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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF CIPROFLOXACIN AND ORNIDAZOLE IN TABLET DOSAGE FORM

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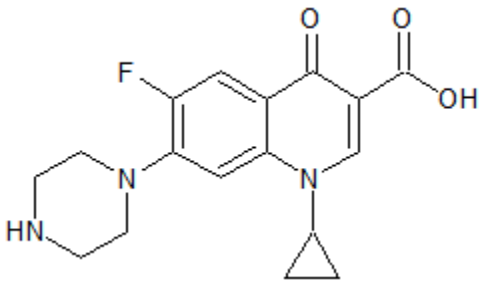
ABSTRACT

Three simple sensitive, rapid, accurate and economical methods were developed for simultaneous estimation of Ciprofloxacin & Ornidazole in bulk and its pharmaceutical formulations. First method was based on the simultaneous equation, second method was based on Q-Analysis (Absorbance ratio method) and third method was based on multicomponent mode. The absorption maxima for Ciprofloxacin and Ornidazole were 272 nm and 319 nm in 0.1 N NaOH respectively. The iso-absorptive point for Ciprofloxacin Hydrochloride and Ornidazole was 292 nm. The calibration curve was linear over the concentration range 2.5 to 22.5 µg/ml of Ciprofloxacin & Ornidazole. The methods had been validated statistically and by recovery study. It can be used in the simultaneous estimation of Ciprofloxacin and Ornidazole in bulk drugs and its pharmaceutical preparations in a routine manner.

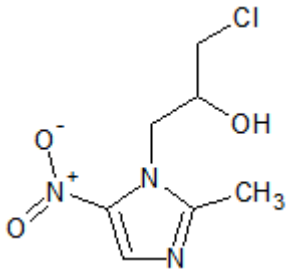
INTRODUCTION: Ciprofloxacin, chemically, 1-cyclopropyl-6-fluoro-1, 4- dihydro-4-oxo- 7 (piperazin-1-yl) quinoline-3-carboxylic acid1; belongs to the group of synthetic fluoroquinolone antibiotics with broad antimicrobial activity. Literature review reveals spectrophotometry [1-11], titrimetry [12], HPLC [13-18] and HPTLC [19] for its determination. Ornidazole, chemically, 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol, is an antimicrobial agent.

Ornidazole is used in the treatment of amoebiasis and other protozoal diseases. This drug is under the category antihelmentics. The combination of two drugs is not official in any pharmacopoeia; hence no official method is available for the estimation of ciprofloxacin and ornidazole in their combined dosage form. Literature review reveals that few methods are available for the determination of ornidazole in bulk and solid dosage form based on spectrophotometry [20-27] and RP-HPLC.

Ciprofloxacin and ornidazole in combined tablet dosage form is available in the market, has gained increasing acceptance in bacterial and protozoal infections. Spectrophotometric [28-29] and HPLC [30] method have been established for their simultaneous estimation in tablet dosage form. This paper presents three simple, sensitive, accurate and reproducible spectrophotometric methods for simultaneous determination of ciprofloxacin and ornidazole in tablet dosage form.



CIPROFLOXACIN



ORNIDAZOLE

FIGURE 1: STRUCTURE OF CIPROFLOXACIN AND ORNIDAZOLE

MATERIALS AND METHODS:

Instrument used: A Shimadzu UV-Vis Double beam spectrophotometer (Pharmaspec-1700) with matched quartz cells was used for all spectral measurements.

Materials: The tablets of the said combination were purchased from a local pharmacy (The label claim contained 500 mg of Ciprofloxacin and 500 mg of Ornidazole). Sodium hydroxide was of analytical grade chemical and distilled water was used to prepare all solutions.

Preparation of 0.1N Sodium Hydroxide Solution: 0.1 N NaOH was prepared by dissolving 4 g of sodium hydroxide in sufficient distilled water to produce 1000 ml.

Preparation of Standard Solutions: The standard stock solution of Ciprofloxacin and Ornidazole were prepared by dissolving 50 mg of each drug in 50 ml of 0.1 N NaOH. Stock solutions of Ciprofloxacin and Ornidazole were further diluted in 0.1 N NaOH to get working standard solutions of concentration 100 µg/ml.

Method-I: Simultaneous equation method (Vierodt's method): If a sample contains two absorbing drugs (X and Y) each of which absorbs at the I_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equation (Vierodt's method) provided that certain criteria apply.

a) **Study of Beer's-Lamberts law:** The standard solutions of Ciprofloxacin Hydrochloride (20 µg/ml) and Ornidazole (20 µg/ml) in 0.1 N NaOH were scanned in the entire UV range to determine λ_{max} of both the drugs. The λ_{max} of Ciprofloxacin hydrochloride and Ornidazole were found to be 272 nm and 319 nm, respectively. A series of standard solutions were prepared having concentration range of 2.5 to 22.5 µg/ml were prepared in 0.1 N NaOH using working standard solution. The absorbance of resulting solution was measured at 278 nm and 319 nm for both Ciprofloxacin HCl and Ornidazole, and calibration curves plotted at these wavelengths. Both the drugs obeyed linearity individually within the concentration range 2.5 - 22.5µg/ml. The overlay UV spectrum of Ciprofloxacin Hydrochloride and Ornidazole is shown in **Fig. 2**.

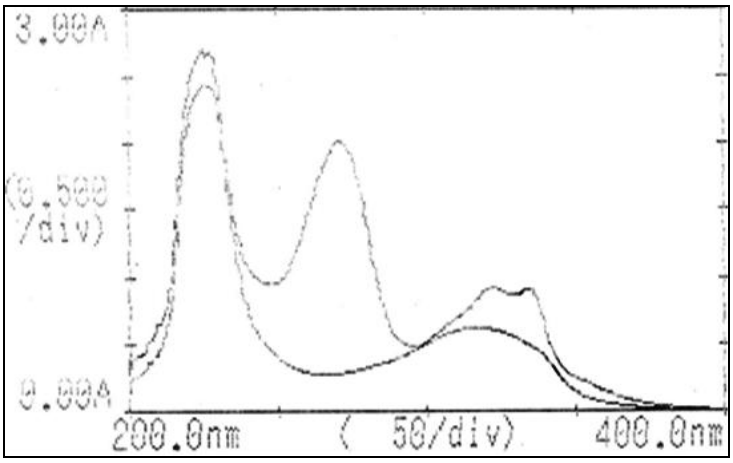


FIGURE 2: THE OVERLAY UV SPECTRUM OF CIPROFLOXACIN HYDROCHLORIDE AND ORNIDAZOLE

b) **Estimation of Absorptivity (E 1%, 1cm) values at selected wavelengths:** The Absorptivity (E 1%, 1cm value) of Ciprofloxacin HCl and Ornidazole drugs were calculated at 272 nm and 319 nm. Two simultaneous equations were formed using these values as given below.

$C_X = 0.0145714\ A_1 - 0.0050635\ A_2$ ..... (1)

$C_Y = 0.039518\ A_2 - 0.0145311\ A_1$ ..... (2)

Where, A_1 and A_2 are the absorbance of sample at 272 nm and 319 nm respectively. C_X and C_Y are the concentration of Ciprofloxacin HCl and Ornidazole in sample respectively.

c) Analysis of standard mixture: The mixture of pure Ciprofloxacin HCl (10 µg/mL) and Ornidazole (10 µg/mL) was prepared from stock solution in 0.1 N NaOH. Their absorbance value at the two selected wavelength was recorded and quantitative estimation of the drugs was carried out by solving simultaneous equations.

d) Analysis of Tablet Formulation: Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to about 50 mg of Ciprofloxacin & Ornidazole; was taken in 50 mL volumetric flask and dissolved in 25 mL of 0.1 N NaOH ; it was further diluted up to the mark with same solvent. The solutions were then filtered and filtrate gets diluted to get final 10 µg/mL concentration of Ciprofloxacin HCl & Ornidazole. The solution was then read at two selected wavelength. The results of tablet analysis are given in **Table 1**.

Method-II: Q - Analysis (Absorbance Ratio Method): Q-Absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelength, the ratio of absorbances at any two wavelengths is a constant value independent of concentration or path length. In the quantitative assay of two components in a mixture by the absorbance ratio method, absorbances are measured at two wavelengths: One being the λ_{max} of one of the component (λ_2) and the other being a wavelength of equal absorptivities of the two components i.e. an iso-absorptive point.

a) **Determination of Iso-absorptive point and selection of suitable Wavelength:** An iso-absorptive point (a wavelength of equal absorptivity of the two components) was determined by taking overlain spectrum of the solutions ciprofloxacin HCl and Ornidazole (20 µg/ml each) in 0.1 N NaOH in UV range against the solvent blank. From the overlain spectra of the two drugs, it was found that ciprofloxacin showed λ_{max} at 272 nm and ornidazole showed λ_{max} at 319 nm. Iso-absorptive point was found out at 292 nm. The two wavelengths 272 nm at which ciprofloxacin exhibits maximum absorption and 292 nm as iso-absorptive point were selected for estimation of drugs simultaneously.

b) Study of Beer-Lambert's law: The solutions having concentrations in range 2.5 - 22.5 µg/ml for both ciprofloxacin HCl and ornidazole were prepared in 0.1 N NaOH using working standard solution. The absorbances of resulting solutions were measured at 272 nm and 292 nm. Calibration curves were plotted at these wavelengths. Both the drugs obeyed linearity individually and combination within the concentration range of 2.5 - 22.5 µg/ml for both ciprofloxacin & Ornidazole.

c) **Estimation of Absorptivity (E 1%, 1cm) values at Selected Wavelengths:** The Absorptivity (E 1%, 1cm value) of Ciprofloxacin HCl and Ornidazole drugs were calculated at 272 nm and 319 nm.

d) **Analysis of Standard Mixture:** The laboratory mixture of Ofloxacin (5 µg/mL) and ornidazole (12 µg/mL) was prepared from stock solution in 0.5 N acetic acid The absorbance value at the two selected wavelength was recorded The contents of Ciprofloxacin and Ornidazole were calculated by substituting values in the formulae given below.

$$C_x = (Q_M - Q_Y)\, A_1 / (Q_X - Q_Y)\, a_{x1}$$

$$C_y = (Q_M - Q_x)\, A_1 / (Q_y - Q_x)\, a_{x1}$$

Where, C_x=concentration of Ciprofloxacin; C_y=concentration of Ornidazole; Q_x= ratio of absorptivity of ciprofloxacin at 292 nm and 272 nm; Q_y= ratio of absorptivity of Ornidazole at 292 nm and 272; Q_M= absorbance ratio of mixture at 292 nm and 272 nm; a_{x1} and a_{y1}=absorptivity of pure Ciprofloxacin and Ornidazole respectively at iso-absorptive wavelength.; A_1=absorbance of mixture at iso-absorptive point.

e) Analysis of tablet formulation: Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to about 50 mg of ciprofloxacin was taken in 50 ml volumetric flask and dissolved in 25 ml of 0.1 N NaOH; it was diluted up to the mark with 0.1 N NaOH. The solutions were then filtered and filtrate gets diluted to get final concentration of ciprofloxacin HCl (10 µg/ml) and Ornidazole (10 µg/ml). The solution was then read at two selected wavelength. The results of tablet analysis are given in Table 1.

Method-III: Multi-component mode: From the overlain spectra of the two drugs, the two wavelengths selected as sampling wavelengths were 272 and 319 nm which are absorbance maxima for Ciprofloxacin HCL and Ornidazole respectively. Now the sampling wavelengths and concentrations of the two

components in each of the mixed standards were fed to the instrument using multi-component mode of the instrument and all the seven mixed standards were scanned in the range of 400 to 200nm. The instrument collects and compiles the spectral data from the mixed standards and gets ready for the quantitative analysis of the samples.

Validation of Analytical Methods: As per I.C.H. guidelines.

Linearity and range: 2.5 – 22.5 µg/ml

Precision: For evaluation of precision of the results a concentration of 10µg/ml was evaluated by 4 replicate determinations. S.D. was found to be less than 0.1. Precision study data is given in **Table 2**.

Accuracy (Recovery study): It was found out by recovery study using standard addition method. Known amounts of standard Ciprofloxacin and Ornidazole was added to pre-analyzed samples at a level from 80% up to 120% and then subjected to the proposed methods. Results of recovery studies are shown in **Table 3**.

TABLE 1: ANALYSIS OF CIPROFLOXACIN AND ORNIDAZOLE IN TABLETS

Method	Label claim (mg/tab)		Amount found* (mg/tab)		% Label claim* ± S.D.	
	CIP	ORD	CIP	ORD	CIP	ORD
I	500	500	502.87	502.48	100.57±0.977	100.49±0.255
II	500	500	502.87	501.45	100.57±0.381	100.50±0.693
III	500	500	502.52	502.57	100.51±0.86	100.52±0.198

*Average of five determinations, CIP-Ciprofloxacin, ORD-Ornidazole

TABLE 2: PRECISION DATA OF CIPROFLOXACIN AND ORNIDAZOLE

Drug used	Conc. (µg/ml)	Absorbance	S.D.
Ciprofloxacin (272 nm)	10	0.763	0.006
	10	0.774	
	10	0.758	
	10	0.768	
Ornidazole (319 nm)	10	0.318	0.007
	10	0.334	
	10	0.328	
	10	0.321	

TABLE 3: RESULT OF RECOVERY STUDY

Method	Level of recovery	% Recovery* ± S.D.	
		CIP	ORD
I	80 %	100.02±1.14	99.83±1.06
	100 %	100.60±0.96	100.75±1.01
	120 %	100.79±1.29	100.45±0.88
II	80 %	100.44±0.88	100.18±0.97
	100 %	100.75±0.72	100.25±1.42
	120 %	100.23±1.73	99.34±1.29
III	80 %	99.44±0.89	100.18±1.55
	100 %	100.25±0.62	100.25±1.42
	120 %	100.73±1.63	101.34±1.29

*Average of five determinations, CIP-Ciprofloxacin, ORD-Ornidazole

RESULTS AND DISCUSSION: The proposed methods were found to be simple, accurate and reproducible for routine simultaneous estimation of ciprofloxacin and ornidazole in combination. In 0.1 N sodium hydroxide solution, ciprofloxacin and ornidazole showed λ_{max} at 272 nm and 319 nm respectively. As their λ_{max} differ more than 20 nm; simultaneous equation method tried for their simultaneous estimation in formulation. Also iso-absorptive point was observed at 292 nm so the absorbance ratio method was tried for simultaneous estimation of drugs in formulation at 272 nm (λ_{max} of ciprofloxacin) and

292 nm (iso-absorptive wavelength). The calibration curve was linear over the concentration range 2.5 to 22.5µg/ml of Ciprofloxacin & Ornidazole. By observing the validation parameters, all the methods were found to be simple, specific, accurate and precise. Recovery experiments indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients.

All the methods were successfully used to determine the amounts of ciprofloxacin and ornidazole present in the tablets. The results obtained were in good agreement with the corresponding labelled amount. Hence, all the three methods can be employed for the routine analysis of these two drugs in combinations.

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