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Application of RP-HPLC for the simultaneous determination of ciprofloxacin hydrochloride and tinidazole in solid dosage form

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ABSTRACT

A simple, precise, accurate, rapid and reproducible reverse phase high performance liquid chromatographic procedure is developed for simultaneous determination of Ciprofloxacin Hydrochloride and Tinidazole in tablet formulations. The mobile phase used was a combination of acetonitrile: water: triethylamine (30:67:3) v/v and the pH was adjusted to 6.0±0.1 by addition of 50% ortho phosphoric acid. The detection of the tablet dosage form was carried out at 296 nm and a flow rate employed was 1ml/min. Linearity was obtained in the concentration range of 2-16 µg/ml of Ciprofloxacin Hydrochloride and 5 -55 µg/ml of Tinidazole with a correlation coefficient of 0.9992 and 0.9996 respectively. The results of the analysis were validated statistically and recovery studies confirmed the accuracy of the proposed method. The developed method has been successfully used for the simultaneous estimation of both drugs in commercial formulation.

Keywords: Ciprofloxacin Hydrochloride, Tinidazole, Simultaneous estimation

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INTRODUCTION

Chemically Ciprofloxacin Hydrochloride (CPH) is (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) is fluoro quinolones and antimicrobials with potent activity against a broad spectrum of bacteria [1-5]. Literature survey revealed that chromatographic method was reported for its estimation from tablet formulation [6,9] and spectrophotometric methods for estimation in combined dosage forms [9-11]. Chemically Tinidazole (TZ) is (1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazo-le), antiprotozoal and anti- bacterial drugs [1-5]. These drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin. Literature survey revealed that chromatographic method was reported for its estimation from tablet dosage form [10] and spectrophotometric methods for estimation in combine dosage forms [9-11]. But so far no chromatographic methods have been reported for simultaneous estimation of CPH and TZ in combined dosage form, hence an attempt has been made to develop simple, sensitive, economical, rapid, precise and accurate methods to analyze the drugs simultaneously.

MATERIALS AND METHODS

Materials

Ciprofloxacin (CPZ) and Tinidazole (TZ) were supplied as gift sample by Cipla Pharmaceutical, Pvt.Ltd, Sikkim (India).All the buffer materials and reagents used were of HPLC grade and purchased from Spectrochem, Mumbai, India. Nylon filter paper of 0.2µm (Ultipor) was purchased from Pall Life Science, Mumbai, India.

Instrumentation

Shimadzu HPLC (LC-10 AT *VP*) system; LC system used consist of pump (Model SHIMADZU; LC-10 AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20 µl. Detector consists of photodiode array detector SPD-10 AVP, SHIMADZU; the reverse phase column used was Luna C_{18} (5µM, 25cm×4.6mm i.d) phenomenex, USA, at ambient temperature.

Preparation and selection of mobile phase

The preliminary isocratic studies on a reverse phase C18 column with different mobile phase combination of acetonitrile and phosphate buffer pH 7.0±0.1 were studied for separation of both drugs. The optimal composition of mobile phase determined to be acetonitrile: water: triethylamine (30:67:3) v/v and the pH was pH was adjusted to 6.0±0.1 by addition of 50% ortho phosphoric acid and was filtered through 0.2 micron membrane filter.

Preparation of standard and sample solutions

CPZ and TZ (10 mg each) were weighed accurately and transferred to separate 100 ml volumetric flasks, both drugs were dissolved in the sufficient amount of mobile phase and final volume made upto 100 ml with mobile phase to get a standard stock solution of 100 µg/ml. Twenty capsules were weighed, with and without shell, their average weight was determined and the content was finely powdered and powder equivalent to weight of one capsule was transferred to a 100 ml volumetric flask and dissolved in 50 ml of mobile phase. The solution was shaken vigorously for 15 min and final volume was made upto 100 ml. The solution was filtered through whatman filter paper # 41.

Preparation of calibration curves

Solutions of both drugs having different concentrations were prepared by dilution of the standard solutions. These solutions (20 µl) were chromatographed and peak areas were measured, peak areas were then plotted against the respective concentrations for both CPZ and TZ.

Analysis of Tablet dosage form

Six replicates of the required dilutions were prepared from capsule stock solution and sonicated for 10 min. These solutions (20 µl) were injected for quantitative analysis. The amounts of CPZ and TZ per tablet were calculated by extrapolating the peak area from the calibration plot. Results of analysis are reported in Table 1. Precision was measured both intra-day and inter-day. In the intra-day study the concentration of both drugs were calculated three times on the same day at intervals of an hour. In the inter-day study the concentrations of both drugs were measured on three different days.

Table 1: Studies of CPZ and TZ in marketed formulation

Drug	Label claim (mg/cap) (n=6)	Amount found (mg)	% of drug content	S.D.	% COV	S.E.
CPZ	300	300.356	101.30	0.586	0.583	0.239
TZ	600	601.453	99.30	0.694	0.693	0.283

S.D.: Standard deviation, COV: Coefficient of variance, S.E.: Standard error

Recovery studies: To perform the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method. The results of the analysis are reported in Table 2.

Table 2: Results from the recovery study

Replicate	**Amount taken (µg/ml)**		**Amount added at**		**(µg/ml)**	**% Recovery**	
	CPZ	**TZ**	**%**	**CPZ**	**TZ**	**CPZ**	**TZ**
1	100	100	80%	80	80	98.30	98.47
2	100	100		80	80	99.12	98.83
3	100	100		80	80	98.23	98.89
1	100	100	100%	100	100	99.96	100.32
2	100	100		100	100	99.93	100.36
3	100	100		100	100	99.97	100.21
1	100	100	120%	120	120	100.01	101.01
2	100	100		120	120	99.99	101.11
3	100	100		120	120	100.10	101.17

Validation of the method

Validation of the optimized HPLC method was done with respect to following parameters as per ICH norms [12-14].

Linearity and range

A stock solution of the drug (1mg ml^{-1}) was prepared in water. From this stock solution seven concentrations of the drug were prepared in water within the concentration range of 5-100 µg ml^{-1}. The solutions were injected in hexaplicate into the HPLC column, keeping all the conditions constant. The results of the analysis are reported in Table 3 and 4.

Table 3: Results of Linearity Calibration Curve for CPZ

Conc(µg/ml)	**50**	**100**	**150**	**200**	**250**	**300**
1	11050.10	11260.80	11990.12	13160.05	13952.56	15780.17
2	10510.13	11971.09	12880.19	13165.98	14958.53	15772.26
3	10826.08	11460.10	12766.18	13178.10	14954.80	15786.46
Mean	10793.16	11560.16	12539.17	13168.04	14614.18	15779.63
SD	221.5071	299.1425	395.4719	7.51193	473.3581	5.809687
RSD	0.2052	0.2587	0.3154	0.0570	0.3239	0.0368

SD.: Standard deviation, SD: Standard error, RSE:Relative Standard error

Table 4: Results of Linearity Calibration Curve for TZ

Conc (µg/ml)	**100**	**200**	**300**	**400**	**500**	**600**
1	7712.25	12814.84	21008.24	27154.22	34987.85	47354.01
2	7780.02	13927.99	21217.68	27066.77	34791.80	47346.51
3	6992.95	13547.85	21457.27	26854.15	33987.99	47865.25
Mean	7486.482	13422.21	21226.94	27024.75	34586.49	47521.3
SD	356.2108	462.0074	183.4538	126.0057	432.5997	242.7881
RSD	0.4634	0.3442	0.0864	0.04662	0.1250	0.05109

SD.: Standard deviation, SD: Standard error, RSE:Relative Standard error

Table 5: Intra-day precision of HPLC study

Intraday precision		
Hour	**CPZ**	**TZ**
After1	15471.18	47542.21
After2	15782.99	47214.25
After3	15472.51	46582.99
After4	14572.15	48521.21
After5	16985.89	47542.25
Afte6	15724.39	46582.15
Mean	15636.47	47331.27
SD	710.861	681.6852
RSD	0.4546	0.1440

SD.: Standard deviation, SD: Standard error,
RSE:Relative Standard error

Precision

Precision of the method [15, 16] was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentration of (30, 50, 80 µg ml^{-1}) drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating the studies on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision). The results of the analysis are reported in Table 5 and 6.

Table 6: Inter day precision of HPLC study

Inter day precision		
Day	**CPZ**	**TZ**
First day	15472.25	48751.22
Second day	14752.15	47841.52
Third day	14241.54	47516.12
Mean	14804.89	48030.63
SD	504.928	522.7049
RSD	0.3410	0.1088

SD.: Standard deviation, SD: Standard error,
RSE:Relative Standard error

Accuracy

Accuracy of the method was tested by fortifying a mixture of decomposed reaction solutions with three concentration of the drug and determining the percentage of recovery of added drug.

Specificity and selectivity

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. Whereas selectivity was established through determination of purity for each degradation product peak using PDA detector. The results of the analysis are reported in Table 7.

Table 7: system suitability study

Property (n=5)	**CPZ**	**TZ**
t_R	8.34	11.42
T_f	0.93	1.07
$k^/$	0.43	.97
N	9708	6016
Rs	2.15	5.51

t_R : Retention time, T_f : Tailing factor, $k^/$:
Capacity factor, N : Number of theoretical plates,
Rs : Resolution factor

RESULTS

The HPLC method was found to be simple, accurate, economic and rapid for routine simultaneous estimation of CPZ and TZ in combined tablet dosage form at 295 nm. The regression: 0.9992 and 0.9996, intercept: 47245 and 228214 and slope: 23525 and 27245 were found to be for CPZ and TZ respectively. Recovery was in the range of 100.42–102.39% and shows the high precision (Table 5, 6) of the developed method.

DISCUSSION

Amongst the various mobile phases used, acetonitrile: water: tri ethylamine (30:67:3) v/v and the pH was adjusted to 6.0±0.1 by addition of 50% ortho phosphoric acid was found to be robust at 1ml/min flow rate. Mobile phase and flow rate selection was based on peak parameters such as height, tailing, theoretical plates, capacity factor, run time, resolutions. A typical chromatogram of CPZ and TZ is shown in Fig.1, 2. The optimum wavelength for detection was 295 nm at which detector response was obtained best. The average retention time for CPZ and TZ was found to be 8.43 ± 0.05 min. and 11.82± 0.05 min respectively. They are used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out and its results are shown in Table 7.

Hence it can be concluded that the developed RP-HPLC method is an accurate, precise and robust method and can be employed successfully for the estimation of CPZ and TZ in bulk and formulation.

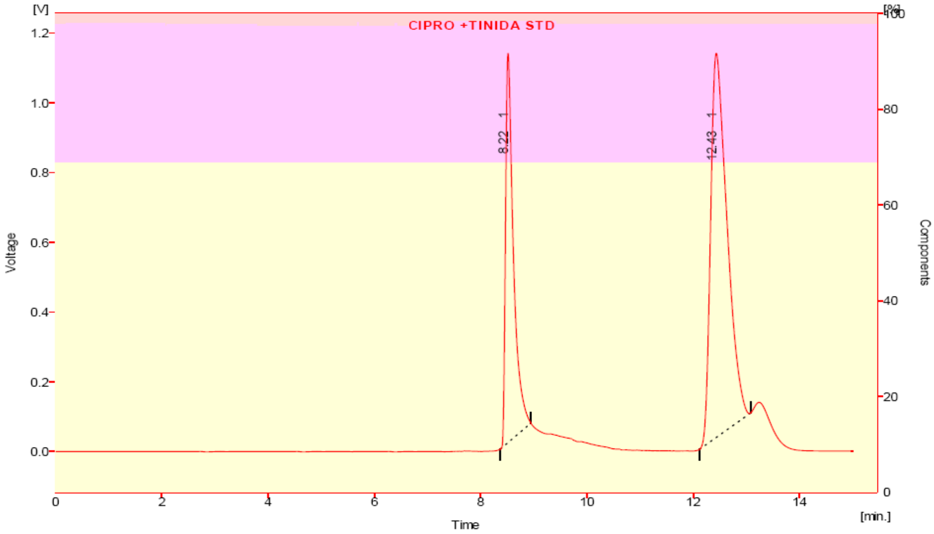

Fig 1: Typical chromatogram of CPZ and TZ in Standard solution

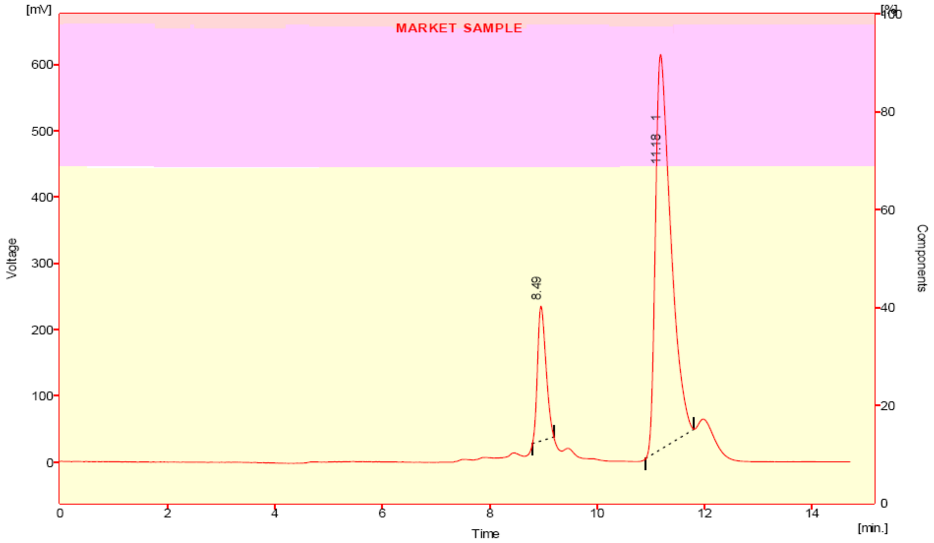

Fig 2: Typical chromatogram of CPZ and TZ in Tablet dosage form

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