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DEVELOPMENT AND VALIDATION OF TWO SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND OFLOXACIN IN SUSPENSION

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ABSTRACT

Two simple, sensitive, rapid, accurate and economical methods were developed for the estimation of Metronidazole and Ofloxacin in two component liquid dosage form. First method is based on the simultaneous equation and second method is based on Q-analysis (absorbance ratio method). Metronidazole has absorbance maxima at 318.0 nm and Ofloxacin has absorbance maxima at 294 nm in acetonitrile and diluent(70:30) solvent P^H of 9.89. The linearity was obtained in the concentration range of 5-30 µg/ml for Metronidazole and 3-18 µg/ml for Ofloxacin. In the first method, the concentrations of the drugs were determined by using simultaneous equations and in second method, the concentration of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of one of the drug. The results of analysis have been validated statistically and by recovery studies.

INTRODUCTION: Metronidazole (MET), an antiprotozoal drug is widely used in treatment of invasive amoebiasis. Chemically it is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol (**fig. 1**) and Ofloxacin (OFL), an antimicrobial drug chemically is (RS)-9-fluoro-3- methyl- 10- (4- methylpiperazin- 1- yl)- 7- oxo- 2, 3-dihydro- 7H- pyrido[1, 2, 3, -de]-1, 4 benzoazeine-6-carboxylic acid (**fig. 2**).

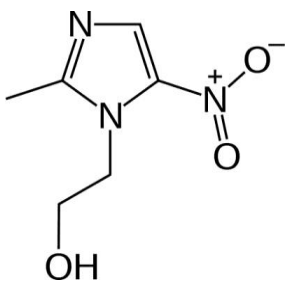

FIG. 1: METRONIDAZOLE (MET)

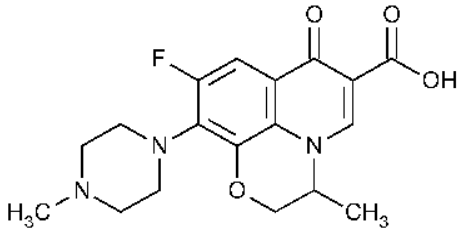

FIG. 2: OFLOXACIN (OFL)

Both drugs are official in Indian pharmacopeia [1], British Pharmacopeia [2] and United States Pharmacopeia [3]. The combination of MET and OFL is widely used in treatment of microbial infections. Literature search reveals that various analytical methods like UV-visible spectrophotometry [4, 5, 6] , conductometry [7], HPLC [8-15], and LC-MS [16-17] have been reported for estimation of MET and OFL in their individual and combined dosage forms with other drugs. There is no reported method for simultaneous estimation of MET and OFL in their combined dosage form [18].

This prompted the present work. The aim of the present work is to develop a simple, rapid, accurate and precise UV-visible spectrophotometric method for simultaneous estimation of MET and OFL in their marketed formulation.

MATERIALS & METHODS:

Apparatus: A Shimadzu model 1700 double beam UV–visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells was used to measure absorbance. Mettler Toledo analytical balance CX-204 was used for weighing and an ultrasonic cleaner (Frontline FS 4) were used in the study.

Reagents and materials: MET and OFL working standards were obtain from Nirlife- healthcare division of Nirma ltd., Sachana, Gujarat, India. The commercial fixed dose combination of MET and OFL (2:1) was procured from local market.

Acetonitrile (spectroscopic grade) and KH_2PO_4 obtained from Finar Chemicals, India was used for the study.

Preparation of Buffer Solution: Take 0.68 gms Dihydrogen ortho phosphate and mix with 100 ml water mix it in 1000ml volumetric flask. Now add 70 ml methanol and mix it. After mixing, make up the volume upto mark with the help of water. The pH of the Buffer is 9.3

Preparation of Standard Solvent Solution: Take 30ml Buffer in 100ml volumetric flask and make the volume upto mark with the help of 70ml Acetonitrile. Now make (acetonitrile and phosphate buffer 70:30) the standard solvent as par requirement. The pH of solvent is 9.89 and this solution used through out study in dilution.

Preparation of Standard Stock Solutions: Accurately weighed portion of MET (10 mg) and OFL (10 mg) were transferred to two different 100 mL volumetric flask. The volume was made up to mark with the prepared diluents solution to obtain standard stock solution having concentration of 100µg/mL of MET and OFL each.

Method I: Simultaneous Equation Method: Working standard solutions (10µg/mL) each of MET and OFL were scanned in range of 200-400 nm to determine the λ-max of both drugs. The λ-max of MET and OFL were found to be 318.0 nm and 294.0 nm respectively (**Fig. 3**).

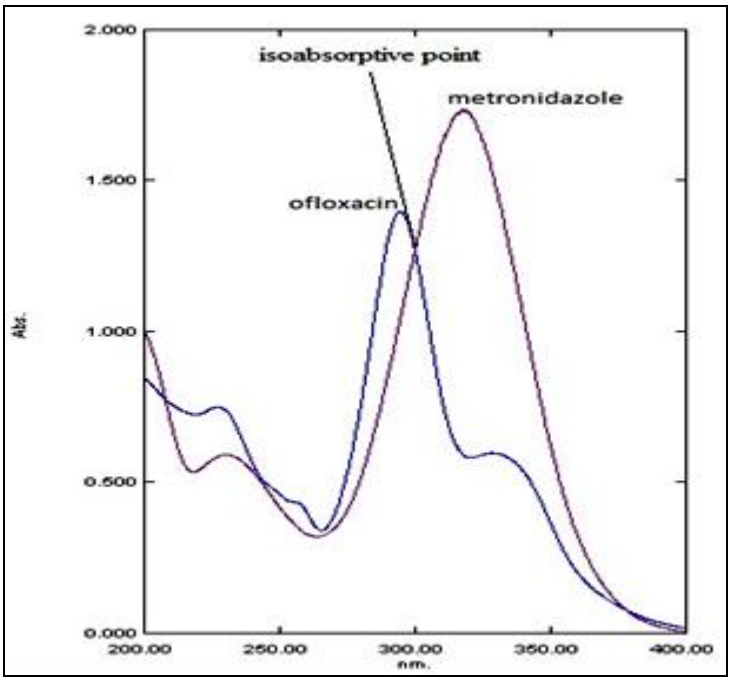

FIG. 3: OVERLAIN ABSORPTION SPECTRA OF MET AND OFL

Six standard solutions having concentration 5, 10, 15, 20, 25 and 30µg/mL for MET and 3, 6, 9, 12, 15 and 18µg/mL for OFL were prepared by appropriate dilutions from their respective standard stock solutions. The absorbances of resulting solutions were measured at 318.0 nm and 294.0 nm and absorptivity coefficients were calculated using Beer Lambert law. The graph of absorbance Vs concentration was plotted at each wavelength and regression coefficients were calculated (**fig. 4 and 5**).

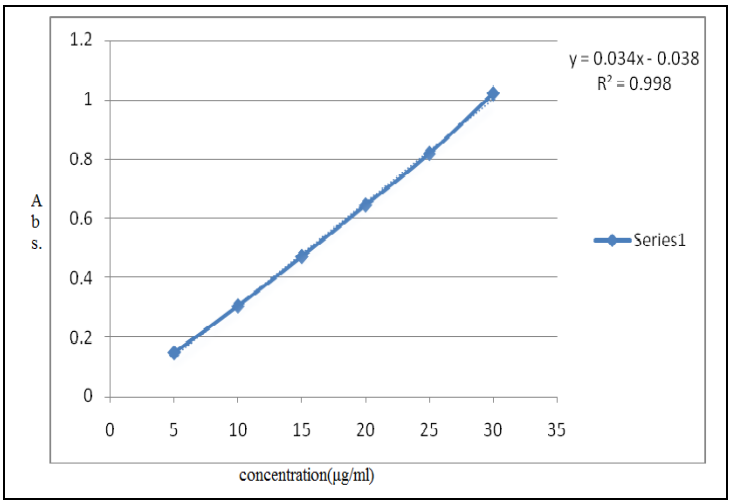

FIG. 4: CALIBRATION CURVE OF MET AT 318.0 nm

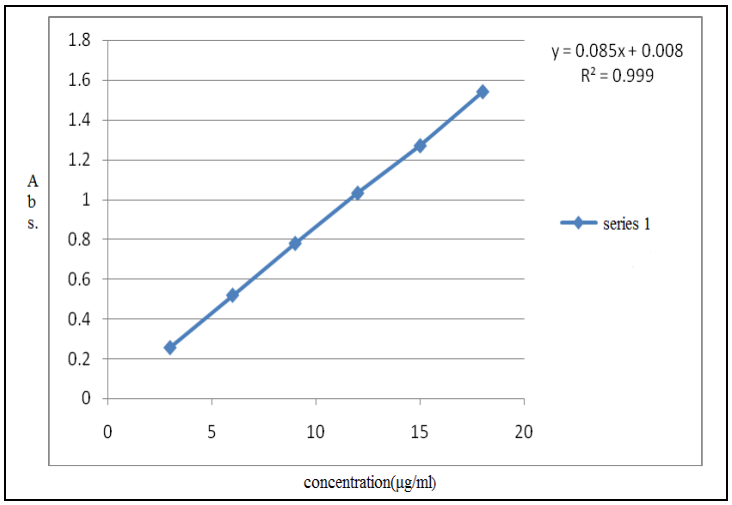

FIG. 5: CALIBRATION CURVE OF OFL AT 294.0 nm

The concentrations of both drugs were calculated by solving these simultaneous equations.

$C_x = (A_1 aY_2 - A_2 Ay_1) / (aX_1 aY_2 - aX_2 aY_1)$..... (1)

$C_y = (aX_1 A_2 - aX_2 A_1) / (aX_1 aY_2 - aX_2 a_{y1})$..... (2)

Where; Cx & Cy are concentrations of MET and OFL respectively in gm/100 ml in the sample solution.

A_1 & A_2 are the absorbances of the mixture at 318.0nm & 294.0 nm respectively

aX_1 and aX_2 =Absorptivity of MET at 318.0nm and 294.0nm

aY_1 and aY_2=Absorptivity of OFL at 318.0nm and 294.0nm

Method II: Absorbance ratio method (Q-Analysis): Absorbance ratio method uses the ratio of absorbances at two-selected wavelength one which is an "isoabsorptive point" and other being the λ-max one of the two components. From the overlay spectra of two drugs (Fig. 3) it is evident that MET and OFL showed an isoabsorptive point at 300 nm. The second wavelength selected as 294.0 nm, λ-max of OFL.

Six standard solutions having concentration 5, 10, 15, 20, 25 and 30µg/mL for MET and 3, 6, 9, 12, 15 and 18µg/mL for OFL were prepared by appropriate dilutions from their respective standard stock solutions. The absorbances of resulting solutions were measured at 300.0 nm and 294.0 nm and absorptivity coefficients were calculated using Beer Lambert law.

The graphs of absorbance Vs concentration were plotted at each wavelength and regression coefficients were calculated (fig. 4 and 5).

The concentration of two drugs in the mixture can be calculated using equations

$C_X = [(Q_M - Q_Y)/(Q_X - Q_Y)] \times A_1/aX_1$ .....(3)

$C_Y = (A_1/aX_1) - C_X$ .....(4)

Where; $Q_m = A_2/A_1$, $Q_x = ax_2/ax_1$, $Q_y = ay_2/ay_1$

1 designates isoabsorptive point and 2 designates λ-max of OFL

ax_1 and ax_2 is absorptivity of MET at 1 and 2 wavelength respectively

$ay_1$and ay_2 is absorptivity of OFL at 1 and 2 wavelength respectively

$A_1$and A_2 are absorbances of the mixture at 1 and 2 wavelength respectively.

Analysis of Marketed Formulation: For determination of the content of MET and OFL marketed formulation, pipette out accurately a volume of suspension equivalent to 100 mg of MET (50 mg OFL) in 100 mL volumetric flask. The volume was made upto the mark with prepared diluent and sonicated for 30 min. Further dilution was made from this solution to get final working concentration of MET (20µg/mL) [OFL (10µg/mL)].

For Method I, the absorbances of the sample solution i.e. A_1 and A_2 were recorded at318.0 nm and 294.0 nm respectively and concentration of two drugs in the sample were determined using above equation (1) and (2).

For method II, the absorbances of the sample solution i.e. A_1 and A_2 were recorded at 300.0 nm (isoabsorptive point) and 294.0 nm (λ-max of Ofloxacin) respectively and ratio of absorbance were calculated i.e. A_2/A_1. Relative concentration of two drugs in the sample was calculated using above equation (3) and (4). The result of analysis of marketed formulation is shown in **Table 1**.

TABLE 1: ANALYSIS OF MARKETED FORMULATION

Suspension	Label claim (mg/ml)		Amount found (mg/ml)		% Label claim* ± S. D.	
	MET	OFL	MET	OFL	MET	OFL
I	20.0	10.0	19.85	10.05	99.25±0.051	100.5±0.09
II	20.0	10.0	19.95	10.09	101.5±0.54	100.9±0.97

*Mean of five determination, I= simultaneous equation method, II= absorbance ratio method

Validation of Developed Methods: The proposed method has been statistically validated for linearity, accuracy, precision, repeatability and reproducibility, limit of detection (LOD) and limit of quantification (LOQ) as per ICH Q2 guidelines [19].

Linearity was observed in range of 5-30 µg/ml for MET (fig. 4) and 3-18µg/ml for OFL (fig. 5). For precision (intra day and inter day), absorbance of three different concentrations were measured for three times within day and for three consecutive days respectively for both developed methods. The % RSD values were found to be less than 2%.

To perform repeatability, absorbance of concentration nearer to assay concentration was measured for six times consecutively and %RSD was calculated. Reproducibility of the developed method was established by calculating %RSD values of the absorbance measured by different analyst. LOD and LOQ values were determined from mathematical equations. Accuracy (recovery) was determined by spiking different concentrations of pure Metronidazole and Ofloxacin standard (80%, 100% and 120%) in pre-analyzed samples. Absorbance was measured at 294, 300, 318 nm and % recovery was calculated (**Table 2**).

TABLE 2: RECOVERY STUDIES

Method	Amount of sample taken (µg/ml)		Amount of standard spiked (%)		Mean % Recovery ± S.D.	
	MET	OFL	MET	OFL	MET	OFL
I	10	6	80%	80%	99.07±0.100	99.44 ± 0.376
I	10	6	100%	100%	99.75 ± 0.484	100.2 ±0.107
I	10	6	120%	120%	99.24 ± 0.151	99.77 ±0.145
II	10	6	80%	80%	99.58 ± 0.99	99.85 ± 0.65
II	10	6	100%	100%	99.31 ± 0.75	100.6 ± 0.39
II	10	6	120%	120%	99.95 ± 1.2	99.6 ± 0.46

*n=3; I= simultaneous equation method, II= absorbance ratio method

The results of validation parameters are summarized in **Table 3**.

TABLE 3: SUMMARY OF VALIDATION PARAMETERS

Parameters	MET	OFL
Linearity range (n=6)	5-30 µg/ml	3-18 µg/ml
Equation	Y=0.034X-0.038	Y=0.085X+0.008
R^2	0.998	0.999
Mean % recovery	99.24-99.95	99.44-100.6
Intra day precision (%RSD) (n=3)	0.97-1.23	0.381-0.812
Inter day precision (%RSD) (n=3)	1.16-1.57	0.570-1.251
Repeatability (%RSD) (n=6)	1.36	0.38
Reproducibility (%RSD) (n=6)	0.97	0.14
LOD	0.1112 mg/ml	0.00446mg/ml
LOQ	0.337225 mg/ml	0.013515 mg/ml

RESULTS AND DISCUSSION: The first method employing simultaneous equation is a very simple method and can be employed for routine analysis of these two drugs. Once the absorptivity values are determined very little time is required for analysis, as would require determination of absorbances of the sample solution at two selected wavelength and few simple calculations. In absorbance ratio method (Method II), the primary requirement for developing a method for analysis is that the entire spectra should follow the beer's law at all the wavelength. This requirement was fulfilled in spectra of both the drugs (Fig. 3).

In this method, the calculations have been minimized by taking one of the measurements as an isoabsorptive point i.e. at 300 nm. As ratio is fixed for a specific mixture, the degree of dilution of two substances does not alter the value of ratio (A2/A1). Moreover, The value of standard deviation and coefficient of variation were satisfactory and recovery studies ranging from 99.24-99.95% (MET) and 99.44-100.6% (OFL) were indicative of the accuracy and precision the proposed methods.

CONCLUSION: The proposed methods for simultaneous estimation of MET and OFL were found to be simple, accurate, economical and rapid. Due to accuracy, precision and sensitivity, the developed simultaneous equation method and Q-ratio method could be used for routine analysis of MET and OFL in their combined dosage forms.

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