A Validated Rp-Hplc Method for Estimation of Ciprofloxacin and Tinidazole in Tablet Dosage Form

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ABSTRACT

A simple, specific, precise and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for estimation of ciprofloxacin and tinidazole in combined tablet dosage form. The separation was carried out by Inertsil C18(250X4.6mm) column with mobile phase phosphate buffers (pH 6.8): acetonitrile (82:18) v/v, at a flow rate of 1.0ml/min. Detection was carried out at 316nm.The retention time of ciprofloxacin and tinidazole was found to be 5.6 and 9.82 min, respectively. The method was validated for linearity, accuracy and precision. Linearity for ciprofloxacin and tinidazole were in the range of 27.5 - 82.5µg/ml for ciprofloxacin, and 33µg/ml - 66µg/ml for tinidazole and the correlation coefficient was found to be 0.9999 for both the drugs. The mean recovery for ciprofloxacin and tinidazole was found to be 99.7 and 100.4. The developed method could be employed for the routine analysis of ciprofloxacin and tinidazole in combine tablet dosage form.

Key Words: *RP-HPLC, Ciprofloxacin hydrochloride, tinidazole, specificity, linearity, precision.*

INTRODUCTION

Ciprofloxacin is 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid is a broad spectrum anti-microbial drug, used as anti-infective agent listed in class 4 of biopharmaceutics classification of drugs used for treatment of variety of infections.[1-3] Tinidazole is a prodrug 1-[2-(ethanesulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole which is used as antiprotozoal agent, listed in class 2 of biopharmaceutics classification of drugs.[4-6] Combination of Ciprofloxacin and Tinidazole are not official in any pharmacopoeia. A Drug X containing 500 mg of ciprofloxacin and 600 mg of tinidazole is available in market. A survey of literature revealed that few chromatographic and spectrophotometric methods are reported for determination of ciprofloxacin[7] and tinidazole in individual[8] and combined dosage form.[9-14] However there is no HPLC method reported for assay development of ciprofloxacin and tinidazole in combined dosage form. The present work describes a simple, precise and accurate HPLC method for the development of ciprofloxacin and tinidazole in combined tablet dosage form.

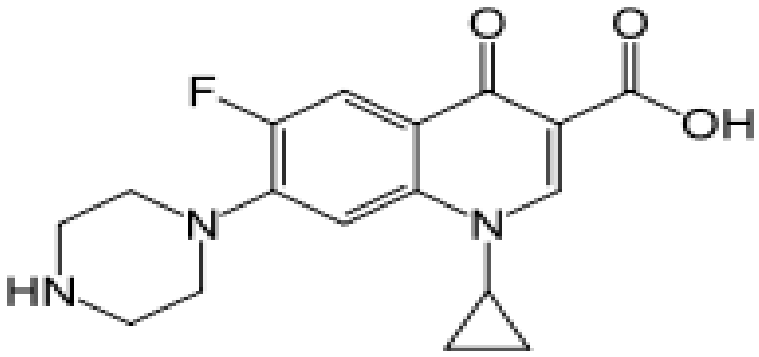

Figure – 1: Structure of Ciprofloxacin

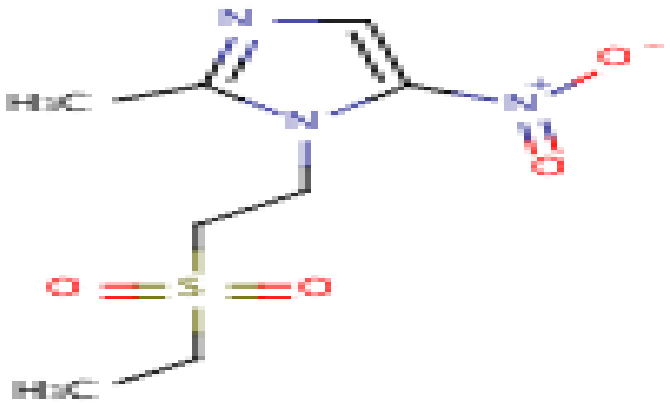

Figure – 2: Structure of Tinidazole

MATERIALS AND METHODS

Drug samples ciprofloxacin hydrochloride and tinidazole were obtained as gift samples from the Gracure pharmaceuticals Pvt. Ltd., Bhiwadi (Rajasthan).

Chemicals and reagents:

Orthophosphoric acid (Analytical grade), Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade), were purchased from Merck Co. Mumbai and S.D. fine chemicals, Mumbai, respectively. All the reagents and chemicals used for analysis were of analytical grade and HPLC grade.

Experimental conditions:

A high performance liquid chromatography (Waters e2695), variable wavelength programmable PDA detector, with empower software was used. The chromatography column used was reverse phase Inertsil C18 column (250mmX4.6) mm, particle size 5 µm. A mixture of phosphate buffers(pH 6.8) and acetonitrile in the ratio 82:18 was used as mobile phase and was filtered through 0.45µ Millipore membrane filter. The flow rate of mobile phase was maintained at 1.0 ml /min. Detection was carried out at 316 nm at room temperature.

Standard solution of ciprofloxacin and tinidazole was prepared in diluent. A quantity of powder equivalent to about 50mg of USP ciprofloxacin hydrochloride and 60mg of tinidazole was weighed and transferred to 100ml volumetric flask containing 60 ml of mobile phase and the mixture was sonicated .The volume was make up to mark (100ml) with mobile phase. The contents were filter through whatmann filter paper. Further dilutions were made to get a concentration of 50µg/ml of Ciprofloxacin and 60µg/ml of Tinidazole. Twenty tablets, each containing 500mg of ciprofloxacin and 600mg of tinidazole were weighed and powdered. A quantity of powder equivalent to 250 mg of ciprofloxacin was weighed and transferred to 200ml volumetric flask containing 120ml of mobile phase. The mixture was sonicated for 30 min. The volume was made upto 200ml with mobile phase. The contents were filter through whatmann filter paper. Further dilutions were made to get a concentration of 50µg/ml of Ciprofloxacin and 60µg/ml of Tinidazole. Twenty microliters of the test and standard solutions were injected separately and chromatograms were recorded upto to 15 minutes.

RESULTS AND DISCUSSION

Ciprofloxacin and tinidazole in combined tablet dosage form was analyzed by using RP-HPLC method. The present investigation was aimed at developing a simple, precise and accurate HPLC method to estimate ciprofloxacin and tinidazole in combine tablet dosage form. Several trials are carried out for selection of column and mobile phase for the method development. After trials the column used in this method Inertsil C18 (250X4.6mm), 5µm and the mobile phase is buffer and acetonitrile in the proportion of 82:18 v/v. The wavelength was set at 316nm as both the drugs showed good absorbance at this wavelength. With the above mentioned composition of mobile phase a good resolution between ciprofloxacin and tinidazole was achieved.

The retention time of ciprofloxacin and tinidazole was found to be 5.6 and 9.82 min,

respectively. Run time was 15 minutes and injection volume was 20µl. A typical chromatogram of the standard and test solution is shown in the fig. 3 and 4 respectively. The peak shape of both drugs were symmetrical and asymmetric factor was lesser than 2.0. The response factor of the standard and test solution was calculated. The proposed method was validated as per ICH guidelines. Each of Samples was injected 6 times and the retention time was observed in all the cases. Precision of proposed method (RSD) for Repeatability was found to be 0.29% for ciprofloxacin and 0.27% for tinidazole and for intermediate precision was found to be 0.39% for ciprofloxacin and 0.33% for tinidazole The low RSD value indicated that proposed method has good precision. Linearity experiments were performed at five different concentrations, thrice for both the compounds and the response was found to be linear in the range of 27.5 - 82.5µg/ml for ciprofloxacin, and 33µg/ml - 66µg/ml for tinidazole. Linearity of ciprofloxacin and tinidazole was plotted by a graph of response factor versus concentration. The correlation coefficient (r) values for ciprofloxacin and tinidazole were 0.9999 and 0.9999, respectively shown in fig.4, 5. Accuracy of the method was calculated by recovery studies at three levels. Amount of drug recovered at each level was calculated. Percent recovery study at each level was calculated. Table 1 shows the data from the recovery study for ciprofloxacin and tinidazole. The average recovery of ciprofloxacin and tinidazole were 99.80%. The sample recovery in the formulation was in good agreement with the label claim. High percentage recovery showed that the method was free from interferences of excipients used in the formulations. The system suitability parameters of ciprofloxacin and tinidazole are given in Table 2. The results of study indicate that proposal method is simple, precise, highly accurate and specific.

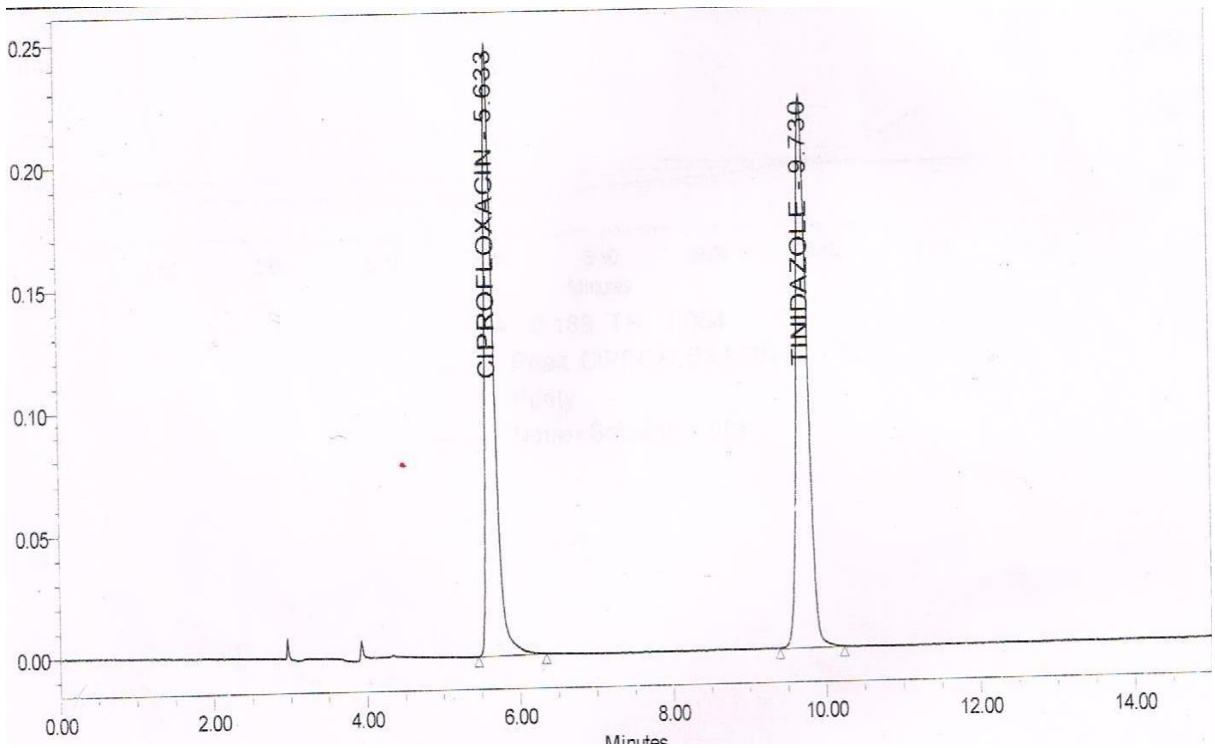

Fig. 3: Chromatogram of the standard drugs

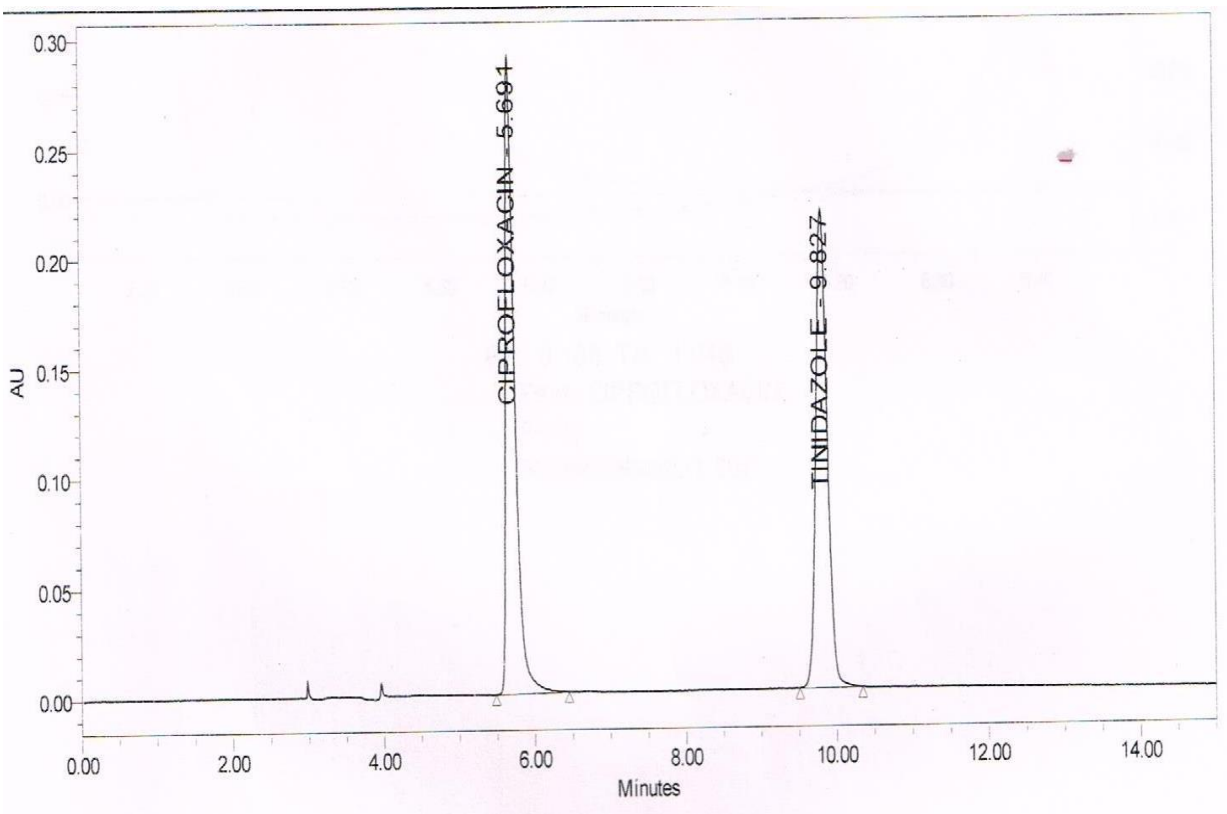

Fig. 4: Typical chromatogram of the sample solution

Typical chromatogram of the sample solution containing ciprofloxacin hydrochloride and tinidazole at the retention time of 5.6 and 9.82 min.

Calibration curve of Ciprofloxacin and Tinidazole

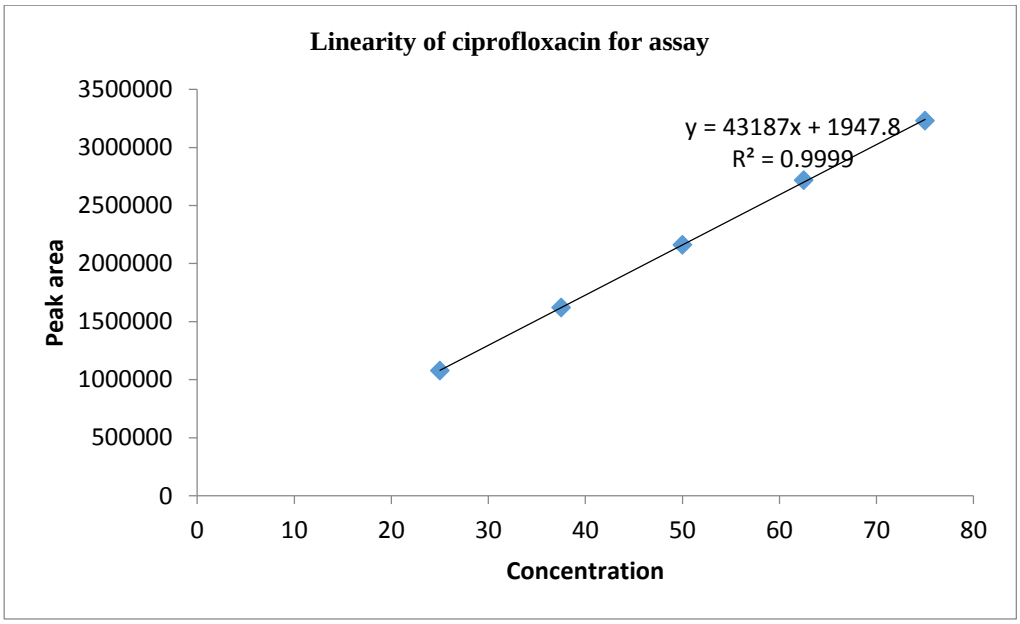

Figure 5: Calibration curve of ciprofloxacin

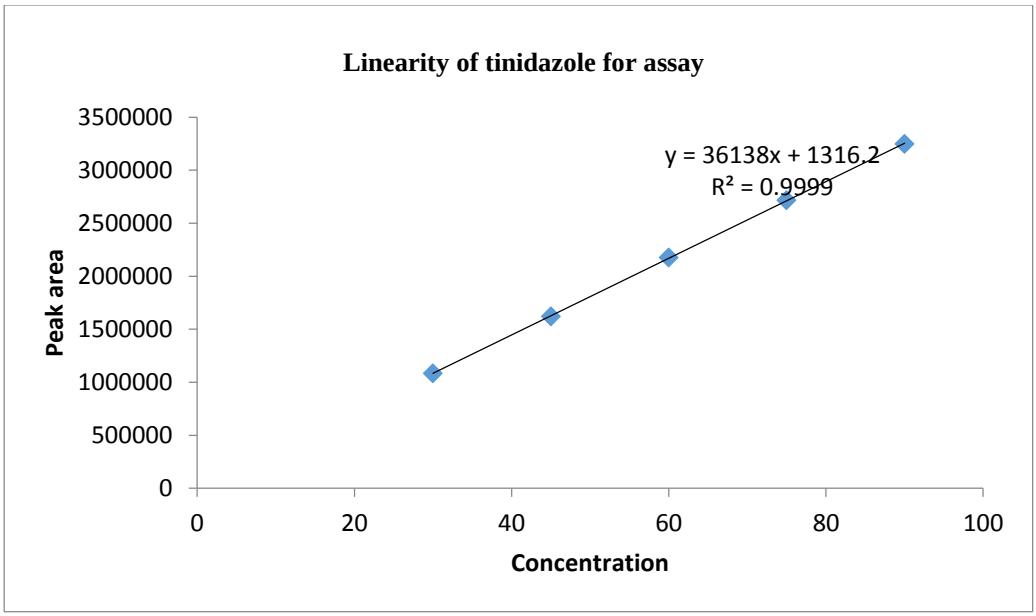

Figure 6: Calibration curve of tinidazole

Table 1: Result of Recovery Study

DRUG	AMOUNT ADDED	AMOUNT RECOVERED	RECOVERY (%)	AVERAGE RECOVERY (%)
CIPROFLOXACIN	126.2	125.6	99.52	99.80
	251.2	250.4	99.68	
	372.4	373.2	100.21	
TINIDAZOLE	150.38	149.98	99.73	99.80
	299.54	298.82	99.75	
	448.64	448.38	99.94	

Table 2: Result of System Suitability Parameter

Parameters	Ciprofloxacin	Tinidazole
Tailing factor	1.36	1.05
Theoretical plates	9841	15816
Calibration range	27.5 - 82.5µg/ml	33- 66µg/ml

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