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Research Article

A SIMPLE AND VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TINIDAZOLE AND CIPROFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Tinidazole and Ciprofloxacin in Tabletdosage form. An Aligant Zorbax Rx-C18 5µ column having 150 x 4.6mm id in Isocratic mode with mobile phase containing Orthophosphoric acid: methanol (70:30 %v/v pH: 3.0) was used. The flow rate was 1.5ml/min and effluents were monitored at 225nm.) The retention time of Tinidazole and Ciprofloxacin was 2.3min and 7.2min respectively.The concentration curves of Tinidazole and Ciprofloxacin were linear in the concentration range of 150-450 µg/mL and 125-375µg/mL respectively. The developed method was validated for specificity, precision, linearity, accuracy, LOD,LOQ, robustness . Recovery of Tinidazole and Ciprofloxacin in formulations was found to be in the range of 97.0% -98.0% and 100%-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision, the proposed HPLC method may be used for the simultaneous determination of these two drugs in pharmaceutical dosage forms.

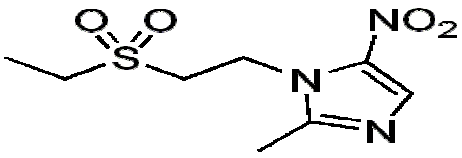
Keywords: RP-HPLC, Ciprofloxacin and Tinidazole.

INTRODUCTION

Tinidazole (TZ), [1-(2-(ethylsulfonyl) ethyl)-2-methyl-5-nitroimidazole] , is used as antiprotozoal/ antibiotic and antibacterial[1] that fights bacteria in the body.

Tinidazole is used for the treatment of trichomoniasis , giardiasis , intestinal amebiasis and amebic liver abscess[2] .

Tinidazole is 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. It is suggested that the toxic intermediates covalently bind to DNA, resulting in DNA damage in the form of loss of helical structure,impaired template function, and strand breakage which eventually lead to cell death[3],[4],[5],[6] . The mechanism by which tinidazole exhibits activity against *Giardia* and *Entamoeba* species is not known, though it is probably similar.



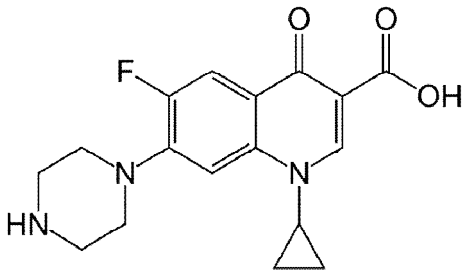
Tinidazole

Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid).

It is a synthetic antibiotic of the fluoroquinolone drug class[8]. It is a second-generation broad-spectrum antimicrobial carboxyfluoroquinoline.

Ciprofloxacin is used for the treatment of the following infections caused by susceptible organisms: urinary tract infections, acute uncomplicated cystitis, chronic bacterial prostatitis, lower respiratory tract infections, acute sinusitis, skin and skin structure infections, bone and joint infections, complicated intra-abdominal infections (used in combination with metronidazole), infectious diarrhea, typhoid fever (enteric fever), uncomplicated cervical and urethral gonorrhea, and inhalational anthrax (post-exposure).

It is a second generation fluoroquinolone antibacterial . It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops DNA and protein synthesis[9] . The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination.



Ciprofloxacin

Several analytical procedures have been proposed for the quantitative estimation of Ciprofloxacin and Tinidazole separately and in combination with other drugs. HPLC[10],[11] , high performance thin layer chromatography [12] and atomic absorption spectrometry [13] methods for estimation of Ciprofloxacin alone in pharmaceutical preparation have been reported. Ciprofloxacin in combination with Ornidazole, Naproxen and Ofloxacin are also available. Tinidazole in combination with other drugs Furazolidine, Norfloxacin and Clotrimazole is estimated by UV and HPLC have also been reported.

To our knowledge simple and economical analytical method for simultaneous determination of Ciprofloxacin and Tinidazole has not been reported so far. So attempt was taken to develop and validate an economic, rapid reversed-phase high performance liquid chromatographic method for the quality control of Ciprofloxacin and Tinidazole in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. The method was validated and found to be accurate, precise and reproducible.

MATERIALS AND METHODS

Apparatus

Waters e2695Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC.

OTHER APPARATUS: Electronic balance, Sonicator, 0.45µ membrane filter

Reagents and Chemicals

Pharmaceutical grade Ciprofloxacin and Tinidazole were kindly supplied as a gift sample by Dr.Reddys Laboratory, Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade and collected from E. Merck, Darmstadt, Germany. Orthophosphoric acid was of analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO water purification system.

Commercial Formulation

Ciprofloxacin and Tinidazole Tablets available in the market as CIPLOX-TZ in composition of Ciprofloxacin hydrochloride (500mg),Tinidazole (600mg). The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Preparation and Selection of Mobile Phase

The preliminary isocratic studies on a reverse phase C18 column with different mobile phasecombination of Orhophosphoric acid of pH 3 and Methanol were studied for simultaneous separation of both the drugs. The optimal

composition of mobile phase determined to be Buffer : Methanol (70:30 v/v) and filtered through 0.45µ membrane filter.

Preparation of Standard Solution

600mg Tinidazole and 500mg Ciprofloxacin was dissolved in 100 ml of Diluent (Methanol) and was further diluted to get stock solution of Tinidazole and Ciprofloxacin (300□µg/ml and 250 µg/ml respectively). This is taken as a 100% concentration. Solution containing mixture of Tinidazole and Ciprofloxacin of different concentrations (50%,75%, 100% 125%, and 150% of target concentration) were prepared in the same way.

Preparation of Sample Solution

Sample solution containing both the drugs was prepared by dissolving tablet powder into Diluent(Methanol). Ten tablets were weighed separately. Their average weights were determined. Powder of tablets equivalent to one tablet weight were weighed and taken in a 100 ml volumetric flask, dissolved in diluent and shaken and sonicated for about 10 minutes then filtered through 0.45µ membrane filter . The filtered solution was further diluted in the diluent to make the final concentration of working sample equivalent to 100% of target concentration.

Chromatographic Conditions

The mobile phase, a mixture of Orthophosphoric acid and methanol (50:50v/v) pumped at a flow rate of 1.5 ml/min through the column (C18; 5 , 4.6 X 150 mm, Agilant Zorbax) at 50°C.The mobile phase was degassed prior to use under vacuum by filtration through a 0.45 membrane filter. Both drugs showed good absorbance at 225 nm, which was selected as wavelength for further analysis.

Development and Validation of HPLC Method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Tinidazole and Ciprofloxacin in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996 and Global Quality Guidelines-2002.The method was validated for the parameters like system suitability , selectivity, linearity, accuracy, precision ,LOD, LOQ, and robustness.

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Tinidazole and Ciprofloxacin. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of Tinidazole and Ciprofloxacin were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of Tinidazole and Ciprofloxacin of different concentrations level (50%, 75%, 100%,125%, and 150%) were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

Accuracy (Recovery Studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Tinidazole and Ciprofloxacin were added to pre-analyzed samples and were subjected to the proposed HPLC method.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2%
for within a day and day to day variations, which proves that method is precise.

Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate,temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by flow and temperature variation.

RESULTS AND DISCUSSION

Results of system suitability study are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

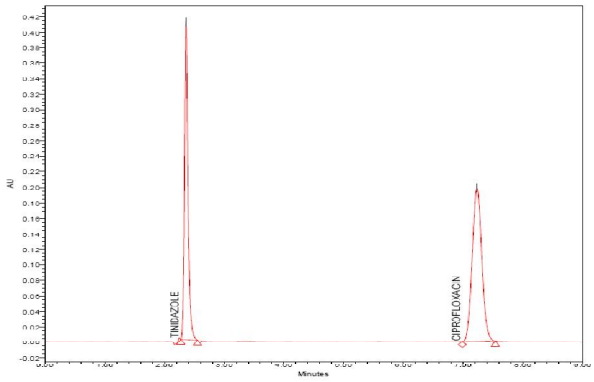


Figure 1: Typical chromatogram of Tinidazole and Ciprofloxacin in marketed formulation.

Parameters	Tinidazole	Ciprofloxacin
Retention Time	2.361	7.232
Area	2344777	3280524
Height	409587	198287
USP Resolution	-	22.62
USP Tailing	1.3	1.1
USP Plate Count	8349	9606

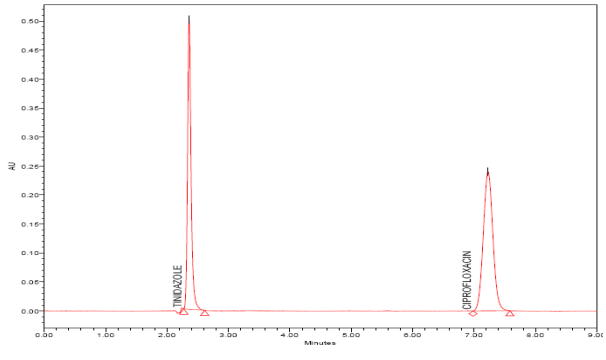


Figure 2: Typical Chromatogram of standard Tinidazole and Ciprofloxacin

Parameters	Tinidazole	Ciprofloxacin
Retention Time	2.359	7.228
Area	2363603	3301853
Height	499354	239207
USP Resolution	-	24.71
USP Tailing	1.3	1
USP Plate Count	9069	10317

System Suitability

Results of system suitability study are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Table 1: Result of system suitability tests of Tinidazole and Ciprofloxacin

Parameters	Tinidazole	Ciprofloxacin
Linearity range	150-450 µg/mL	125-375 µg/mL
Correlation coefficient	0.999	0.999
Slope	23427x+5164	32502x+8561
Retention time	2.3	7.2
Resolution Factor	-	24.71
USP plate count	9069	10317
Tailing factor*	1.3	1
Limit of Detection(LOD)	1 µg/mL	1.5 µg/mL
Limit of quantification(LOQ)	3 µg/mL	5 µg/mL

*=%Mean

Chromatograms shown in figure1 and figure 2 explain that retention time for standard sample and commercial product of Tinidazole and Ciprofloxacin are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. A linear relationship between peak areas (average peak areas of six replicates) versus concentrations was observed for Tinidazole and Ciprofloxacin in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both the drugs which prove that the method is linear.

Table2: Intraday and inter day precision result of Tinidazole and Ciprofloxacin

Drugs	%RSD (intra-day)	%RSD (inter-day)
Tinidazole	0.14	0.4
Ciprofloxacin	1.12	1.5

Table 3: Accuracy (%recovery) results of Tinidazole and Ciprofloxacin

Tinidazole

Sample No.	Spiked Amount (mg)	Recovered Amount (mg)	%Recovered	%Average recovery
1	15mg	14.70mg	98	
2	30mg	29.10mg	97	97.66%
3	45mg	44.10mg	98	
		Ciprofloxacin		
1	12.5mg	12.87mg	103	
2	25mg	25mg	100	101.66%
3	37.5mg	38.25mg	102	

Table 4: Results for robustness test of Tinidazole and Ciprofloxacin

		Tinidazole		
Parameters	**Changes**	**RT**	**USP Tailing**	**USP Plate count**
Flow rate(ml/min)	1	2.831	1.2	6609
	1.8	2.02	1.1	6302
Temperature	45°c	2.560	1.3	6775
	55°c	2.120	1.40	6001
		Ciprofloxacin		
Parameters	**Changes**	**RT**	**USP Tailing**	**USP Plate count**
Flow rate (ml/min)	1	8.645	1	8309
	1.8	6.160	1	7258
Temperature	45°c	7.522	1	7311
	55°c	6.9193	1	9193

Results of Intraday and inter day variability were summarized in table 2. Intraday variability was done from 9.00 am to 6.00 pm on the same day. % RSD of peak areas was calculated for various run .The method is highly precise as % RSD of peak area was less than 2% in all tests.

CONCLUSION

The new HPLC method developed and validated for simultaneous determination of Tinidazole and Ciprofloxacin pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies

and can be employed for bioequivalence studies for the same formulation.

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