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Original Research Article

Simultaneous Determination of Ciprofloxacin and Tinidazole in Tablet Dosage Form by Reverse Phase High Performance Liquid Chromatography

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Abstract

Purpose: *To develop a simple, sensitive and specific liquid chromatographic method with PDA detection for the simultaneous estimation of ciprofloxacin and tinidazole in tablet dosage form.*
Methods: *Separation was achieved with an Agilent XDB C18, 250 × 4.60 mm 5 µ column, low pressure gradient mode with a ambient temperature and mobile phase comprising acetonitrile water containing 0.1 % orthophosphoric (20:80). The flow rate was 1 ml/min and eluent was monitored spectrophotometrically at 316 nm.*
Results: *The selected chromatographic conditions effectively separated ciprofloxacin and tinidazole with retention time of 3.036 and 4.224 min, respectively. Linearity for ciprofloxacin and tinidazole was in the range 50 - 100 and 60 - 120 µg/ml, respectively. Regression coefficient was 0.999 for both ciprofloxain and tinidazole while recovery waas 100.19 - 100.92 and 99.36 - 100.48 % for ciprofloxacin and tinidazole, respectively. Relative standard deviation (RSD) of intra- and inter-day precision was < 2 % for both drugs.*
Conclusion: *The developed method is precise, accurate, reproducible and specific and it can also be used for routine simultaneous quality-control analysis of ciprofloxacin and tinidazole in combination tablets.*

Keywords: *High performance liquid chromatography, Ciprofloxacin, Tinidazole, Simultaneous determination.*

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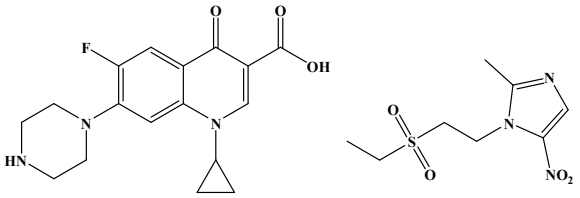
INTRODUCTION

Ciprofloxacin, 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid is a second generation fluoroquinolone. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are essential for bacterial transcription, repair, DNA replication, strand supercoiling repair, and recombination. Literature search reveals various methods for the estimation of ciprofloxacin alone and its combination by high performance liquid chromatography [1-4], UV [5-7], capillary zone electrophoresis [8]. Tinidazole, 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole, is an anti-protozoal agent. Tinidazole is a prodrug and the anti-protozoal action of tinidazole results from reduction of nitro group of tinidazole in *Trichomonas* by a ferredoxin-mediated electron transport system. As a result of this reduction, a free nitro radical is generated and is believed to be responsible for the antiprotozoal activity. These toxic free radicals covalently bind to DNA,

resulting in DNA damage and leads to cell death. The activity of tinidazole against *Giardia* and *Entamoeba* species is not known, though it is probably similar. Literature search reveals various methods for the estimation of tinidazole alone and its combination by high performance liquid chromatography [9-12] and UV [13].

The focus of the present study was to develop and validate a simple precise RP-HPLC method for the simultaneous estimation of ciprofloxacin and tinidazole.



(a) (b)

Fig 1: Structures of (a) ciprofloxacin and (b) tinidazole

EXPERIMENTAL

Chemicals and reagents

Working standards of ciprofloxacin and tinidazole were obtained from Aurbindo Laboratories, Hyderabad, India. Orthophosphoric acid AR grade was purchased from Merck. HPLC grade solvents - acetonitrile, methanol and water - were obtained from Ranchem, Mumbai, India. The pharmaceutical dosage form containing 500 mg ciprofloxacin and 600 mg tinidazole, Alcipro-TN 500 mg/600 mg (Alkem) was purchased from a local drug store.

Instrumentation

The development and validation of the assay was performed on an Alliance WATERS 2695 with high speed auto sampler, column oven, degasser, 2996 PDA detector and a class Empower-2 software. Chromatographic analysis was performed on Agilent XDB (C18) RP Column, 150 mm × 4.6 mm particle size 5 micron size column. The flow rate was 1 mL min-1, injection volume was 10 µL, and UV detection was performed at 316 nm. Peak identity was confirmed by both retention time comparison and comparison of spectra obtained from the UV detector.

Standard preparation

Accurately, 25 mg of Ciprofloxacin and 30 mg of Tinidazole were taken into a 25 ml volumetric flask. Then 20 mL of diluents was added to it and sonicated for 2 min and made up to volume (i.e., 25 mL) with mobile phase and filtered through 0.45 µ membrane filter and diluted to get the stock solution (1000 µg/mL ciprofloxacin and 1200 µg/mL tinidazole) as per formulation composition.

Preparation of calibration plot

Into a series of 10ml volumetric flasks containing aliquots of ciprofloaxcin and tinidazole, standard stock solutions equivalent to 25 - 125 µg/mL ciprofloxacin and 30 - 150 µg/mL tinidazole were prepared with mobile phase, sonicated and filtered through 0.45 µ membrane filter. Each solution was injected in triplicate and chromatographed under the chromatographic conditions specified above. Linear relationships were obtained when standard peak area ratios were plotted against the corresponding concentrations for each drug.

Sample preparation

Twenty tablets of commercial formulation containing ciprofloxacin and tinidazole (Alcipro-TN) were taken and powered. The powder equivalent to 10 mg and 12 mg of ciprofloxacin and tinidazole, respectively, was dissolved in 10 ml of diluent to get a stock solution of 1000 and 1200 µg/mL of ciprofloxacin and tinidazole, respectively, and then sonicated for 30 min. This solution was filtered through a Whatman filter paper no. 4. From the filtrate, 0.5 mL was taken and further diluted with diluent up to 10 ml, which contains 50 and 60 µg/mL of ciprofloxacin and tinidazole, respectively, was used for the analysis.

Method validation

Linearity

The linearity of the method was evaluated by analyzing different concentration of the drugs. According to International Conference on Harmonisation (ICH) recommendations, at least five concentrations must be used [14]. In the present study, five concentrations were chosen, in the ranges of 25 – 125 and 30 – 150 µg/mL for ciprofloxacin and tinidazole, respectively.

Accuracy and precision

The accuracy of the method was determined by recovery experiments using the standard addition method. The solutions were injected in triplicate and percent recovery was calculated. The precision of the method was determined by studying intra-day and inter-day variation. In the

inter-day studies, standard and sample solutions were analysed in triplicate on three consecutive days, and percent relative standard deviation RSD calculated. In the intra-day studies, standard and sample solutions were analyzed in triplicate on the same day and percentage RSD was calculated.

Limit of detection (LOD) and limit of quantitation (LOQ)

In accordance with ICH recommendations, the method based on the standard deviation of the response and the slope of the calibration plots was used to determine the detection and quantification limits [15]. LOD and LOQ values were estimated as [(standard deviation of repeatability)/(Slope of the regression equation)] by multiplying with 3.3 and 10, respectively.

Specificity

The specificity of the method was evaluated by assessing whether excipients present in the pharmaceutical formulations interfered with the analysis. Excipients for each tablet were mixed in order to prepare a placebo and solutions were prepared by following the procedure described in the section on sample preparation.

Robustness

Robustness is a measure of capacity of analytical methods to remain unaffected by small but deliberate variation of the operating conditions. This was tested by studying the effect of changing mobile phase pH by 0.2, the amount of buffer in the mobile phase by 2 % and detector wavelength by 2 nm.

Stability

The sample and standard solutions injected at 0 h (comparison sample) and after 24 h (stability sample) by keeping at ambient room temperature. Stability was determined by determining RSD for sample and standard solutions.

Statistical analysis

Wherever applicable, results were expressed as the Mean ± SD, % RSD and data were analyzed statistically by using t-test with aid of Microsoft excel-2007 software and data were considered significantly different at 5 % significance level of probability ($p \leq 0.05$).

RESULTS

To establish and validate an efficient method for analysis of these drugs in pharmaceutical formulations and with the objective of selecting optimum chromatographic conditions, some preliminary tests were performed. The separation was tried using either columns described previously in the literature or alternative stationary phases. Lack of resolution between ciprofloxacin and tinidazole was encountered as the main problem during these investigations. To solve these problems, three different types of C18 columns with different configurations, were used for simultaneous determination of the drugs. Good peak shape without excessive tailing and best resolution were obtained by using the Agilent XBD C18 column.

The effects of mobile phase composition, pH and flow rate were also studied. The best resolution along with reasonable retention time was obtained with mobile phase containing acetonitrile, and 0.1 % of ortho phosphoric acid buffer pH 3.0 (20:80) with 1.0 mL/min flow rate. A major reason for using a concentration of 0.1 % was to achieve maximum sensitivity of UV detection at low wavelengths. The detector was set at 316 nm to increase the sensitivity of the method. The separation of the compounds was complete, which indicates that the method is specific as illustrated in Figs. 3 and 4. Average retention times for ciprofloxacin and tinidazole were, 3.036 and 4.224 min, respectively, for six replicates.

System suitability

The RSD values of peak area and retention time for drugs are within 2 indicating the suitability of the system (Table 1).

Linearity

The calibration curves were prepared by plotting the peak areas of the drug against concentration which were linear in the range of 25 - 125 and 30 – 150 µg/mL for ciprofloxacin and tinidazole, respectively. The calibration equations and correlation coefficients were calculated by subjecting peak area ratios and concentrations to least square linear regression analysis. The mean regression equations were found as $y = 11604x + 15949$ and $r^2 = 0.9991$ for ciprofloxacin and $y = 15038x + 22878$ and $r^2 = 0.9995$ for tinidazole, respectively. Based on the linearity equation, $y = a x + b$, “y” is the peak area ratio of

drugs, “a” is the slope, “b” is the intercept and “x” is the concentration of the measured solution in μg/mL. The result shows that there is excellent correlation between the peak area ratio and the concentration of drugs in the range tested.

LOD and LOQ

The LOD was 0.0587 μg/mL for ciprofloxacin and 1.0667 μg/mL for tinidazole. The limit of quantification was determined as 0.1779 μg/mL for ciprofloxacin and 3.232 μg/mL for tinidazole.

Precision

Intra-day precision was performed by relative standard deviation of five repeated assays of samples at the three concentration levels. Inter-day precision was determined by analyzing the same set of samples for five different days. The RSD values were found to be 0.226 – 1.928 % for ciprofloxacin and 0.420 – 1.187 % for tinidazole, respectively, indicating good precision (Table 2).

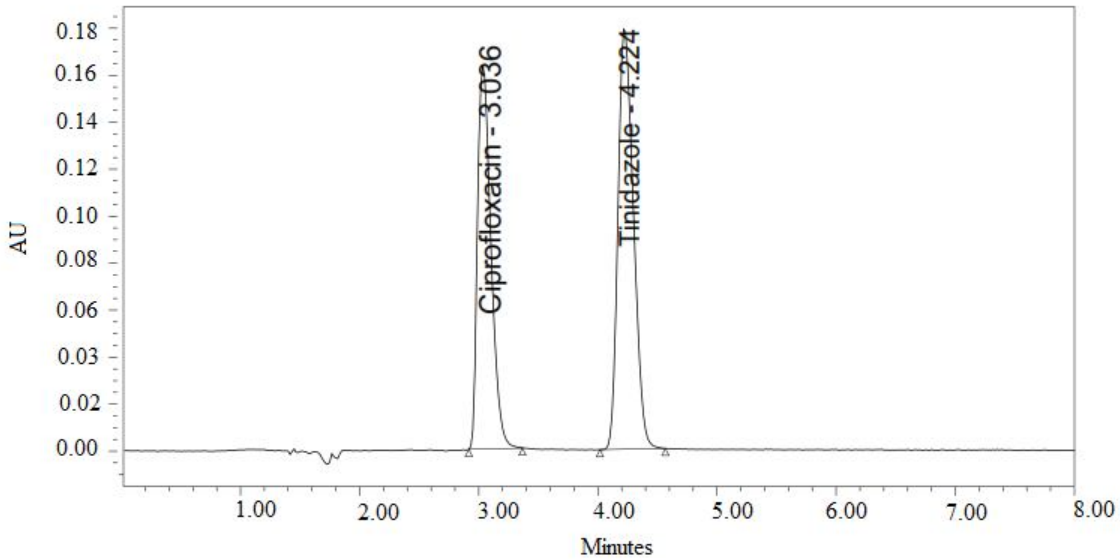


Fig 3: HPLC chromatogram of pure ciprofloxacin and tinidazole

Table 1: Results of system suitability study

	Ciprofloxacin		Tinidazole	
	Retention time (min)	***Peak area***	***Retention time (min)***	***Peak area***
Mean	3.1673	596289	4.2208	925708
SD	0.035	1686	0.0523	3562
RSD	1.13	0.35	1.24	0.48

Table 2: Intra- and inter-day precision and accuracy of ciprofloxacin and tinidazole

Drug	**Actual concentration (μg/mL)**	**Intra-day**		**Inter-day**	
		Found concentration (μg/mL) ± SD	***RSD (%)***	***Found concentration (μg/mL) ± SD***	***RSD (%)***
CIPRO	25	25.144 ± 0.177	0.707	24.888 ± 0.479	1.928
		19.910 ± 0.383	1.928	20.115 ± 0.142	0.707
	50	50.657 ± 0.381	0.752	50.130 ± 0.113	0.226
		40.104 ± 0.090	0.226	40.525 ± 0.305	0.752
	75	75.050 ± 0.967	0.967	75.573 ± 0.470	0.622
		60.458 ± 0.376	0.622	60.040 ± 0.580	0.967
TINI	30	29.805 ± 0.210	0.705	29.678 ± 0.352	1.187
		23.742 ± 0.282	1.187	23.844 ± 0.168	0.705
	60	59.666 ± 0.250	0.420	59.768 ± 0.404	0.677
		47.815 ± 0.323	0.677	47.733 ± 0.200	0.420
	90	90.824 ± 0.525	0.578	90.884 ± 0.766	0.843
		72.707 ± 0.613	0.843	72.659 ± 0.420	0.578

Table 3: Student t- test data for precision results of ciprofloxacin and tinidazole

Validation parameter	Ciprofloxacin		Tinidazole	
	Mean response	Probability (≥ 0.05)	Mean response	Probability (≥ 0.05)
Day 1	596649.8	0.491	925554.7	0.018
Day 2	596516.8		925120.5	

Table 4: Results of recovery studies by standard addition method

Name of the drug	Amount of drug in tablet (µg/mL)	Amount of pure drug added (µg/mL)	Total found(µg) (Mean ± SD)	RSD (%)	Recovery (%)
Ciprofloxacin	50	25	75.1575 ± 0.194	0.323	100.21
	40	20	60.0421 ± 0.194		
	50	50	100.19 ± 0.662	0.827	100.19
	40	40	80.0763 ± 0.662		
	50	75	126.15 ± 0.592	0.588	100.92
	40	60	100.5535 ± 0.592		
Tinidazole	60	30	90.432 ± 0.262	0.364	100.48
	48	24	72.1158 ± 0.262		
	60	60	120 ± 0.014	0.015	100.00
	48	48	96.0011 ± 0.014		
	60	90	149.04 ± 0.660	0.552	99.36
	48	72	119.5463 ± 0.660		

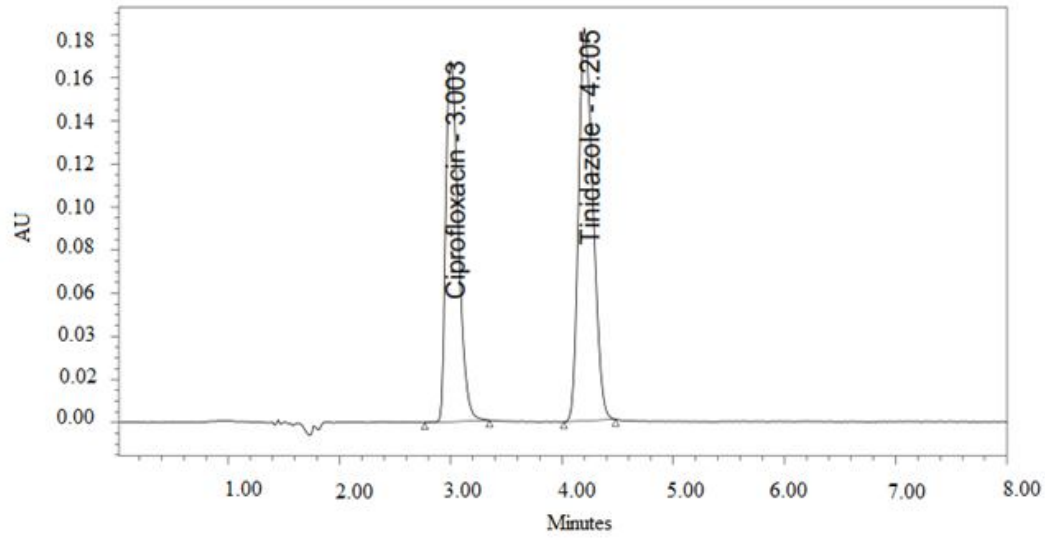


Fig. 4: HPLC chromatogram for tablet formulation

Statistics

The probability value (P) for ciprofloxacin and tinidazole at 5 % significance level was 0.209 and 0.229, respectively. The p values were > 0.05 and hence there was no significant difference between the precision results carried out on two consecutive days. The results are shown in Table 3.

Recovery

To examine the accuracy of the method, recovery studies were carried out by standard addition method. The percent recovery of the added standard to the assay samples was calculated from:

Recovery % = {(C1 – Cu)/Ca}100 …… (1)
where C1 is the total concentration of analyte found; Cu is the concentration analyte present in the formulation; and Ca is the concentration added to the formulation. The average percent recoveries obtained as 99.36 - 100.92 % indicate good accuracy of the method (Table 4)

Specificity

The specificity was determined by the complete separation of ciprofloxain and tinidazole as

shown in Figs. 3 and 4 with parameters like retention time (Rt), resolution (Rs) and tailing factor (T). The peaks obtained showed that there is no blank and placebo interference with that of the main peaks.

Robustness

To ensure the insensitivity of the HPLC method to minor changes in the experimental conditions, it is important to demonstrate robustness of the method. None of the modifications caused a significant change in the resolution between the drugs, peak area RSD, USP tailing factor, peak width or theoretical plates.

Stability of sample solution

The sample and standard solutions were injected at 0 h (comparison sample) and after 24 h (stability sample) and keeping at ambient room temperature 30 ^{o}C. RSD for 0 and 24 h for sample and standard solutions of ciprofloxacin are 1.23, 0.94 and1.87, 0.64, respectively. RSD for 0 and 24 h for sample and standard solutions of tinidazole is 1.27, 0.97 and 1.73, 0.66, respectively. RSD results for both ciprofloxacin and tinidazole are within the limit of ≤ 2 and hence the sample and standard stock are stable for 24 h at ambient room temperature

Assay

The proposed method was used to estimate the total drug content in commercially available pharmaceutical dosage forms. The results obtained are in agreement with the claimed amounts of the tablet dosage form (Alcipro-TN) and results were shown in Table 5.

Table 5: Assay results for commercial tablets (Alcipro-TN)

Drug	Claimed (mg)	Found ± SD (mg)
Ciprofloxacin	500	497.32±0.19
Tinidazole	600	601.35±0.11

DISCUSSION

Recovery of ciprofloxacin and tinidazole was 100.44 and 99.94 %, respectively, and it shows that the proposed method is accurate. The regression coefficient value is 0.999 for both ciprofloxacin and tinidazole and thus the response is linear. Repeatability and intermediate precision values were within the acceptable limits. This indicates that the method is precise. The resolution between two peaks is always > 2. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive. The solution stability studies indicate that both the drugs were stable up to 24 h. Change in flow rate, temperature and mobile phase composition did not cause any significant change in results shows reliability of the development method.

CONCLUSION

A simple, rapid, and reliable LC method has been established for the simultaneous determination of ciprofloxacin and tinidazole either alone or in their binary formulations. The method has several advantages, including rapid analysis, a simple mobile phase, simple sample preparation, and improved sensitivity. It is suitable for analysis of these drugs in their binary formulations in a single isocratic run, in contrast with previous methods. This makes the method suitable for routine analysis of the combination product in quality-control laboratories.

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