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DEVELOPMENT AND VALIDATION OF CIPROFLOXACIN, TINIDAZOLE AND ORNIDAZOLE BY CHROMATOGRAPHIC TECHNIQUES

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ABSTRACT

Ciprofloxacin is an antibiotic effective against many different bacteria, both Gram-positive (Gram +) e. g. Pneumonia and Gram-negative (Gram -) e.g. H. pylori used in the treatment of common urinary tract infections, and certain gastrointestinal infections, gynecological infections / STDs, skin infections, and upper and lower respiratory infections (such as sinusitis, pneumonia, bronchitis).Tinidazole is a drug used against protozoan infections. It is widely effective against amoebic and parasitic infections. Its antibacterial and antiprotozoal activity works against G.vaginalis, T.vaginalis and E. histolytica.Ornidazole is an antibiotic used to treat protozoan infections and prevention of infections caused by anaerobic bacteria, during operative treatment, gynecological surgeries, severe intestinal ameobiasis and giardiasis. This article aims to study accurately about various analytical methods used for determination of ciprofloxacin, tinidazole and ornidazole without excipients and in marketed product as well.

The various analytical techniques used are spectroscopy, electrochemical methods, capillary electrophoresis, high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), and gas chromatography.

Keyword: Ciprofloxacin, tinidazole, ornidazole, antiprotozoal, analytical method.

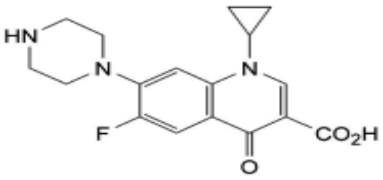
1. INTRODUCTION

Ciprofloxacin is effective against many different bacteria, both Gram-positive (Gram +) e. g. Pneumonia and Gram-negative (Gram -) e.g. H. pylori. It is used in the treatment of common urinary tract infections, in certain gastrointestinal infections, gynecological infections / STDs, skin infections, upper and lower respiratory infections (such as sinusitis, pneumonia, bronchitis).

Mode of Action: That they kill bacteria. The use of these antibiotics is, they work by inhibiting

the bacterial DNA gyrase enzyme. Where DNA gyrase enzyme is important for the replication of DNA in bacteria. For the each time of dividing cells copying of DNA is necessary, and they interrupt in replication which makes difficult for the bacteria to multiply

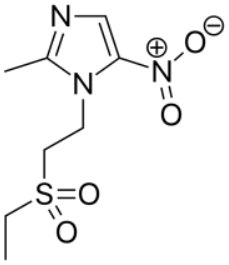
Clinical use: Include the treatment of common urinary tract infections, and certain gastrointestinal infections, gynecological infections / STDs, skin infections, and upper and lower respiratory infections (such as sinusitis, pneumonia, bronchitis).



Tinidazole is a drug used against protozoan infections. Its antibacterial and antiprotozoal activity is similar to that of metronidazole. It works against G.vaginalis; the hydroxy metabolite is significantly more active than that of metronidazole. H. pylori is inhibited by 0.5 mg/L. T.vaginalis and T. fetus at 2.5 mg/L and E. histolytica is inhibited by about 0.3–2.5 mg/L.

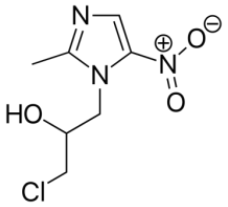
Mode of action: Tinidazole has a mechanism of action that parallels that of metronidazole as well as a similar metabolic pathway leading to hydroxylation at the 2-methyl group catalyzed by

CYP3A4.generally, tinidazole works and acts as like metronidazole but, some time they reported that it also effective against some of protozoa which are resistant to metronidazole.



Clinical use: Anaerobic bacterial infections (prophylaxis and treatment) Trichomoniasis Giardiasis (single dose) Ameobiasis (including amebic liver abscess) Bacterial vaginosis Gastric colonization with H. pylori (in combination with other agents).

Ornidazole is an antibiotic used to treat protozoan infections. Antimicrobial spectrum is quite similar metronidazole and is tolerated better. First time ornidazole was introduced for the treatment of trichomoniasis which already recognized working against broad anti-protozoan and anti-anaerobic bacteria capacities. Previously found for the treatment of Crohn's disease. It was also shown to be active in vivo against coliforms despite in vitro resistance. It is suggested that in the presence of anaerobes the drug is active in-vivo against aerobic Gram-negative microorganisms.



Clinical uses: used in the treatment of anaerobic systemic infections caused by ornidazole-sensitive microflora, prevention of infections caused by anaerobic bacteria, during operative treatment (especially middle and straight intestine surgeries), gynecological surgeries, severe intestinal ameobiasis, all extra-intestinal ameobiasis forms, giardiasis.

2. METHODOLOGY

Sample preparation is an essential and vital step has used during analytical procedures. It is found that approximately 30% errors were contributed from sample analysis during sample preparation.

Balance	Mettle Toledo, AB 204-S
HPLC pump	Shimadzu LC 10AT VP
Detector	Shimadzu UV-10 VP
Injector	SIL-10AD VP Auto Injector
Control module	Shimadzu system controller SCL-10A VP
Software	Shimadzu class VP
pH meter	Lab India PN 05130711

Name of reagent used	Make
Acetonitrile	HPLC Grade (Rankem)
Triethylamine	AR Grade (Thomas baker) AR Grade (Thomas baker)
Phosphoric acid	
Sodium hydroxide pellets	AR grade (Arnols Thried)

All the chemicals and reagents used were of analytical grade. Sodium hydroxide pellets and hydrochloric acid were procured while distilled water used for the dissolution of reagents was prepared in the laboratory for tinidazole.

Name of Standard	Batch no.	Purity
Ciprofloxacin hydrochloride	WS408024	93.56%
Ethylenediamine	J0A030	100%
Ciprofloxacin 500mg	1330106	93.56%
Ciprofloxacin 250mg	1290106	100%
Tinidazole 250 mg		98.00%
Tinidazole 500 mg	1764434	96.00%
Ornidazole 250 mg		99.06%
Ornidazole 500 mg	1433276	95.76%
	4327698	
	4323198	

Buffer preparation for mobile phase: Dissolve 1.44 ml of orthophosphoric acid in 1000 ml of distilled water and adjust pH to 3.0 ± 0.1 with triethylamine.

Diluent preparation: 80 volumes of buffer and 20 volumes of acetonitrile. Buffer preparation for diluent: Dissolve 1.44 ml of orthophosphoric acid in 1000 ml of distilled water and adjust pH to 2.0 ± 0.1with triethylamine.

Standard Preparation: Transfer accurately weighed about 20mg of all three tablets is working standard to a 100ml volumetric flask, add about 80 ml of diluent to dissolve, dilute up to the mark with diluent and mix.

Resolution solution: Transfer about 5mg of ethylenediamine analog RS and about 20mg of ciprofloxacin, tinidazole and ornidazole hydrochloride working standard to a 100 ml volumetric flask, add about 80 ml diluent and sonicate to dissolve. Dilute up to the mark with diluent and mix.

Sample Preparation: Transfer 5 tablets to a 500ml volumetric flask, add 400ml of diluent and sonicate for about 20 min with intermittent shaking. Dilute to volume the diluent, mix the contents and filter through 0.45 µm membrane filter. Transfer 2ml of the filtrate to 25ml volumetric flask and dilute to volume with the diluents.

Relative data for ciprofloxacin, tinidazole and ornidazole:

No. of tablets	Test area	% Assay
5	9075578	99.6
5	9101550	99.9
5	9111620	100.0
5	8969377	98.5
5	9104438	100.0
5	9045930	99.3
Mean	9068082	99.6
SD	54086.97	0.58
% RSD	Limit NMT 2.00%	0.58

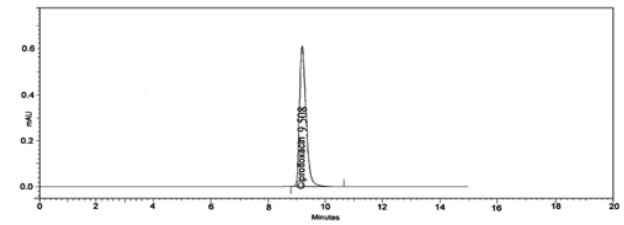
Level	Std. wt. (mg)	Area	% Recovery	Mean recovery	SD	% RSD
80% 1	1110	6979124	99.9			
80% 2	1111	6982530	99.8	99.9	0.06	0.06
80% 3	1111	6985916	99.9			
100% 1	1388	8693324	99.5			
100% 2	1385	8700318	99.8	99.7	0.17	0.17
100%3	1380	8672490	99.8			
120% 1	1666	10405583	99.2			
120% 2	1667	10503520	100.1	99.8	0.49	0.49
120% 3	1666	10493202	100.0			

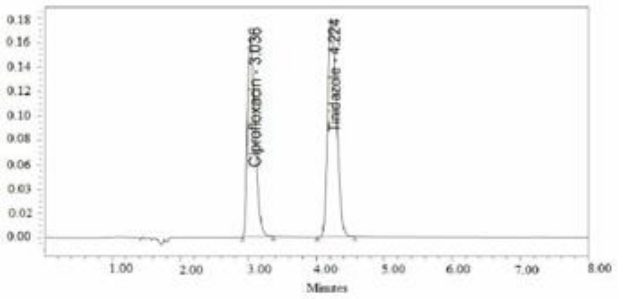
Analytical methods:

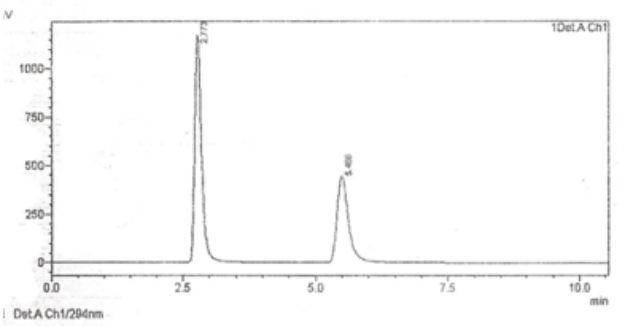
Chromatography is probably the most powerful and versatile analytical technique available to the modern chemistry, its power arises from its capacity to determine quantitatively many individual components present in mixture in one, single analytical procedure. The sample can range in complexity from a single substance to multi component mixture containing widely differing chemical species. HPLC Is a method of chemical separation in which stationary phase contained in column and mobile phase in pumped at high pressure from one end. The pressure used here is about 350 atmosphere (5000 psi = pounds / Sq. inch).

In this method separation of mixture from microgram to gram is done by passing the sample through a column of 5 mm diameter containing a stationary solid bed by means of pressurized flow of liquid mobile phase. The component of the mixture migrates through the column at different rates, due to their solubility or depends upon molecular charge separation of the mixture occur.

Dosage forms	k/min	$t_{1/2}$ (min)	t_{90} (min)
Tablet ciprofloxacin 500	4.81,10–2	201. 56	40.30
Tablet ciprofloxacin 250	5.38, 10–2	183.83	36.75
Tablet tinidazole 250	4.90, 10-8	156.76	20.98
Tablet tinidazole 500	7.0, 10-7	178.78	45.96
Tablet ornidazole 250	7.8, 10-6	149.98	26.56
Tablet ornidazole 500	6.8 , 10-9	156.89	43.98







3. DISCUSSION

The development of an analytical method for the estimation of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The objective of this study was to develop a rapid and sensitive HPLC method for the analysis of all three formulation in bulk drug and its formulations. In this study, the HPLC method for assay of ciprofloxacin, tinidazole and ornidazole tablets 250mg and 500mg has been validated. The validation is performed according to the current requirements as laid down in the ICHguidelines.

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiply samplings of a homogenous sample. Precision may be a measure of the degree of repeatability or reproducibility of the analytical method under normal operating conditions. Here determine the %RSD for peak area, retention time and system suitability parameters i.e. theoretical plates, asymmetry & resolution. From the data %RSD for the assay values of ciprofloxacin, tinidazole and ornidazole tablets is within the acceptable limit.

The robustness of analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations in the method parameters and provides an indication of its reliability during normal usage. % RSD for robustness study is not more than 2%. It can be concluded that the Analytical method is robust towards the above designed changes.

Check the stability of drug substance in solution form at different time intervals. As per test, it can be concluded that the solution is stable after 24 hrs within experimental conditions.

4. CONCLUSION

The results obtained in this study demonstrate that the HPLC method described in the protocol is valid for the determination of assay of ciprofloxacin, tinidazole and ornidazole tablets 250mg, 500 mg. Therefore the method is suitable for its intended use.

- The present study describes a highly sensitive, accurate and reproducible HPLC method for determination of ciprofloxacin, tinidazole and ornidazole. This method has several advantages. Sample preparation is

simple and the chromatographic column is available commercially.

- The procedure for sample preparation is rapid and inexpensive. Retention time for ciprofloxacin hydrochloride is less.
- The very low quantification limit obtained with a UV detector helped to avoid use of fluorimetric detection, which demands more expensive equipments. UV detectors give more reproducible and stable responses than fluorimetric detectors.
- Another advantage of this method is the use of an isocratic mobile phase of very simple composition, which gives the column a longer lifetime.
- From the results, it can be concluded that proposed method has been successfully applied for the analysis of all three types of tablets and can be used for the routine analysis of formulations containing any one of the above drugs without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations.

5. ACKNOWLEDGEMENT: XYZ

6. CONFLICT OF INTEREST: - NONE

7.DECLERATION

I hereby declare that the work incorporated in the present project report entitled.

"DEVELOPMENT AND VALIDATION OF CIPROFLOXACIN, TINIDAZOLE AND ORNIDAZOLE BY CHROMATOGRAPHIC TECHNIQUES" is my own work and is original. This work (in part or full) has not been previously submitted to any university for the award of degree.

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