and 57.5 are absorptivities at 319nm respectively. The molar absorptivity for ofloxacin at 288nm was 1.0×10^{-3} and for ornidazole it was 4.6×10^{-5}. By applying Cramer's rule and matrices to equation (1) and (2) concentrations C_1 and C_2 obtained as.

$$C_1 = \frac{A_1 \times 57.5 - A_2 \times 23.2}{9999} \quad (3)$$

$$C_2 = \frac{A_2 \times 97.0 - A_1 \times 37.5}{9999} \quad (4)$$

TABLE 1 : Absorptivity values for ofloxacin and ornidazole

Concentration (µg/ml)		Absorptivity at 319nm		Absorptivity at 288nm	
Ofloxacin	Ornidazole	Ofloxacin	Ornidazole	Ofloxacin	Ornidazole
5	10	0.056	0.057	0.133	0.023
10	20	0.037	0.048	0.097	0.029
15	30	0.037	0.042	0.094	0.052
20	40	0.037	0.045	0.094	0.037
25	50	0.037	0.041	0.086	0.019
Mean		0.040	0.046	0.100	0.032

Analysis of tablet formulation

Twenty tablets were weighed and crushed to obtain a fine powder. An accurately weighed sample equivalent to 25mg of OFX and 20mg ORZ was taken in a stoppered volumetric flask (100ml) 20ml of phosphate buffer was added and ultrasonicated for 10minutes. Finally the volume was made up to the mark with phosphate buffer. Then the solution was filtered through Whatmann filter paper (No-41). Appropriate dilutions were made to obtain mix sample solutions in Beer-Lambert's range for each drug in the ratio of 1:2.5 from 5,10,15,20 and 25µg/ml of OFX and 10,20,30,40 and 50µg/ml of ORZ. The absorbances of mixed sample solutions were measured at 288nm and 319nm. Recovery study was carried out at 80%, 100% and 120% level of labeled claim.

Method II: Absorbance ratio (Q-Analysis) Method

In the quantitative assay of two components by Q-analysis method absorbances are measured at two wavelengths, one being the isobestic point and other being the wavelength of maximum absorption of one of the two components. From the overlain spectra (Figure 1) of OFX and ORZ, absorbance's were measured at the selected wavelengths i.e. 304nm (isobestic point) and 319nm (Wavelength of maximum absorption of Ornidazole).The concentration of each component can be calculated by mathematical treatment of mentioned equation.

For Ofloxacin

$$C_1 = Q_0\text{-}Q_1/Q_1\text{-}Q_2 \times A/a \quad (5)$$

For Ornidazole

$$C_2 = Q_0\text{-}Q_1/Q_2\text{-}Q_1 \times A/a \quad (6)$$

Where C_1 = Concentration of Ofloxacin
C_2 = Concentration of Ornidazole
A = Absorbance of sample at isobestic wavelength (304 nm)
a = Absorbity of Ofloxacin and Ornidazole at isobestic wavelength (304 nm)
Q1 = Absorbance of Ofloxacin at 319 nm/ Absorbance of Ofloxacin at 304 nm
Q2 = Absorbance of Ornidazole at 319 nm/Absorbance of Ornidazole at 304 nm
Q_0 = Absorbance of sample solution at 319 nm/ Absorbance of sample solution at 304 nm

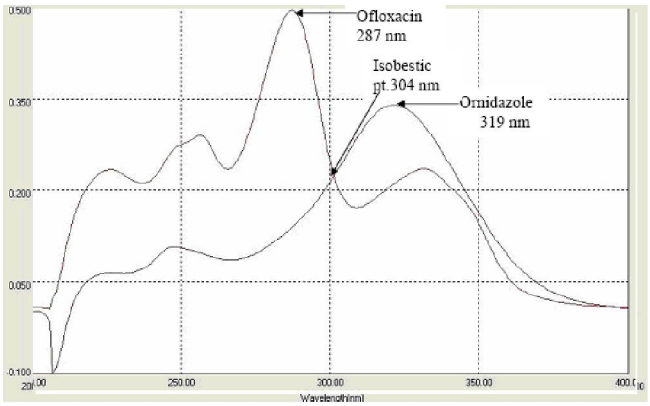

Figure 1 : Overlain UV spectra of ofloxacin and ornidazole in phosphate buffer pH 6.8

Analysis of sample solution

Tablet solution was prepared in phosphate buffer pH 6.8 as described earlier and was further diluted with the same solvent. Appropriate dilutions were made to obtain mix sample solutions in Beer-Lambert's range, for each drug in the ratio of 1:2.5 from 5,10,15,20 and 25µg/ml of OFX and 10,20,30,40 and 50µg/ml of ORZ. The absorbances of mixed sample solutions were measured at 304 nm and 319 nm. These values are then equated in the above mentioned equations (5) and (6) and the concentration of each drug was calculated.

RESULT AND DISCUSSION

To study accuracy, reproducibility, reliability and the interference from excipients used in the formulation, recovery experiment was carried out by standard addition method. The recovery of added standard was found at five different concentrations levels for each drug. For each determination 20 tablets were taken. From the total amount of drug found, the percentage recovery was calculated. The results of recovery analysis are presented in TABLE 2.

TABLE 2 : Recovery studies

Sr. No.	Drug name	Amount label claimed µg/tablet	Amount standard added µg/tablet	Total amount recovered µg/tablet	% Recovery
		10	0	10.26	102.6
		10	10	19.45	97.25
1.	Ofloxacin	10	20	31.05	103.49
		10	30	39.93	99.82
		10	40	49.46	98.99
				Mean Recovery	100.43
		25	0	24.41	97.64
		25	5	29.18	97.05
2.	Ornidazole	25	10	35.32	100.91
		25	15	40.82	102.05
		25	20	45.14	100.31
				Mean Recovery	99.59

Linearity obtained for both OFX and ORZ was in the concentration range 5-25µg/ml and 10-50 µg/ml respectively. The value of correlation coefficient suggests the level of precision of the method. Furthermore, the mean recoveries of OFX and ORZ were 100.43% and 99.59% respectively. Thus, the proposed method for simultaneous estimation of OFX and ORZ in tablet dosage form was found to be rapid, simple, accurate, sensitive, and economical and validated statistically TABLE 3.

TABLE 3 : Optical characterization of ofloxacin and ornidazole

Sr. No.	Parameters	Ofloxacin	Ornidazole
1	λmax (nm)	288nm	319nm
2	Linearity range	5-25µg/mL	10-50µg/mL
3	Mean absorptivity	$1.\times10^{-3}$	4.6×10^{-5}
4	Regression values		
	Slope	0.0852	0.0414
	Intercept	-1.208	-1.796
	Correlation-coefficient	0.9939	0.9948
	Squared coefficient correlation	0.987	0.989

CONCLUSION

Thus proposed method is fairly simple and rapid

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and do not involve use of complex instruments. High percentage of recovery shows that the method is free from the interference of excipient(s) used in formulation. Therefore the method can be useful in routine quality control analysis of these drugs.

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