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Method Development and Validation for Rapid Simultaneous Estimation of Tinidazole and Diloxanide Furoate in Pharmaceutical Dosage Form by Using RP-HPLC

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Abstract

A new method was established for simultaneous estimation of Tinidazole and Diloxanide furoate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tinidazole and Diloxanide furoate by using Thermosil RP C18 (4.5×100 mm) 5.0µm, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol. The retention times were found to be 2.408mins and 3.016mins. The % purity of Tinidazole and Diloxanide furoate was found to be 99.24% and 101.27% respectively. The system suitability parameters for Tinidazole and Diloxanide furoate such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Tinidazole and Diloxanide furoate was found in concentration range of 50ppm-250ppm and 5ppm-25ppm and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.56% and 101.47%, %RSD for repeatability was 0.1 and 0.3, % RSD for intermediate precision was 0.19 and 0.57respectively. The precision study was precise, robust, and repeatable. LOD value was 4.27 and 6.80, and LOQ value was 0.0272 and 0.3125 respectively.

Keywords: Thermosil RP C18 column, Tinidazole and Diloxanide furoate, RP-HPLC

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Contents:

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1. Introduction

Tinidazole is an antisporezole and protozole medication. [2-(ethanesulfonyl)ethyl] is the IUPAC nomenclature 1. 1-H-imidazole-2-methyl-5-nitro. Tinidazole's chemical formula is $C_{15}H_{10}CL_2N_2O_2$. Trichomonas uses a ferredoxin-mediated electron transport mechanism to decrease the tinidazole's NITR group.The free nitroradicals produced by this reduction are thought to be responsible for anti-protozole activity. Toxic free radicals bind to DNA, causing DNA damage, and are thought to lead to cell death. The mechanism by which tinidazole is active against Giardia and Enteroeva species is unknown, but is likely similar. Tinidazole is a synthetic anti-protozoal treatment. Tinidazole is active in both in vitro and clinical infections against the following protozoa: Trichomonas vagina, Giardia doodenaris (also known as G. lambria), and Enterteva histolitica. Tinidazole does not appear to be active against most strains of vaginal lactic acid bacteria. Food hospitalization leads to a delay in TMAX of about 2 hours and a decrease in CMAX of about 2 hours. 901.6Â±126.5 mcg 10% and AUC 2. Diloxanide is lumbar gait with the IUPAC name of 4-(2.2-dichloro-N-methylacetamide) phenylfuran-2-carboxylic acid. The

chemical formula for diloxanide is C14H11Cl2NO4 However, the mechanism of action of diloxanide is unknown. Diloxanide kills E. histolytica's vegetative spores, which eventually develop into cysts. People who are infected with asymptomatic ambiguity expel cysts. In the gastric intest region, the diloxanide furoate is delayed and hydrolyzed to yield the active component, diloxanide. Dyloxanation can inhibit protein synthesis.

Bioavailability is 90% (drioxanized form of parental form), but dirioxanide floats are slowly absorbed by the stomach intestines. It produces comprehensive glucuronation (comprehensive glucuronation as glucuronide as free dilioxanide in the whole body cycle). Simple and specific, accurate anti-phase high performance fluid chromatography (RP-HPLC) has been developed and verified. shape. Anju Goyal et al. , separation was performed by Inertsil C18 (250 Ã°Â¥4.6 mm) containing mobile phase phosphate buffer (pH 6.8): acetonitrile (82:18) v/v. Malathi Raghunath et al., Ciprofloxacinhydroclide (CPX) is fluoroquinolone antibacterial. Tinidazole (TNZ) is nitroimidazole-anti-protozoal, while dicyclominhydrohydrochloride (DIC) is an anticholinergic anti-stage treatment. Three drugs in the solid spinal cord combination are often administered in infections of mixed origin with typhoid fever.

The aim of this study was to develop an inverse phase high-performance liquid chromatography method for the simultaneous estimation of CPX, TNZ and DIC in tablet formulations combined with bulk products. Isocratic's inverse liquid-liquid method was created by Ravi Varma et al. to quantify ciprofloxacin and tinidazole quantitatively at the combination points. Acetonitrile was utilized in a mobile phase with water pH 2.4 that contained orthophosphate, in a ratio of inertsil C18 (150*4.6*5 mineral) column (850:150, v/v). Wastewater was measured at 275 nm, the column temperature was 30Â°C, and the flow rate was 1.0 ml/min. Tinidazole and ciprofloxacin had respective retention durations of 5.119 and 2.419 minutes.

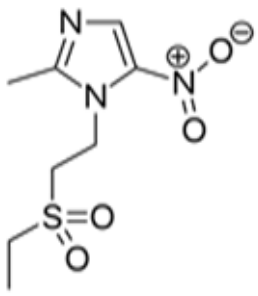

Fig.1 Tinidazole

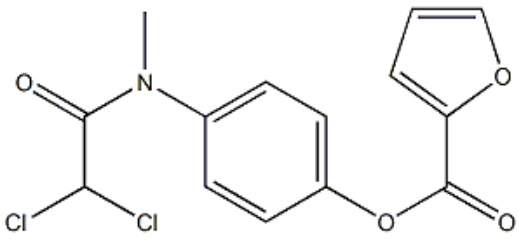

Fig.2 Diloxanide Furoate

Inference: We developed and validated a method for the quick simultaneous measurement of tinidazole, diloxanide furoate in medication dosage form using RP-HPLC based on the literature review.

2. Materials and Methods

2.1 Materials

The study's materials included potassium di hydrogen phosphate (KH2PO4) from MERCK and tinidazole and diloxanide furoate from torrent pharmaceuticals. MERCK is the source of water, methanol, acetonitrile, and orthophosphoric acid.

1.2 Instruments

A Waters HPLC system (Model No. Separation module 2695, UV detector 2487) running Empower software version -2 was one of the study's instruments. For spectroscopic analysis, a Lab India UV 3000+ UV/VIS spectrophotometer was used. It was an Ascoset digital weighing balance. ADWA was the PH meter. The Enertech sonicator was utilized.

1.3 Preparation of Solutions: Weighing out 2.95 grams of sodium acetate, we put it in a 1000 ml beaker, dissolved it, diluted it with 1000 ml of HPLC water, and used orthophosphoric acid to bring the pH down to 4.5. After that, the solution was filtered and sonicated.

Preparation of Mobile phase: Combine 30 milliliters (30%) of the aforesaid buffer with 70 milliliters of methanol (HPLC grade 70%), then degas for five minutes in an ultrasonic water bath. Filter under vacuum filtering using a 0.22 µ filter. Tinidazole and Diloxanide Furoate Standard and Sample Solution Preparation

Preparing a sample solution:

After precisely weighing and transferring 10 mg of tinidazole and 1 mg of powdered Diloxanide furoate tablets into a 10 ml clean, dry volumetric flask, add roughly 2 ml of diluent, sonicate to dissolve it fully, and top off the volume with the same solvent (stock solution). 10 ml of the stock solution mentioned above was then pipetted into a 100 ml volumetric flask and diluted with diluent to the appropriate level.

Standard solution preparation: After precisely weighing and transferring 10 mg of tinidazole and 1 mg of diloxanide furoate working standard into a 10 ml clean, dry volumetric flask, add roughly 2 ml of diluent, sonicate to dissolve it fully, and then use the same solvent (stock solution) to get the volume up to the required level.Additionally, 1 milliliter of the stock solution mentioned above was pipetted into a 10-milliliter volumetric flask and diluted with diluent to the appropriate level.

2.4 Methodology

An RP HPLC technique for the simultaneous measurement of diloxanide furoate and tinidazole was developed as part of this investigation. Tinidazole or dilioxanide furoate were diluted to separate the standard solutions of diloxanide furoate 500Ã®veng/ml and tinidazole 500 Ã®Â¼g/ml. According to II criteria, this approach was validated for system budget, linearity, medium accuracy, accuracy, robustness, LOQ, and LOD. For drug analysis, this approach demonstrated good resolution, linearity, and reproducibility.

1. Specificity:

To find out if there are any contaminants interfering with the analytical peak's retention time, the system appropriateness for specificity was tested. Blank injection was used to achieve specificity.

2. Linearity

As long as the analyte concentration falls within the specified range, the approach can generate a linear response. The linearity was investigated for 5% to 25% of Diloxanide furaote and 10% to 50% of Tinidazole concentrations. The coefficient of determination for both analytes.

3. Range: The assay method is exact, linear, and accurate in the range of 50 ppm-250 ppm and 5 ppm-25 ppm of tinidazole and diloxanide furoate, respectively, according to data on precision, linearity, and accuracy.

4. Accuracy

In the chromatographic system, standard solutions with accuracy levels of 50%, 100%, and 150% were introduced. Determine the amounts of tinidazole and diloxanide furoate that were identified and added, as well as the recovery percentages for each and the mean recovery percentage.

5. Precision

Five injections of the standard solution were made, and the area of each injection was measured in an HPLC. It was discovered that the area of five replicate injections' %RSD fell within the predetermined bounds.

6. Limit of detection (LOD)

According to the formula, LODs can be computed using the slope of the calibration curve (S) at levels that approximate the LOD and the standard deviation of the response (SD). The standard deviation of the regression lines' y-intercepts can be used to calculate the response's standard deviation.

7. Limit of quantification

According to the formula, LOQs can be computed using the slope of the calibration curve (S) and the standard deviation of the response (SD). Once more, the standard deviation of the y-intercepts of regression lines can be used to calculate the standard deviation of the answer.

8. Robustness

Deliberate adjustments to the flow rate and mobile phase composition were conducted as part of the robustness test to assess the effect on the procedure.

a. The flow rate was adjusted between 0.4 and 0.6 milliliters per minute. A standard solution containing 150 parts per million of tinidazole and 15 parts per million of ramipril was made and examined using a range of flow rates in addition to the procedure flow rate.

b. The mobile phase's organic content ranged from 65% to 75% of the reference solution. In addition to the real mobile phase composition in the procedure, 150 µg/ml of tinidazole and 15 µg/ml of diloxanide furoate were produced and analyzed using the altered mobile phase composition.

9. System suitability

The theoretical plates for the peaks caused by tinidazole and diloxanide furoate in standard solution should not be less than 2000, and the tailing factor for these peaks should not be greater than 1.5.

3. Results and Discussions

The purpose of the investigation and validation presented in this work is to use the RP HPLC method to estimate tinidazole, diloxanide furoate simultaneously. According to the literature, no analytical techniques were provided for the RP HPLC method's simultaneous estimation of tinidazole, diloxanide furoate. Consequently, there was a belief that a novel analytical technique was needed for the simultaneous determination of tinidazole and dilioxanide furoate in medicinal dose versions. Both diloxanide furoate and tinidazole were retained, and their isobotic points demonstrated maximum absorption at 270 nm.

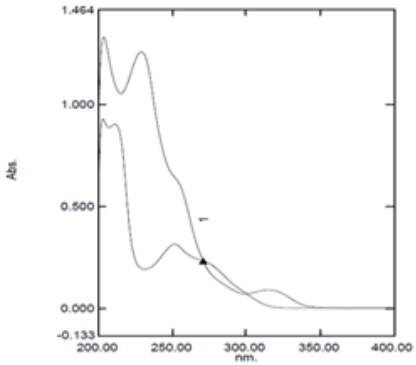

Figure.3 Spectrum showing overlapping spectrum of Tinidazole and Diloxanide furoate

Linearity

The concentrations of 50 ppm to 250 ppm and 5 ppm to 25 ppm were the subjects of the linearity research. The chromatographic system was injected with each level. The correlation coefficient was calculated using the area of each level. The outcomes are displayed below.

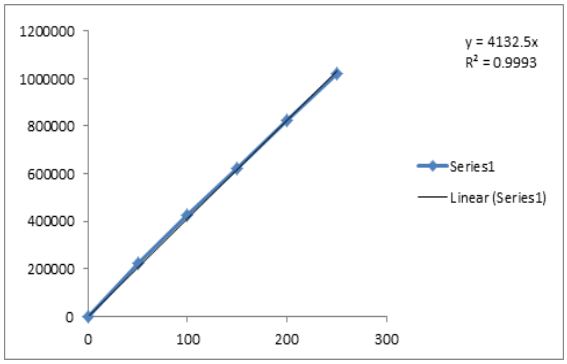

Figure.4 Calibration graph for Tinidazole

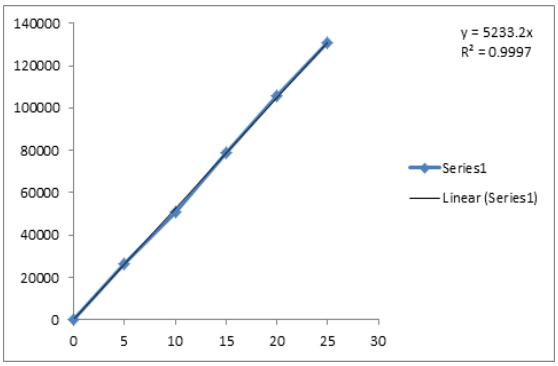

Figure.5: Calibration graph for Diloxanide furaote

Precision

Five injections of the standard solution were made, and the area of each injection was measured in an HPLC. It was discovered that the area of five replicate injections' %RSD fell within the predetermined bounds.

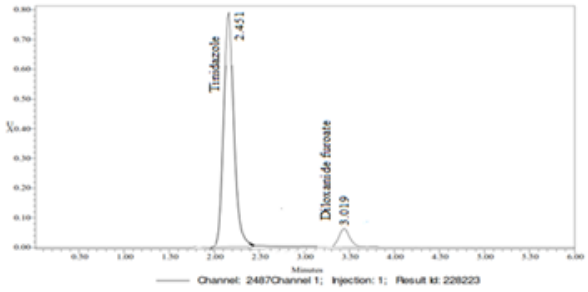

Figure.6: Chromatograph for precision

Table: 1 Results of precision

Name : Tinidazole

	Name	RT	Area	Height (µV)
1	Tinidazole	2.451	596886	63755
2	Tinidazole	2.452	597766	63808
3	Tinidazole	2.453	600318	61988
4	Tinidazole	2.453	600832	65724
5	Tinidazole	2.454	600884	64272
Mean			599337	
Std. Dev.			1875.2	
% RSD			0.31	

Table: 1 Results of precision

Name : Diloxanide furoate

	Name	RT	Area	Height (µV)
1	Diloxanide	3.019	6423669	779071
2	Diloxanide	3.018	6418299	791461
3	Diloxanide	3.017	6435957	781924
4	Diloxanide	3.016	6426016	810297
5	Diloxanide	3.015	6425928	799359
Mean			6425974	
Std. Dev.			6400.9	
% RSD			0.10	

5. Intermediate precision

Five injections of tinidazole and diloxanide furoate were the subject of the intermediate precision study. The chromatographic system was injected with each standard injection. The percentage RSD was calculated using the area of each standard injection.

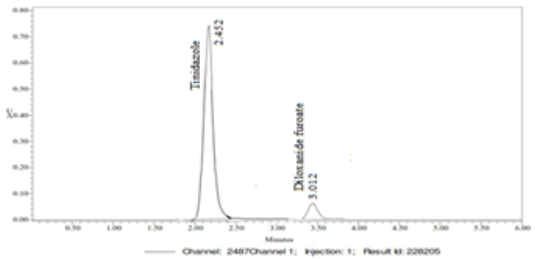

Figure.7: Chromatograms showing intermediate precision injections -1to 5

Table: 3 Results of Intermediate precision

Name : Tinidazole

	Name	RT	Area	Height (µV)
1	Tinidazole	2.452	628573	69298
2	Tinidazole	2.451	624731	63015
3	Tinidazole	2.453	619076	65274
4	Tinidazole	2.456	622317	66090
5	Tinidazole	2.455	625203	68413
Mean			623980	
Std. Dev.			3534.5	
% RSD			0.57	

Table: 4 Results of Intermediate precision

Name : Diloxanide furoate

	Name	RT	Area	Height (µV)
1	Diloxanide	3.012	6609039	864770
2	Diloxanide	3.013	6625558	816850
3	Diloxanide	3.014	6633630	815380
4	Diloxanide	3.015	6643244	828530
5	Diloxanide	3.016	6628255	851393
Mean			6627945	
Std. Dev.			12545.9	
% RSD			0.19	

Detection limit

According to the formula, LODs can be computed using the slope of the calibration curve (S) at levels that approximate the LOD and the standard deviation of the response (SD). The standard deviation of the regression's y-intercepts can be used to calculate the response's standard deviation.

Quantitation limit

According to the formula, LOQs can be computed using the slope of the calibration curve (S) and the standard deviation of the response (SD). Once more, the standard deviation of the y-intercepts of regression lines can be used to calculate the standard deviation of the answer.

Accuracy

The accuracy research was conducted for tinidazole and diloxanidefuroate at 50%, 100%, and 150%.Three injections of each level were made into the chromatographic apparatus. The percentage recovery was calculated using the area of each level.

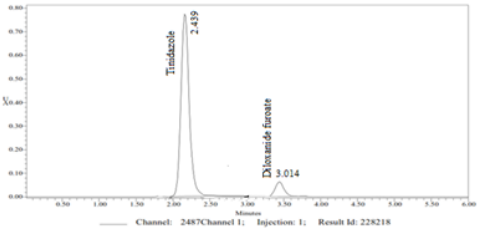

Figure.8: Chromatograms showing accuracy-50% injection

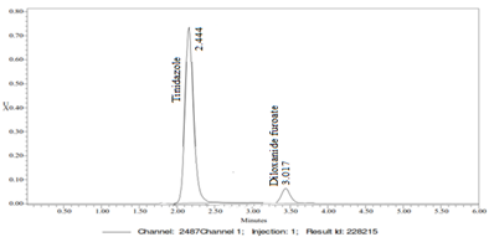

Figure.9: Chromatogram showing accuracy -100% injection

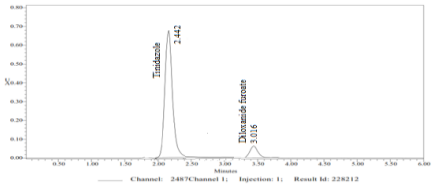

Figure.10: Chromatogram showing accuracy -150 % injection

Robustness

For tinidazole and diloxanide furoate, the robustness was tested for changes in the mobile phase ratio from a more organic to a less organic phase ratio and changes in the flow rate from 0.4 to 0.6 milliliters per minute. Only under low flow conditions is the approach resilient, and it remains so even when the mobile phase changes by ±5%.

Table 5: Results of Tinidazole and Diloxanide

S.No	Tinidazole		Diloxanide Furoate	
	Amount	**Peak Area response (mV)**	**Amount**	**Peak Area response (mV)**
1	50ppm	221543	5ppm	26472
2	100ppm	426277	10ppm	50841
3	150ppm	624999	15ppm	78655
4	200ppm	826124	20ppm	105541
5	250ppm	1022139	25ppm	130567
R^2		0.999	0.999	

Table 6: Results of LOD

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Tinidazole	382625.50	572175863	4.27
Diloxanide furoate	5862.40	467579210	0.0272

Table 7: Results of LOQ

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Tinidazole	381727.80	583265980	6.80
Diloxanide furoate	5681.30	469828490	0.312

Table 8: Accuracy results of Tinidazole

	Name	RT	Area	Height(µV)
1	Tinidazole	2.439	499058	64425
2	Tinidazole	2.438	499571	63112
3	Tinidazole	2.440	499134	61925
Mean			499254	
Std. Dev			276.86	
% RSD			0.05	

Table 9: Accuracy results of Diloxanide

	Name	RT	Area	Height(µV)
1	Diloxanide	3.014	5236293	773132
2	Diloxanide	3.015	5279758	763591
3	Diloxanide	3.016	5281105	764285
Mean			5265719	
Std. Dev			25492.2	
% RSD			0.48	

Table 10: System suitability results for Diloxanide furoate (change in Flow rate)

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	3543	1.2
2	1	3452	1.2
3	1.2	3226	1.2

Table 11: Showing system suitability results for Tinidazole **(**change in Flow rate)

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2430	1.1

2	1	2453	1.2
3	1.2	2369	1. 1

Table 12: Showing system suitability results for Diloxanide furoate (Change in organic composition)

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	3187	1.2
2	*Actual	3452	1.3
3	5 % more	2569	1.4

Table 13: Showing system suitability results for Tinidazole (Change in organic composition)

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2195	1.1
2	***Actual**	**2356**	**1.1**
3	5 % more	2170	1.0

4. Conclusion

Simple, sensitive, fast and economic stability indicates that the RP HPLC method was developed and validated for assays with tinidazole and diloxanide furoate in tablet combinations. This method has resulted in high recovery with good linearity and accuracy. The proposed method can be drawn to the conclusion that it is a suitable approach to achieving reliable results and is suitable for routine analysis of tablet formulations with tinidazole and dilioxanide furoate layers. From these studies we reported % of decomposition products under a variety of conditions, including acid hydrolysis, alkali hydrolysis, oxidation, heat, UV, and neutral hydrolysis. Therefore, the proposed RP HPLC method can be used for routine analysis of tinidazole and diloxanide furoate in API and drug dosage forms.

Acknowledgement

I am very thankful to Director, JNTUA-OTPRI, Ananthapuramu for providing the laboratory facilities, chemicals to carryout entire research work.

Conflict of Interest

We affirm that there are no conflicts of interest.

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