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Research Article

DEVELOPMENT OF UV-SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF OFLOXACIN AND ORNIDAZOLE IN COMBINED LIQUID ORAL DOSAGE FORM BY SIMULTANEOUS EQUATION METHOD

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ABSTRACT

A simple, sensitive and accurate UV-Spectrophotometric method for the quantitative estimation of Ofloxacin and Ornidazole in bulk and combined liquid oral dosage form has been developed and validated. Methanol was used as a solvent for estimation of ofloxacin and ornidazole in combined liquid dosage form. Both the standard solutions were scanned over the range of 400-200 nm in spectrum mode of spectrophotometer at medium scanning speed using U.V spectrophotometer 119, Systronics. The maximum absorbance for Ofloxacin and Ornidazole was found at 295.6 nm and 310.8 nm respectively. Estimation was carried out by simultaneous equation method. Both the drugs obeyed the Beer-Lambert's law and were found to be linear over the concentration range of 2-10 µg/ml for Ofloxacin and 5-25 µg/ml for Ornidazole. Percentage recoveries for Ofloxacin and Ornidazole were found to be in the range of 99.58 – 100.69 % and 99.86 – 101 %, respectively. The results of analysis were validated statistically.

Keywords: Ofloxacin, Ornidazole, UV-Spectrophotometric method, Quantitative Estimation.

INTRODUCTION

Ofloxacin (OFL) is a second-generation fluoroquinolone acting as antimicrobial agent. Chemically it is known as 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. Ornidazole (ORN) belongs to nitroimidazole class of drugs mainly used as tissue amoebicides, 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol[1-2]. The chemical structure of Ofloxacin and Ornidazole are shown in Figure 1 and 2 respectively. Literature review reveals that only a few analytical methods are reported for estimation of Ofloxacin and Ornidazole as a single component and in combination with other drugs[3-9] and it also reveals that there is no analytical method reported for estimation of Ofloxacin and Ornidazole in combined liquid oral dosage form. In this present work, the aim was to develop simultaneous equation (Vierodt's method) method for simultaneous estimation of both the drugs in combined liquid oral dosage form.

MATERIAL AND METHODS

Instrumentation

UV–Visible Spectrophotometer (UV spectrophotometer 119, Systronics, Software Version 1) with 1 cm matched quartz cuvettes were used for all absorbance measurements. Electronic Weighing Balance (Tapson's Analytical Balance) was used.

Chemicals and reagents used

Ofloxacin and Ornidazole reference substances were provided by Dodel Analytical Lab., Vapi, Gujarat, India. Suspension dosage forms (O2-Suspension, Medley) were procured from the local market, each 5 ml containing 50 mg of Ofloxacin and 125 mg of Ornidazole. All reagents and solvents used for study were of analytical grade.

Preparation of stock solution of Ofloxacin (OFL)

50 mg of standard OFL was weighed and transferred to 100 ml volumetric flask. OFL was dissolved in 35 ml methanol by gentle shaking and volume was made up to the mark with same solvent to obtain final concentration of 500 µg/ml and labeled as 'Std Stock OFL-A'.

From the 'Std Stock OFL-A' solution 2 ml of aliquot was pipetted out in a 25 ml volumetric flask and the volume was made up to the mark with methanol to obtain final concentration of 40 µg/ml and labeled as 'Std Stock OFL-B'.

Preparation of stock solution of Ornidazole (ORN)

125 mg of standard ORN was weighed and transferred to 100 ml volumetric flask. ORN was dissolved in 35 ml methanol by gentle shaking and volume was made up to the mark with same solvent to obtain final concentration of 1250 µg/ml and labeled as 'Std Stock ORN-A'.

From the 'Std Stock ORN-A' solution 2 ml of aliquot was pipetted out in a 25 ml volumetric flask and the volume was made up to the mark with methanol to obtain final concentration of 100 µg/ml and labeled as 'Std Stock ORN-B'.

Preparation of stock solution of Mix Standard Solution

50 mg of standard OFL and 125 mg of standard ORN was weighed and transferred to 100 ml volumetric flask. Dissolved in 35 ml methanol by gentle shaking and volume was made up to the mark with same solvent to obtain final concentration of 500 µg/ml OFL and 1250 µg/ml of ORN and labeled as 'Mixed Std Stock-A'.

From the 'Mixed Std Stock-A' solution 2 ml of aliquot was pipetted out in a 25 ml volumetric flask and the volume was made up to the mark with methanol to obtain final concentration of 40 µg/ml OFL and 100 µg/ml ORN and labeled as 'Mixed Std Stock-B'.

Preparation for Calibration Curve of OFL

From the 'Std Stock OFL-B' (40 µg/ml) solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml of aliquot was pipetted out in a series of 10 ml volumetric flasks. The volume was made up to the mark with methanol to obtain the concentration of 2, 4, 6, 8 and 10 µg/ml (OFL). Absorbance of the above solutions (2-10 µg/ml) was measured at 295.6 nm and a calibration curve was constructed by plotting absorbance *vs* concentration.

Preparation for Calibration Curve of ORN

From the 'Std Stock ORN-B' (100 µg/ml) solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml of aliquot was pipetted out in a series of 10 ml volumetric flasks. The volume was made up to the mark with methanol to obtain the concentration of 5, 10, 15, 20 and 25 µg/ml (ORN). Absorbance of the above solutions (5-25 µg/ml) was measured at 310.8 nm and a calibration curve was constructed by plotting absorbance *vs* concentration.

Analysis of O2-Suspension

Amount equivalent to about 5 ml of O2-Suspension containing 50 mg of OFL and 125 mg of ORN, was accurately weighed and transferred carefully to 100 ml volumetric flask containing 35 ml methanol, kept in a shaker for 20 min and then the volume was made up with the same to give a solution of 500 µg/ml of OFL and 1250 µg/ml of ORN. The resulting solution was filtered through Whatmann filter paper No. 41 and this solution was used as 'Sample Stock A'. From the 'Sample Stock A' solution 2 ml of the aliquot was pipetted out and transferred to a 25 ml volumetric flask. The volume was made up to the mark with methanol to obtain a solution with concentration of 40 µg/ml of OFL and 100 µg/ml of ORN and labeled as 'Sample Stock-B'. From this solution 1.5 ml of the aliquot was transferred to a 10 ml volumetric flask. The volume was made up to the mark with methanol to obtain a solution with concentration of 6 µg/ml of OFL and 15 µg/ml of ORN. The absorbance of above solution was measured at 295.6 nm and 310.8 nm.The amount of OFL and ORN present in the suspension were calculated using following equations,

$$c_x = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$c_y = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Where, c_x and c_y are the concentration of OFL and ORN respectively, A_1 and A_2 are the absorbances of mixture at 295.6 nm and 310.8 nm respectively, ax_1 and ax_2 are the absorptivities of OFL at 295.6 nm and 310.8 nm respectively, ay_1 and ay_2 are the absorptivities of ORN at 295.6 nm and 310.8 nm respectively.

Validation of Spectrophotometric Method

The method was validated by various parameters as recommended by ICH Guidelines[10-11].

(a) Accuracy

To study the accuracy, % recoveries has to be calculated, recovery studies were carried out by standard addition method by adding the known amount of OFL and ORN (reference standard) to the pre-analyzed sample at three different concentration levels i.e. 80%, 100%, and 120% of assay concentration and percentage recoveries were calculated.

From the above 'Sample Stock B' (OFL – 40 µg/ml and ORN – 100 µg/ml) solution 1.5 ml of the aliquot was pipetted out and transferred to three different 10ml volumetric flasks separately along with 1.2, 1.5, 1.8 ml of aliquot from the 'Mixed Std Stock-B' (OFL – 40 µg/ml and ORN – 100 µg/ml) solution. The volume was made up to the mark with methanol. All solutions were scanned over the spectrum mode in the range of 380 nm to 200 nm, the absorbance at 295.6 nm and 310.8 nm is taken into calculation and likewise from these data obtained, % recoveries were calculated.

(b) Precision

The precision of an analytical method was studied by performing Repeatability and Intermediate precision.

Repeatability

Six different standard mixture solution of the same concentration of OFL and ORN (6:15 µg/ml) and scanned over the range of 380 nm-200 nm in spectrum mode of spectrophotometer at medium scanning speed and the absorbance at 295.6 nm and 310.8 nm is taken into calculation. The standard deviation and % Relative standard deviation were also calculated.

Intermediate precision

(i) Intra-day Precision

Variation of results within the same day was analyzed. Intra-day precision was determined by measuring the standard mixture solution of OFL and ORN (6:15 µg/ml) at three different time intervals on the same day.

(ii) Inter-day Precision

Variation of results between the days was analyzed. Inter-day precision was determined by measuring the standard mixture solution of OFL and ORN (6:15 µg/ml) on three consecutive days.

(c) Linearity and Range

The linearity of analytical method for OFL and ORN were determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the log curve.

(d) Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of six calibration curves and average slope of six calibration curves.

$$LOD = 3.3 \times \frac{\text{Standard Deviation of intercept}}{\text{Slope}}$$

$$LOQ = 10 \times \frac{\text{Standard Deviation of Intercept}}{\text{Slope}}$$

RESULTS AND DISCUSSION

In this method, methanol was used as a solvent because both the drug's showed absorbance in the same. Ofloxacin was found to be absorbing prominently at 295.6 nm (λmax) while Ornidazole absorbed at 310.8 nm (λmax) and are describe in Figure 3. The linearity of analytical method at five concentration levels was ranging from 2-10 µg/ml and 5-25 µg/ml for Ofloxacin and Ornidazole respectively and is presented in Table 3. The regression equation of calibration curves were Y= 0.0803x + 0.184 and Y= 0.0391x+0.0448 for Ofloxacin and Ornidazole and are shown in Figure 4, Figure 5 respectively. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range. The correlation coefficient (r^2) was found to be 0.9994 and 0.9994 for OFL and ORN respectively. Thus these above data represents that simultaneous equation method obeyed Beer-Lambert's Law. The LOD was found to be 0.2985 µg/ml for OFL and 0.4557 µg/ml for ORN. LOQ was found to be 0.9103 µg/ml for OFL and 1.3809 µg/ml for ORN, respectively. The developed method was found to be accurate from % recovery studies, the results are shown in Table 2. The mean % Assay shown in Table 1 was found to be 96.48 % and 95.54 % for OFL and ORN respectively, it was obtained by comparing with the stated label claim. The results obtained had satisfactorily fulfilled the criteria.

CONCLUSION

The experimental results demonstrate that the proposed UV-Spectrophotometric method using simultaneous equation method is simple, rapid, sensitive, accurate, precise and economical. Thus this method can be used for the determination of Ofloxacin and Ornidazole either in bulk or in the combined liquid oral

dosage form. The excipients usually present in the pharmaceutical formulation did not interfere with determination of Ofloxacin and Ornidazole. Any of the developed method can be successfully used for routine quality control of Ofloxacin and Ornidazole in their combined dosage form.

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Table 1: Assay results of O2 suspension by Simultaneous Equation method

S. No.	Amount Present (mg/5ml)		Amount Found (mg/5ml)		% Assay	
	OFL	ORN	OFL	ORN	OFL	ORN
1	50	125	47.74	120.33	95.48	96.26
2	50	125	48.33	118.99	96.66	95.19
3	50	125	48.66	118.99	97.32	95.19
Mean			48.24	119.43	96.48	95.54
± SD			0.4660	0.7736	0.9321	0.6177
% RSD			0.9661	0.6477	0.9661	0.6465

OFL is *Ofloxacin*, ORN is *Ornidazole*, SD is *Standard Deviation* for n=3 observation, RSD is *Relative Standard Deviation*.

Table 2: Validation data for accuracy study

Level of % Recovery	Mean* (% Recovery)		± SD		% RSD	
	OFL	ORN	OFL	ORN	OFL	ORN
80%	100.06	100.02	0.5126	0.2112	0.5123	0.2112
100%	100.2	100.19	0.2645	0.4163	0.2640	0.4155
120%	100.18	100.38	0.4869	0.5493	0.4860	0.5471

Mean is Mean of 3 estimations, SD is *Standard Deviation* for n=3 observations, RSD is *Relative Standard Deviation*.

Table 3: Results of Validation and System Suitability Parameters

Parameters	OFL	ORN
Linearity Range (µg/ml)	2-10	5-25
Regression Equation (y = mx+c)	0.0803x+0.184	0.0391x+0.0448
Correlation Coefficient (r^2)	0.9994	0.9994
LOD (µg/ml)	0.2985	0.4557
LOQ (µg/ml)	0.9103	1.3809
Analysis of O2- suspension (% Assay)	95.48-97.32	95.19-96.26
% Recovery	99.58-100.69	99.83-101
Intra Day Precision (% RSD)	0.2398-0.6155	0.1846-0.5447
Inter Day Precision (% RSD)	0.2498-0.3142	0.4253-0.6448
Repeatability (% RSD)	0.3137	0.5254

OFL is *Ofloxacin*, ORN is *Ornidazole*, y = mx+c where y is *absorbance*, m is *slope*, c is *intercept*, LOD is *Limit of Detection*, LOQ is *Limit of Quantitation*, RSD is *Relative Standard Deviation*, min is *minute*, <3% is *Less than three percentage*.

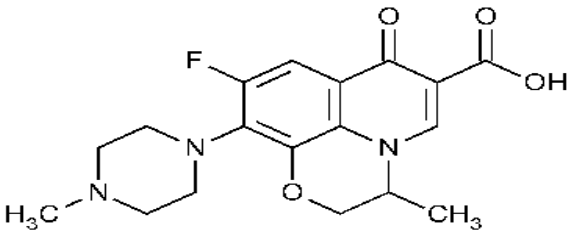

Fig. 1: Chemical structure of Ofloxacin (OFL)

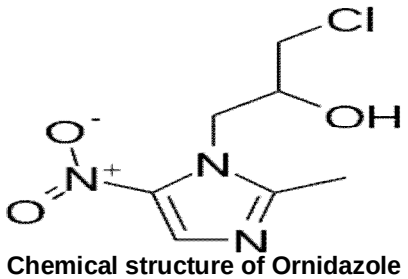

Fig. 2: Chemical structure of Ornidazole (ORN)

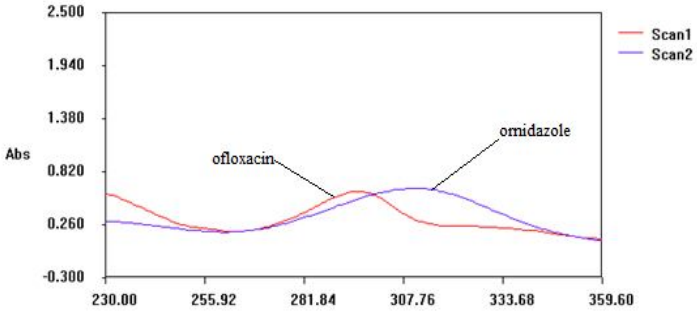

Fig. 3: Overlain Spectra of mixed standard solution of OFL (6 µg/ml), ORN (15µg/ml)

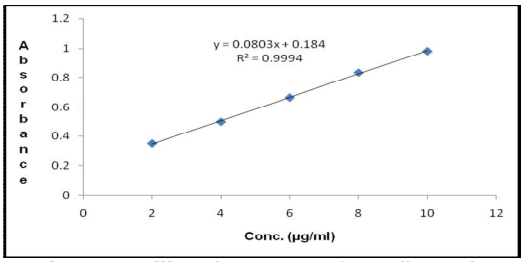

Fig. 4: Calibration Curve for Ofloxacin

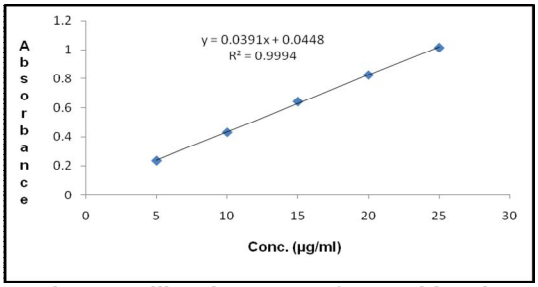

Fig. 5: Calibration Curve for Ornidazole

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