Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

APPLICATION OF UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF CIPROFLOXACIN AND TINIDAZOLE IN COMBINED TABLET DOSAGE FORM

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Received: 13 March 2012, Revised and Accepted: 21 April 2012

ABSTRACT

Two simple, sensitive, rapid, accurate and precise simultaneous UV-spectrophotometric methods have been developed and validated for the estimation of ciprofloxacin and tinidazole in bulk and combined tablet dosage form. The first method is based on simultaneous equation, for the determination of sampling wavelengths, each of ciprofloxacin and tinidazole were scanned in the Wavelength range of 200-400 nm in spectrum mode and sampling wavelengths were selected for analysis were 280 (λ max of ciprofloxacin) and 310 (λ max of tinidazole) respectively in methanol: water (50:50%v/v). Beer's law obeyed in the concentration range of 2-16µg/ml for the both drugs. The second method based on measurement of absorbance at isoabsorptive point at 291 nm. Beer's law obeyed in the concentration range of 2-16µg/ml for the both drugs. The correlation coefficients and mean recoveries were found satisfactory by both methods. The proposed methods can be successfully applied for simultaneous estimation of ciprofloxacin and tinidazole.

Keywords: Ciprofloxacin hydrochloride, Tinidazole, UV-Spectroscopy, Simultaneous equation, Q analysis.

INTRODUCTION

Ciprofloxacin hydrochloride **(CIP)** [1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone-carboxylic acid], is a recently developed fluoroquinolone antibacterial compound with a broad spectrum of activity .It has particularly enhanced activity against gram-positive organisms[1, 2, 3]. It is rapidly absorbed with a time to maximum plasma concentration (Tmax) of 1- 2h and displays linear pharmacokinetics over the dosage range studied (250 to 750 mg), with half-life (t1/2) after single or repeated administration of about 3-5 h. A minimum of 70% of the oral dose is excreted unchanged in the urine. Plasma protein binding of ciprofloxacin is about 30%. Tinidazole **(TIN)** (1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole) antiprotozoal and antibacterial drug.[4,5]The structures of these two drugs are shown in Fig. 1.These drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin[6]. There are several methods reported for estimation of drug in single or in combination by HPLC[7, 8, 9], UV-spectrophotometry[10, 11,12,13,14]. In present methods the use of methanol: water: 50:50%v/v is used as a solvent that is not used earlier methods for simultaneous of CIP and TIN by simultaneous equation and Q method. The present method is relying on the use of simple chemicals than reported and technique provides sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC and HPTLC.

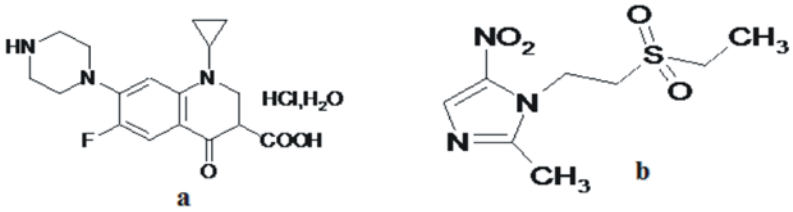

Fig. 1: Structure of Ciprofloxacin hydrochloride a and Tinidazole b.

MATERIALS AND METHODS

A double beam, Shimadzu 2450 UV/Vis spectrophotometer connected to HCL computer loaded with UV probe 2.21 software was used in the current investigation. CIP and TIN were supplied by Aarti drugs Mumbai. A methanol (Fischer Scientific (India)) and doubled distilled water were used for this work. A commercial pharmaceutical preparation Ciplox-TZ of CIPLA (containing CIP 500 mg and TIN 600 mg /tablet) was used for analysis.

Standard stock solution

Standard stock solutions containing CIP and TIN were prepared individually by dissolving 10 mg of CIP and TIN in 50:50%v/v methanols: water in 100 ml volumetric flask to dissolve the content and further diluted with same. This gave concentration of 100µg/ml. Respectively for CIP and TIN and these solutions were used as standard stock solution for the further analysis.

Selection of analytical wavelength

Using appropriate dilution of working standard stock solutions, 12µg/ml of CIP and 12µg/ml of TIN were separately prepared and scanned in the UV range 200-400 nm. The overlain zero-order absorption spectra of CIP and TIN were obtained showing absorption maxima at 280 nm (λ1max of CIP) and 310 nm (λ2max of TIN). The overlain spectra showed isoabsorptive point at 291 nm (fig.2).

Determination of absorptivity at analytical wavelength

For each drug appropriate aliquots were pipette out from standard stock solution and a series of dilutions of different concentrations were made for CIP and TIN, the concentration range taken was 2-16 µg/ml for both drugs. The absorbances were measured at respected wavelength. The absorbance was then divided by concentration in gm/l to get absorptivity.

Absorptivity values (Method I Simultaneous Equation Method)

$$C_{CIP} = \frac{(A_2a_{y1} - A_1a_{y2})}{(a_{x2}a_{y1} - a_{x1}a_{y2})} \text{ -------- (1)}$$

$$C_{TIN} = \frac{(A_1a_{x2} - A_2a_{x1})}{(a_{x2}a_{y1} - a_{x1}a_{y2})} \text{ -------- (2)}$$

Where,

C_{CIP} = concentration of CIP in gm/100 ml.

C_{TIN} = concentration of TIN in gm/100 ml.

ax1 = absorptivity value of CIP at 280 nm.

ax2 = absorptivity value of CIP at 310 nm.

ay1= absorptivity value of TIN at 280 nm.

ay2= absorptivity value of TIN at 310 nm.

A1 = absorbance of tablet mixture at 280 nm

A2 = absorbance of tablet mixture at 310 nm.

Absorptivity values (Method II Absorption ratio/ Q Method)

The overlain zero-order absorption spectra of CIP and TIN were obtained showing isoabsorptive point at 291 nm (fig.2). The two wavelengths were selected one as 291 nm (isoabsorptive point) and other 310 nm (wavelength absorption maxima of TIN), series of dilutions of different concentrations were made for CIP and TIN, the concentration range taken was 2-16 µg/ml for both drugs. The absorbances were measured at respected wavelength. The absorbance was then divided by concentration in gm/l to get absorptivity. The Q value is used for the determination of concentrations of drugs in sample solution. The following formulae are used in the method.

$$C_{CIP} = \frac{Qm - Qy \times A}{Qx - Qy \; Ax1} \text{ -------- (3)}$$

$$C_{TIN} = \frac{Qm - Qx \times A}{Qy - Qx \; Ay1} \text{ -------- (4)}$$

Cx = concentration of CIP in gm/100 ml.

Cy = concentration of TIN in gm/100 ml.

ax1 = absorptivity value of CIP at 291 nm.

ax2 = absorptivity value of CIP at 310 nm.

ay1= absorptivity value of TIN at 291 nm.

ay2= absorptivity value of TIN at 310 nm.

A1 = absorbance of laboratory mixture at 291 nm.

A2 = absorbance of laboratory mixture at 310 nm.

In method I, the concentration of both CIP and TIN were determined by measuring the absorbance of the sample solution at 280 nm and 310 nm. Values were substituted in the respective formula to obtained concentrations (Table 2).

In method II, the concentration of both CIP and TIN were determined by measuring the absorbance of the sample solution at 291 nm and 310 nm. Values were substituted in the respective formula to obtained concentrations (Table 2).

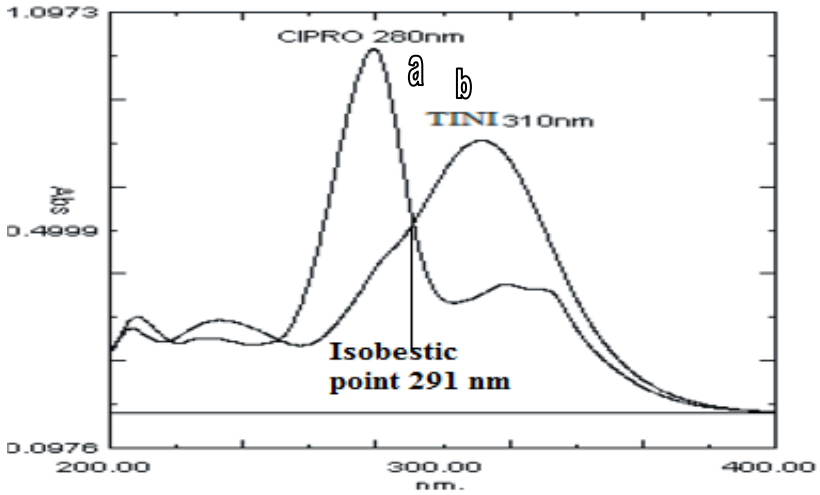

Fig. 2: Overlain UV Spectra of CIP (12µg/ml) and TIN (12µg/ml) in methanol: water 50:50%v/v

Table 1: Optical characteristics of proposed methods

Parameters	Method I		Method II	
	CIP	TIN	CIP	TIN
Beer's law range (µg/ml)	2-16	2-16	2-16	2-16
Correlation coefficient (r2)	0.9997	0.9995	0.9978	0.9998
Slope	0.1661	0.0304	0.0655	0.0536
Intercept	0.0017	0.0815	-0.0028	0.0171
DL (µg/ml)	0.011	0.014	0.039	0.039
QL (µg/ml)	0.033	0.042	0.13	0.11

Table 2: Result of analysis of samples

Analyte	Method I		Method II	
	CIP	TIN	CIP	TIN
% Conc. estimated* (mean±RSD)	99.60±0.53	100.03±0.17	99.94±0.13	99.36±0.91

Where, *Average of four determinations, RSD= Relative standard deviation

Analysis of tablet formulation

Twenty tablets of marketed formulation containing 600 mg of CIP and 500 mg of TIN were finely powdered. The powder equivalent to 10 mg of CIP is weighed and required amount of TIN was added to get concentration of TIN in linearity range. Sample solutions were analyzed to get the spectra, absorbance measured at 280 nm and 310 nm for method I and 291 and 310 nm for method II. The concentration of each drug was then calculated by using equations (1) and (2), for simultaneous equation method and equation (3) and (4) used for Q method. Procedure was repeated four times for analysis of homogenous sample (Table.3).

Table 3: Result of analysis of tablet samples and recoveries study

Method	Drug	Label claimed	% Label claimed*±RSD	% Recovery*±RSD
I	CIP	600 mg	100.18±0.32	100.34±0.35
	TIN	500 mg	99.39±0.86	99.09±0.63
II	CIP	600 mg	99.48±0.67	100.04±0.68
	TIN	500 mg	99.53±0.71	99.94±0.50

Method Validation

Linearity

The linearity was evaluated by analyzing different concentration of standard solution of CIP and TIN. For simultaneous equation and Q method, beer's law obeyed in the concentration range of 2-16µg/ml for the both drugs

Precision

Precision of the method was evaluated by using tablet powder equivalent to 100% of the label claim of CIP and TIN. Method repeatability was obtained from R.S.D. value by repeating assay of four replicates of single concentration three times in a same day. Intermediate precision was assessed by assay of four replicates of single concentration of CIP and TIN on three consecutive days (Table.4).

LOD and LOQ

The limit of detection and the limit of quantitation were calculated on basis standard deviation of y-intercept and slope,

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Where σ = Standard deviation. S = slope of calibration curve of analyte.

Recovery

A recovery study was carried out by addition of known amount of standard drug in the preanalyzed tablet formulation, in 80%, 100% and 120% of label claim. At each level of amount three determinations was performed (Table.3).

Statistical evaluation

The ANOVA test was applied to determine whether there is significant difference between the results of analysis by two different analysis methods. The p value was found for CIP and TIN which is greater than theoretical p value ($p>0.5$) showing there is no statically significant difference between two methods.

Table 4: Result of analysis of Intra and Inter day precision.

Day	Method I		Method II	
	CIP	TIN	CIP	TIN
% Label claimed estimated (mean±RSD)				
Intra day	99.59±0.25	99.66±0.62	100.31±0.22	99.61±0.58
Inter day	99.93±0.095	99.55±0.26	100.13±0.38	99.53±0.46

RESULT AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of CIP and TIN. In simultaneous equation method, wavelengths selected for analysis were 280.0 nm (λmax of CIP) and 310.0 nm (λmax of TIN), and in Q analysis wavelengths selected for analysis were 291.0 nm (isobestic point) and 310.0 nm (λmax of TIN) The method linearity for detector response was observed in the concentration range of 2-16 µg/ml for both CIP and TIN. Absorptive coefficients were calculated for both the drugs at selected wavelengths and substituted in equations for determining concentration of CIP and TIN in tablet sample solution. Percent label claim for CIP and TIN in tablet analysis was found 100.18% and 99.39 % respectively for method I and 99.48% and 99.53 % respectively for method II. Standard deviation and coefficient of variance for four determinations of tablet sample was found to be less than ± 2.0 indicating the precision of the method. Accuracy of proposed methods was ascertained by recovery studies. The mean percent recovery for CIP and TIN by both the methods was found in the range of 100.04-100.34 % and 99.09-99.94 % respectively. The proposed methods can be employed for routine quality control of Ciprofloxacin and Tinidazole in combined dosage tablet formulation.

CONCLUSION

The developed methods proved to be simpler in procedure and it produced more accurate results. Hence method is effective for the routine analysis of Ciprofloxacin and Tinidazole in bulk and tablet dosage form.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Aarti drugs, Mumbai, for providing gift sample of ciprofloxacin and tinidazole. The authors are also thankful to Principal and Management of MGV's Pharmacy College, Panchavati, Nashik for providing necessary facilities and constant support for research work.

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