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RESEARCH ARTICLE

ESTIMATION OF TINIDAZOLE AND CIPROFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP HPLC METHOD

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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 Tablet dosage form. An Aligant Zorbax Rx-C18 5µ column having 150 x 4.6mm id in Isocratic mode with mobile phase containing phosphate buffer : acetonitrile (70:30 %v/v pH: 3.0) was used. The flow rate was 1.2ml/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.

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.

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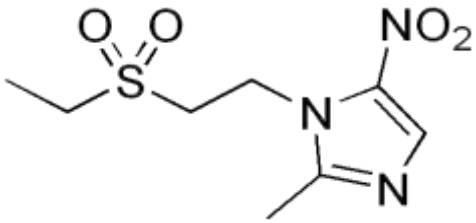
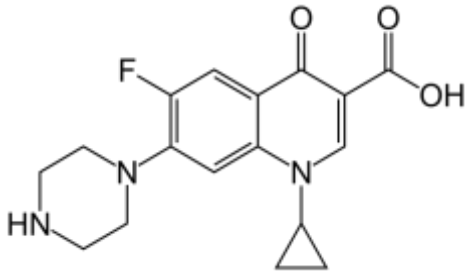


Tinidazole[7]

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 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. Acetonitrile was of HPLC grade and collected from E. Merck, Darmstadt, Germany. Potassium dihydrogen ortho phosphate 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 phase combination of phosphate buffer of pH 3 and acetonitrile were studied for simultaneous estimation of drugs. The optimal composition of mobile phase determined to be Buffer:acetonitrile (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 (acetonitrile) 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. twenty tablets were weighed separately. Their average weight was determined. Powder of tablets equivalent to one tablet weight was weighed and taken in a 100 ml volumetric flask, dissolved in diluent and sonicated for about 10 minutes then filtered through 0.45µ membrane filter . The filtered solution was further diluted to make the concentration of working sample equivalent to 100% of target concentration.

Chromatographic Conditions
The mobile phase, a mixture of Orthophosphoric acid and acetonitrile (50:50v/v) pumped at a flow rate of 1.2 ml/min through the column (C18; 5µ, 4.6 X 150 mm, Agilant Zorbax) at 50ºC.The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ 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 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, 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.

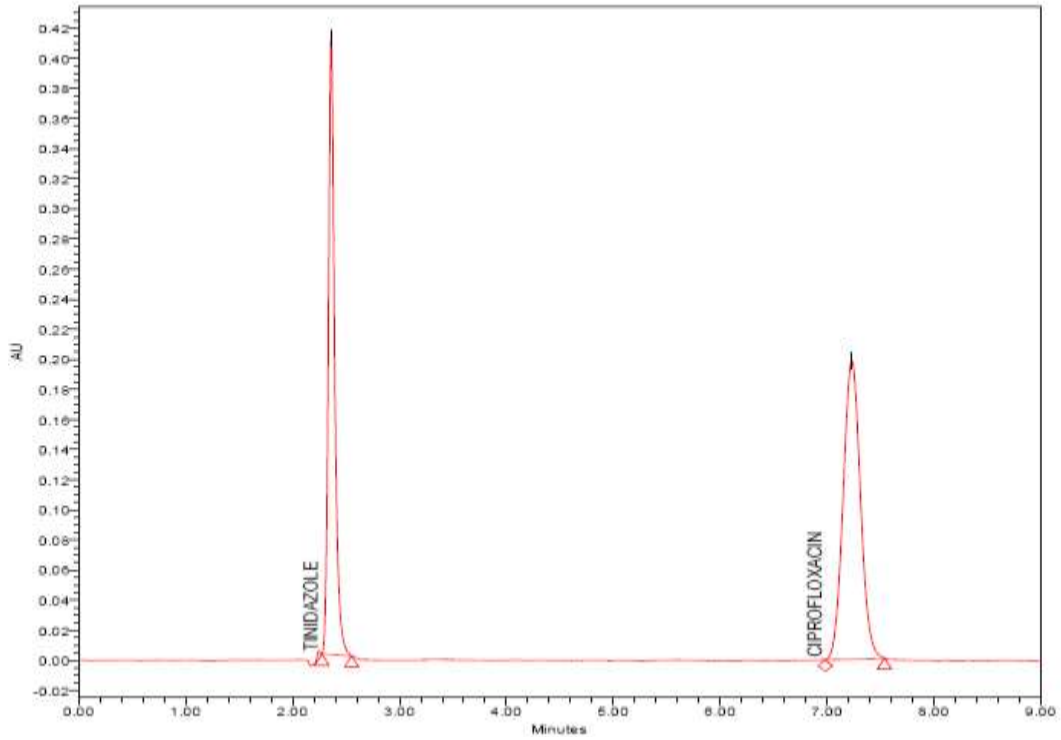


Figure 1:- Typical chromatogram of Tinidazole and Ciprofloxacin in marketed formulation.

	Name	Retention Time	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	TINIDAZOLE	2.361	2344777	409587		1.3	8349
2	CIPROFLOXACIN	7.232	3280524	198287	22.62	1.1	9606

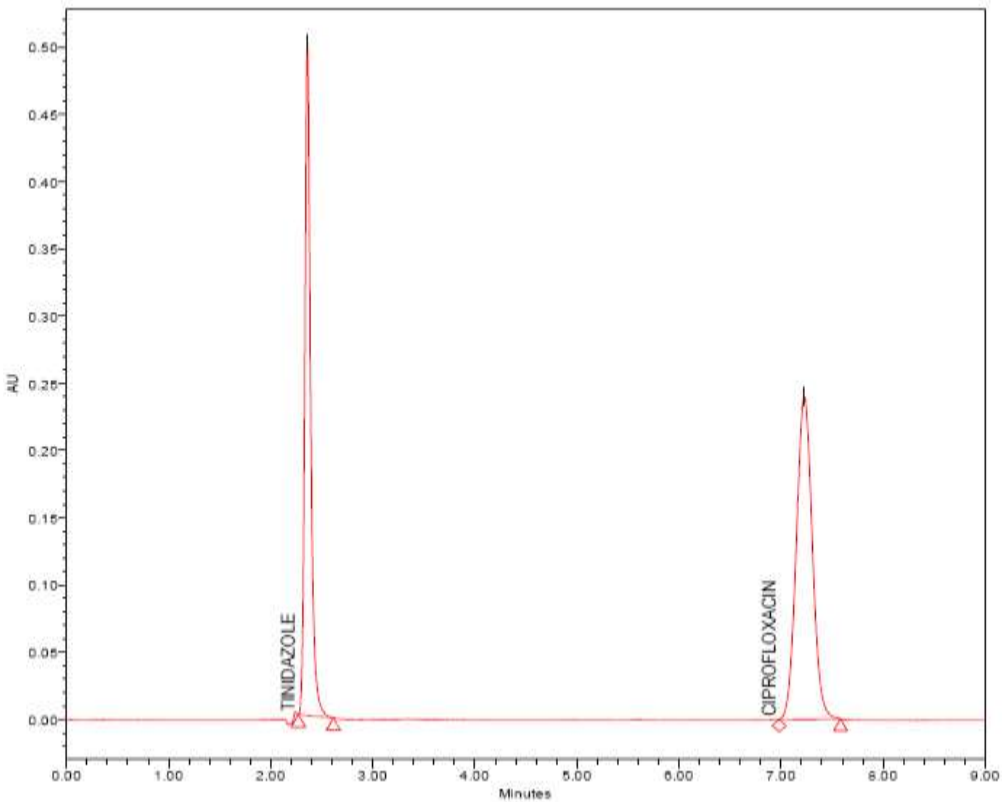


Figure 2:- Typical Chromatogram of standard Tinidazole and Ciprofloxacin.

	Name	Retention Time	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	TINIDAZOLE	2.359	2363603	499354		1.3	9069
2	CIPROFLOXACIN	7.228	3301853	239207	24.71	1.0	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	1.0
Limit of Detection(LOD)	1µg/mL	1.5µg/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

Results of Intra day and inter day variability were summarized in table 2. Intra day 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

Table2:- Intra day and inter day precision result of Tinidazole and Ciprofloxacin.

Drug	%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.

Sample No.	Spiked Amount (mg)	Recovered Amount (mg)	%Recovered	%Average recovery
		Tinidazole		
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	

Results of accuracy study are presented in table 3. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 97.66% for Tinidazole and 101.66% for Ciprofloxacin. All the results indicate that the method is highly accurate.

The results of robustness of the present method showed that small changes were made in the flow rate and temperature did not produce significant changes in analytical results which are presented in Table 4 . As the changes are not significant we can say that the method is robust

Table 4:- Results for robustness test of Tinidazole and Ciprofloxacin.

Parameters	**Changes**	**RT**	**USP Tailing**	**USP Plate count**
		Tinidazole		
Flow rate(ml/min)	1	2.831	1.2	6609
	1.8	2.029	1.1	6302
Temperature	45ºc	2.560	1.3	6775
	55ºc	2.120	1.40	6001
		Ciprofloxacin		
Flow rate(ml/min)	1	8.645	1	8309
	1.8	6.1601	7258	
Temperature	45ºc	7.522	1	7311
	55ºc	6.9193	1	9193

Conclusion:-

The new HPLC method developed and validated for simultaneous determination of Tinidazole and Ciprofloxacin in pharmaceutical dosage forms. 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.

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