Research Article

Development and Validation of Analytical Methods for Simultaneous Estimation of Ciprofloxacin and Tinidazole in Bulk and Marketed Formulation

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ABSTRACT

The present work deals with the aim of simultaneous estimation of Ciprofloxacin(CIPRO) and Tinidazole(TINI) in tablet dosage form by using two methods that is simultaneous equation by matrix & absorption correction. For simultaneous equation by matrix method and absorption correction method the wavelengths selected were 272nm i.e. maximum absorbance of CIPROand 317nm i.e. maximum absorbance of TINI. Both the drugs obey Beer-Lamberts law within the range of 2-10 μg/ml respectively. The % recovery was found in within limit for both drugs. Calibiration curve gaves good linearity in the range of 0.99-0.94 .The method was validated with respect to linearity,accuracy,precision and ruggedness. Recovery study confirmed the accuracy of the proposed methods. From the studies it can be concluded that UV spectrophotometric methods are accurate, precise, simple and sensitive.

Keywords : Absorption correction method,Ciprofloxacin, Tinidazole.

INTRODUCTION

CIPRO is 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid, (**Figure 1**).It is a broad-spectrum antibiotic active against both Gram-(+) and Gram-(-)bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division.

TINI chemically is 1-[2-(ethanesulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole, (**Figure 2**). Itis a prodrug and antiprotozoal agent. The nitro group of tinidazole is reduced in *Trichomonas* by a ferredoxin-mediated electron transport system. The free nitro radical generated as a result of this reduction is believed to be responsible for the antiprotozoal activity.

The literature reveals that several UV spectrometric method are available for EBS and MON in individual[3,4] respectively and simultaneous estimation with other drugs[5-9] respectively. A number of RP-HPLC methods are reported for MON in individual[10,11]. Also number of RP-HPLC methods are reported for simultaneous estimation of EBS and MON with other drug[12,13] respectively. But no method has been reported for their simultaneous estimation of EBS and MON in combined tablet dosage form for proposed methods.

MATERIALS AND METHODS

Apparatus

The instrument used for the analysis was UV double beam spectrophotometer (Thermo Japan) having two matched cells with 1-cm light path wavelength accuracy of ± 0.5nm with automatic wavelength correction with a pair of 10mm quartz cells. A Citizen Digital Balance (CY 104) analytical balance was used for weighing the sample and an ultrasonic bath were used in the study.

Reagents and materials

Giftsample of CIPROand TINIwere procured from Leben Laboratories ofAkola respectively . Whereas their formulation (Ciprowin TZ-250) obtained from local market.

Preparation of standard stock solutions

An accurately weighed quantity of CIPRO (10 mg) and TINI (10 mg) were transferred to a separate 10 ml volumetric flask and dissolved and diluted to the mark with distilled water. Take 1 ml of above solution into 10 ml volumetric flask and dilute the mark with distilled water to obtain standard solution having concentration of CIPRO (100 µg/ml)

and TINI (100 µg/ml). This solution was used as working standard solution.

METHODS

Ten tablets were weighed. Average weight was calculated. They were triturated in glass mortar. The accurately weighed quantity of powder was transferred into 50 ml volumetric flask, dissolved and diluted up to mark with distilled water. The solution was sonicated for 15minutes and centrifuged for another 15 min at 100 rpm. Filter the solution through Whatman filter paper no.42 and discard first few drops of filtrate. Pipette out 1ml of the above solution in 10ml volumetric flask and diluted to mark with distilled water. Absorbance of the resulting solution was measured at 272.0 nm, 317.0nm and 300.0 nm against distilled water and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations[14]:

Equation for absortion correction :

$$Cx = \frac{A1}{ax1} \text{.....(1)}$$

$$Cy = \frac{A2 - ax2\ Cmax}{ay2} \text{.....(2)}$$

Where,

A1 and A2 are the absorbances of mixture at 272.0 nm and 317.0 nm respectively,

ax1 and ax2 are absorptivities of CIPRO at 272.0 nm and 317.0 nm respectively,

ay1 and ay2 are absorptivities of TINI at 272.0 nm and 317.0 nm respectively.

Equation for simultaneous equation by matrix method :

$$A_1 = ax_1\ b\ C_x + ay_1\ b\ C_Y \text{.....(3)}$$

$$A_2 = ax_2\ b\ C_x + ay_2\ b\ C_Y \text{.....(4)}$$

Where,

A_1 and $A_2$are absorbances of CIPRO and TINI at 272.0 nm &317.0 nm respectively,

$ax_1$and $ax_2$are absorptivity of CIPRO at 272.0 nm &317.0 nm respectively,

ay_1 and ay_2 are absorptivity of TINI at 272.0nm &317.0nm respectively,

C_X,C_Y = Concentration at CIPRO and TINI respectively.

b = pathlength

VALIDATION OF PROPOSED METHODS

Linearity

Different dilutions were prepared from the working stock solution of 100µg/ml of CIPRO and TINI respectively by dissolving 6 to 14µg/ml of each drug in 10ml of GR grade methanol it become 60% to 140% concentration respectively. Then absorbance of solutions was then measured at the respective analytical wavelengths such as 252.0 nm, 344.0 nm and 280.0 nm for both methods. The calibration curves were plotted between the value of the observed absorbance and respective concentration (**Figure 3 and Figure 4).**

Precision

Intraday Precision

Mixed solutions containing 10 µg/ml CIPRO and 10 µg/ml TINI was analyzed 3 times on the same day and % RSD was calculated on respective wavelength for both methods.

Interday Precision

Mixed solutions containing 10µg/ml CIPRO and 10µg/ml TINI was analyzed on 3 different day and % RSD was calculated on respective wavelength for the both methods.

Accuracy

The accuracy of the both methods was determined by calculating recoveries of CIPRO and TINI in mixture by the standard addition method. Known amount of standard solutions of CIPRO (0.1, 0.2, 0.3and 0.4 µg/ml) and TINI (0.1, 0.2, 0.3 and 0.4 µg/ml) were added to a pre-quantified sample solution of 100 µg/ml CIPRO and 100 µg/ml TINI mixtures. The absorbance of CIPRO and TINI were recorded at 272.0 nm, 317.0 nm and 300.0 nm for both methods . The percentage recovery was calculated by measuring the absorbance of both drug at their absorbance maxima and fitting these values into simultaneous equation by matrix and absorption correction equations. Each response was average of three determinations.

Analysis of EBS and MON in combined tablet

Ten tablets were weighed. Average weight was calculated. They were triturated in glass mortar. The accurately weighed quantity of powder was transferred into 50 ml volumetric flask, dissolved and diluted up to mark with distilled water. The solution was sonicated for 30 minutes and centrifuged for another 20 min at 100 rpm. Filter the solution through Whatman filter paperno.42 and discard first few drops of filtrate. Pipette out 1ml of the above solution in 10ml volumetric flask and diluted to mark with distilled water. Absorbance of the resulting solution was measured at 272.0 nm, 317.0nm and 300.0 nm against distilled water, relative concentration of two drugs in the sample was calculated using above equation (1),(2),(3) and (4) for the both methods.

RESULT AND DISCUSSION

In simultaneous equation by matrix and absorption correction method, the primary requirement for developing methods for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs**Figure 5 and 6**. The two wavelengths were used in case of absorption correction method for the analysis of the drugs were 252 nm (λmax of EBS) and 344 nm (λmax of MON) and two another wavelength were used for simultaneous equation by matrix method for the analysis of the drugs were 252 nm (λmax of EBS) and 280 nm (isobestic point) at which the calibration curves were prepared for both the drugs respectively. The overlain UV absorption spectra of EBS (252 nm) and MON (344 nm) in methanol is shown in **Figure 7**. Regression analysis data for both proposed methods for both the drugs are given in **Table 1, Table 2**. The validation parameters were studied at all the wavelengths for the proposed methods. Accuracy was determined by calculating the recovery and the mean was determined **Table 3, Table 4**. The method was successfully used to determine the amounts of EBS and MON present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labeled amount **Table 5, Table 6**.

CONCLUSION

All these factors leads to conclusion that proposed absorption correction method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of CIPRO and TINI as compare to the developed simultaneous equation by matrix . The developed methods was validated as per ICH guidelines[15]. Statistical analysis proved that the methods is repeatable and selective for the analysis of CIPRO and TINI in their combined pharmaceutical formulations.

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Table 1: Regression analysis data and summary of validation parameters for 1.1) Absorption correction method

S. No	Parameters	CIPRO	TINI
1	Wavelength range (nm)	272	317
2	Beer's law limit (µg/ml)	2 - 10	2–10
3	Regression equation (y = mx + c)	Y = 0.019x + 0.049	Y = 0.038x + 0.032
4	Slope	0.019	0.038
5	Intercept	0.049	0.032
6	Correlation Coefficient (r2)	0.963	0.992
7	Intraday precision	102.1-104.4	100-98.3%
8	Interday precision	103-104.9%	100.1-98.9%
9	Accuracy (% recovery)	100.2 - 103.1 %	100.1 - 104.2 %

1.2) Simultaneous equation by matrix method

S. No	Parameters	MON	EBS
1	Wavelength range (nm)	272	317
2	Beer's law limit (µg/ml)	2 - 10	2–10
3	Regression equation (y = mx + c)	Y = 0.019x + 0.049	Y = 0.038x + 0.032
4	Slope	0.019	0.038
5	Intercept	0.049	0.032
6	Correlation Coefficient (r2)	0.963	0.992
7	Intraday precision(n=3)	104-106.2%	100-101.1%
8	Interday precision(n=3)	100.1-103.2%	99.1-98.8%
9	Accuracy (% recovery)(n=3)	105.1 - 111.6 %	101 - 104.2 %

Table 3: Recovery data for absorption correction method

S. No	Average weight of tablet Powder in mg	Amount added in µg/ml		Amount Recovered in µg		% Recovery	
		EBS	MON	MON	EBS	MON	EBS
1	149.09	10	10	10.4	11.5	104.9	115
2		20	20	19.9	17.09	99.58	82.45
3		30	30	30.1	34.5	105	115.3
4	MEAN					103.1%	104.2%

Table 4: Recovery data for simultaneous equation by matrix method

S. No	Average weight of tablet Powder in mg.	Amount added inµg/ml		Amount Recovered in µg		% Recovery	
		EBS	MON	EBS	MON	EBS	MON
1	149.08	10	10	11.5	11.3	115	113.9
2		20	20	17.09	22	82.45	110
3		30	30	34.5	33.3	115.3	111.1
4	MEAN					104.2%	111.6

Table 5: Analysis of MON and EBS by Absorption correction method

S. No	Formulation	Labeled claim (mg)		% label claim	
		EBS	MON	EBS	MON
1	Ebast - M			98.0	106.8
2		10	10	98.2	95.1
3				98.1	110.4
4	MEAN			98.1	104.1

Table 6: Analysis of MON and EBS by simultaneous equation by matrix method

S. No	Formulation	Labeled claim (mg)		% label claim	
		EBS	MON	EBS	MON
1	Ebast - M			105.4	103.3
2		10	10	98.6	101.8
3				94.9	109.6
4	MEAN			99.6	104.9

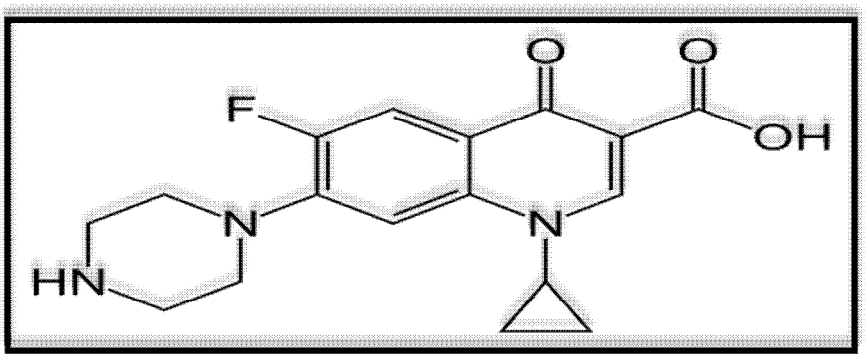

Fig. 1: Chemical structure of CIPRO

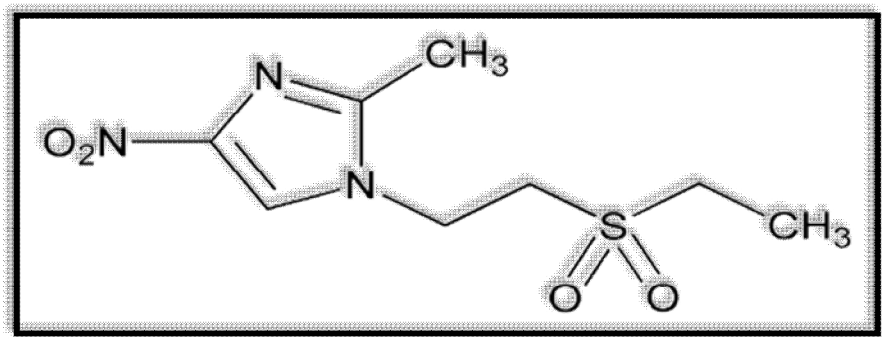

Fig. 2: Chemical structure of TINI

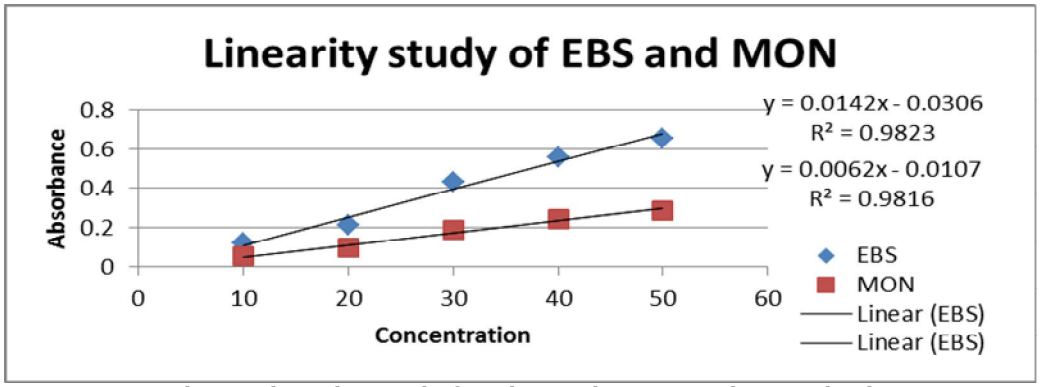

Fig. 3: Linearity study for absorption correction method

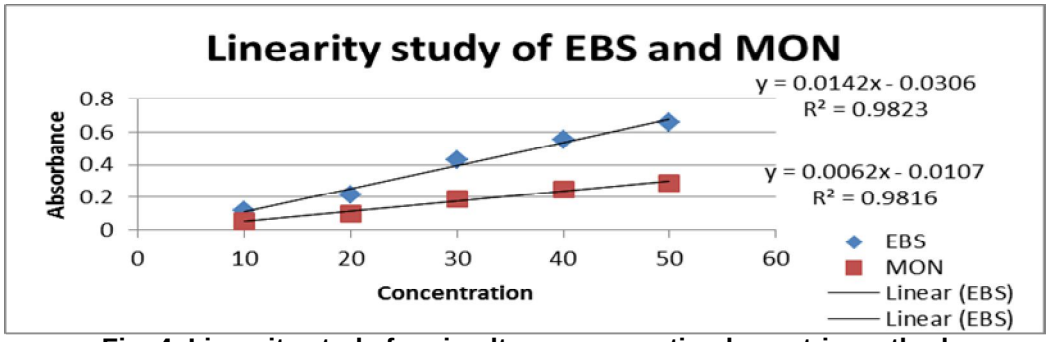

Fig. 4: Linearity study for simultaneous equation by matrix method

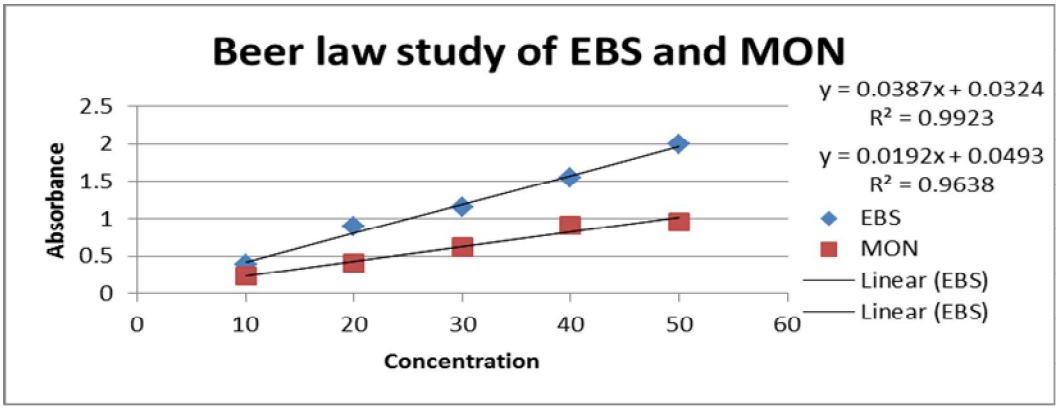

Fig. 5: Beer's law obeyed in Absorption correction method

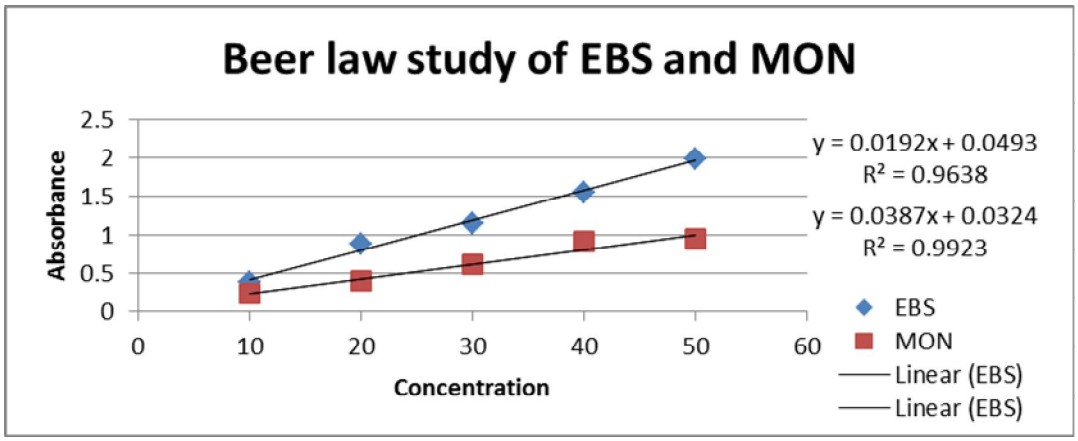

Fig. 6: Beer's law obeyed in Simultaneous equation by martix method

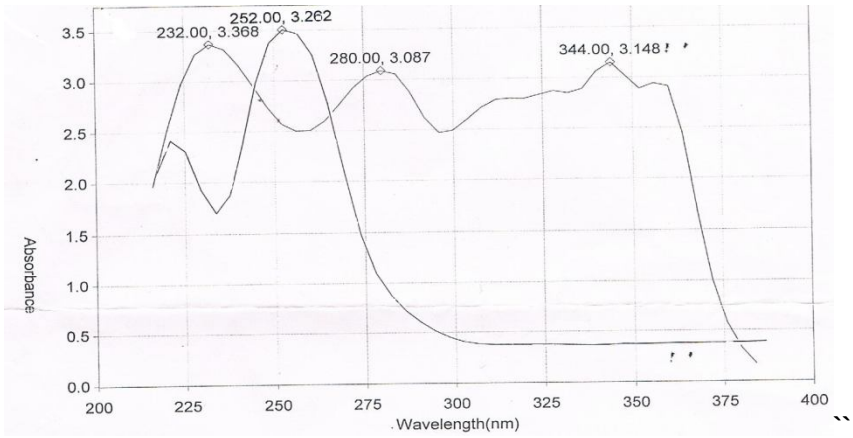

Fig. 7: Overlain absorption spectra for MON (344 nm) and EBS (252 nm) in methanol

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