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RP-HPLC Method Development and Validation for Tinidazole and Diloxanide Furose Pharmaceutical Formulation and Its Forced Degradation Studies

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Abstract

For the simultaneous measurement of tinidazole and diloxanide furoate in pharmaceutical dosage form, a straightforward, accurate, and exact approach was created. A Waters 2695 HPLC system with an auto sampler, UV detector, and Empower 2 software was used to conduct the chromatography. The Hypersil OSD C18 column (250 x 4.6 mm, 5 µ particle size) was used for the analysis, which was conducted at 278 nm and at 250C. Acetoitrile (45:55V/V) and Phosphate Buffer (KH2PO4) make up the optimum mobile phase. A constant flow rate of 1 ml/min was maintained.Tinidazole and Diloxanide Furoate were determined to have respective purity percentages of 100.24% and 99.64 percent. The theoretical plates, tailing factor, and other system suitability parameters for tinidazole and diloxanide furoate were determined to be 5630, 7362, 1.32, and 1.24, respectively. According to the linearity investigation, the correlation coefficient (r2) for tinidazole and diloxanide furoate was 0.999 and 0.999, respectively, and the mean recovery percentages were 99.85% and 100.21%.The corresponding RSDs for repeatability and intermediate precision were 0.6 and 0.6, respectively. The precision study was repeatable, reliable, and accurate. The study's findings demonstrated the simplicity, accuracy, precision, robustness, speed, and reproducibility of the suggested RP-HPLC approach, which could be helpful for routinely estimating the amounts of tinidazole and diloxanide furoate in pharmaceutical dosage forms.

Keywords: Tinidazole, Diloxanide Furoate, RP-HPLC, Simultaneous estimation.

INTRODUCTION:

Tinidazole is a nitroimidazole that is used to treat bacterial vaginosis, amebiasis, trichomoniasis, and giardiasis. An antitrichomonal nitroimidazole that works well against infections caused by Giardia lamblia, Entamoeba histolytica, and Trichomonas vaginalis. for the treatment of male and female patients suffering from trichomoniasis brought on by T. vaginalis. For the treatment of intestinal amebiasis and amebic liver abscess produced by E. histolytica in adults and children older than three years, as well as for the treatment of giardiasis caused by G. duodenalis in both adult and pediatric patients. Tinidazole is an antiprotozoal and prodrug. Trichomonas uses a ferredoxin-mediated electron transport mechanism to decrease the nitro group of tinidazole. The antiprotozoal action is thought to be

caused by the free nitro radical that is produced as a result of this reduction. It has been proposed that the harmful free radicals bond to DNA covalently, damaging it and ultimately resulting in cell death. Although the exact mechanism of tinidazole's action against Giardia and Entamoeba species is unknown, it is most likely comparable.[1–3] Tinidazole's IUPAC name is 1-[2-(ethanesulfonyl)ethyl].-2-methyl-5-nitro-1H-imidazole. The formula for molecules is C8H13N3O4S. 247.2 is the molecular weight.

Entamoeba histolytica and certain other protozoal infections are treated with the anti-protozoal medication diloxanide (also known as diloxanide furoate). Despite not being authorized for usage in the US at this time, a CDC research from 1977 to 1990 found that it was effective in treating 4,371 cases of Entamoeba histolytica. When treating asymptomatic (cyst passers) intestinal amebiasis brought on by Entamoeba histolytica, diloxanide is the sole therapy option.[4]. When treating invasive or extraintestinal forms of amebiasis, diloxanide can also be used either concurrently or sequentially with other medications, such as nitroimidazoles (like metronidazole). Diloxanide Furoate is known by its IUPAC designation, 2,2-dichloro-N-(4-hydroxyphenyl)-N-methylacetamide. The formula for molecules is C14H11Cl2NO4. 328.1 is the molecular weight.

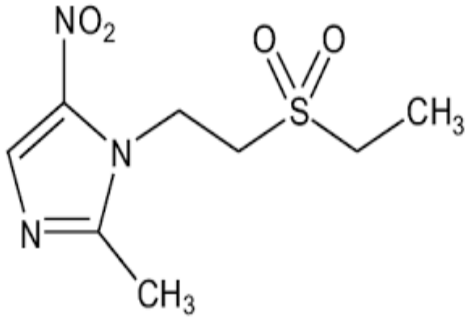

Figure 1: Structure of Tinidazole

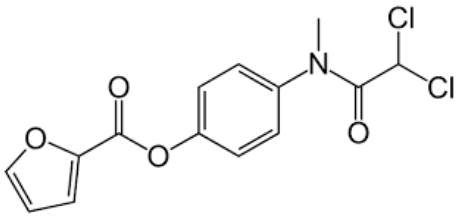

Figure 2: Structure of Diloxanide Furoate

Literature survey shows that a number of methods have been reported for estimation of Tinidazole And Diloxanide Furoate individually or in combination with other drugs. As found from the literature, diloxanide furoate has been reported to be estimated in combination with other drugs by HPLC[5–7] methods. Tinidazole has been reported to be estimated by HPLC[8–10] methods. Tinidazole and diloxanide furoate have been simultaneously determined by spectrometric methods[11-14]. The aim of the present study was A New Rp-Hplc Method for Simultaneous Estimation of Tinidazole and Diloxanide Furoate in Its Bulk and Tablet Dosage Form and Its Force Degradation Studies as Per Ich.

MATERIALS AND METHODS:

Chemicals and Reagents: Tinidazole and Diloxanide Furoate were Purchased from Hetero drugs. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 278 nm with column Hypersil OSD C18 (250 x 4.6 mm, 5 µ particle size), dimensions at 25^0C temperature. The optimized mobile phase consists of Phosphate Buffer (KH2PO4) : Acetoitrile (45:55V/V). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Solvent Selection

In order to select suitable solvent for determination of Tinidazole and Diloxanide Furoate various solvents were selected for the solubility studies and it was found that they were freely soluble in methanol. Hence in the present work, methanol was used for all the dilutions.

Preparation of Standard Stock Solution of Tinidazole

Accurately weighed and transferred 10 mg of Tinidazole working standard into a 100 ml clean dry volumetric flask and it was dissolved by using methanol and the volume was made up to the mark with methanol (100 µg/ml).

1 ml of the above standard stock solution was pipetted into 10 ml volumetric flasks and diluted up to the mark with methanol to get concentration of 10 µg/ml.

Preparation of Standard Stock Solution of Diloxanide Furoate

Accurately weighed and transferred 10 mg of Diloxanide furoate working standard into a 100 ml clean dry volumetric flask and it was dissolved by using methanol and volume was made up to the mark with methanol (100 µg/ml).

1 ml of the above standard stock solution was pipetted into 10 ml volumetric flasks and diluted up to the mark with methanol to get concentration of 10µg/ml.

Preparaion of buffer:

Transferred an accurately weighed 1.36gm of Potassium di-hydrogen Ortho phosphate into a 1000 ml volumetric flask, 900 ml of milli-Q water was added and degassed. Finally make up the volume with water the added 1ml of triethylamine and then pH adjusted to 3.3 with dil.Orthophosphoric acid solution

Mobile phase:

Buffer and Acetonitrile taken in the ratio 45:55% v/v.

Diluent preparation: ACN: Water (50:50 % v/v)

Accurately 50 ml of acetonitrile and 50 ml of milli-Q water were measured and mixed well.

RESULTS AND DISCUSSION

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Hypersil OSD C18 (250 x 4.6 mm, 5 µ particle size), the mobile phase of composition Phosphate Buffer (KH2PO4) : Acetoitrile (45:55V/V)

was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Table 1: System suitability parameters

Parameter	Tinidazole	Diloxanide Furoate
Theoretical plates	5630	7362
Retention time	2.4	3.6
Tailing factor	1.32	1.24

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Tinidazole and Diloxanide Furoate in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Table 2: Assay results for Tinidazole and Diloxanide Furoate

		TINIDAZOLE		DILOXANNIDE FUROATE	
S No		RT	Area	RT	Area
1	Standard-1	2.439	450640	3.640	600062
2	Standard-2	2.440	455090	3.642	592891
	Average		452865		596476.5
1	Assay-Sample	2.439	447916	3.642	595854
2	Assay-Sample	2.441	448928	3.644	595903
	Average		448422		595878.5

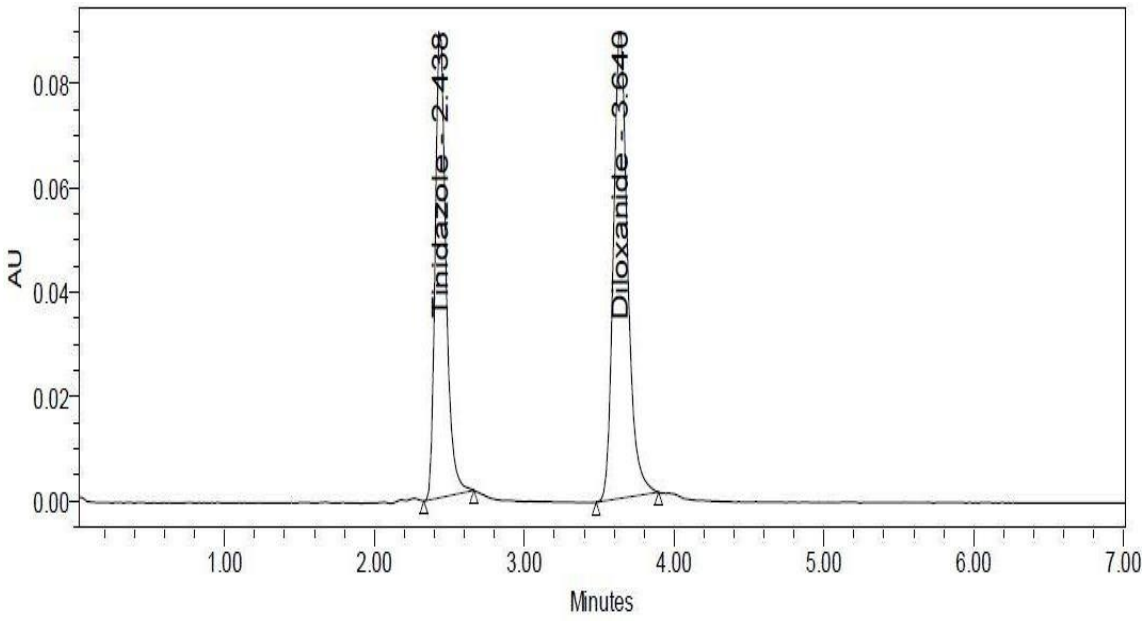

Figure 3: Standard chromatogram

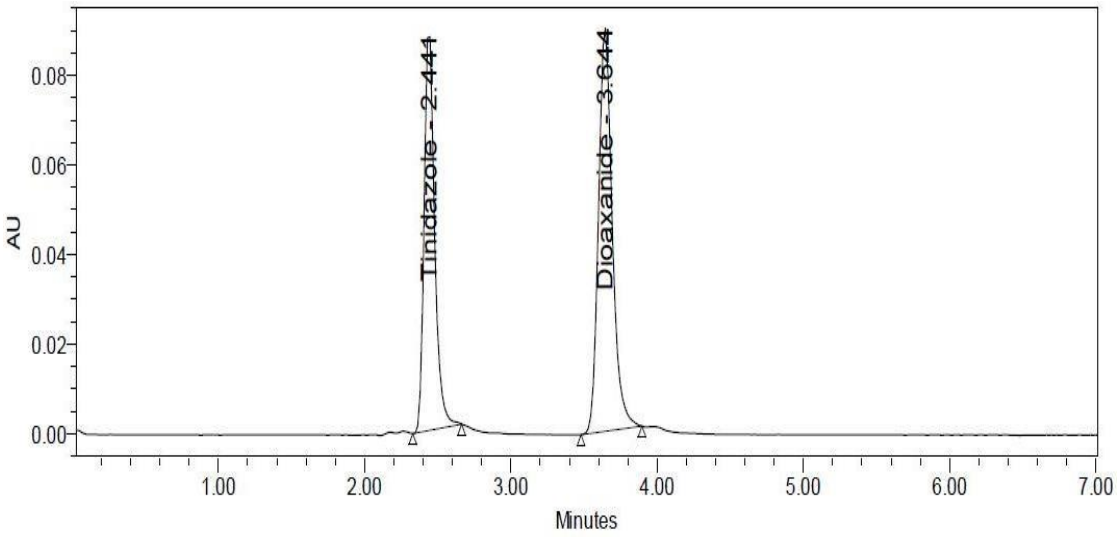

Figure 4: Sample chromatogram

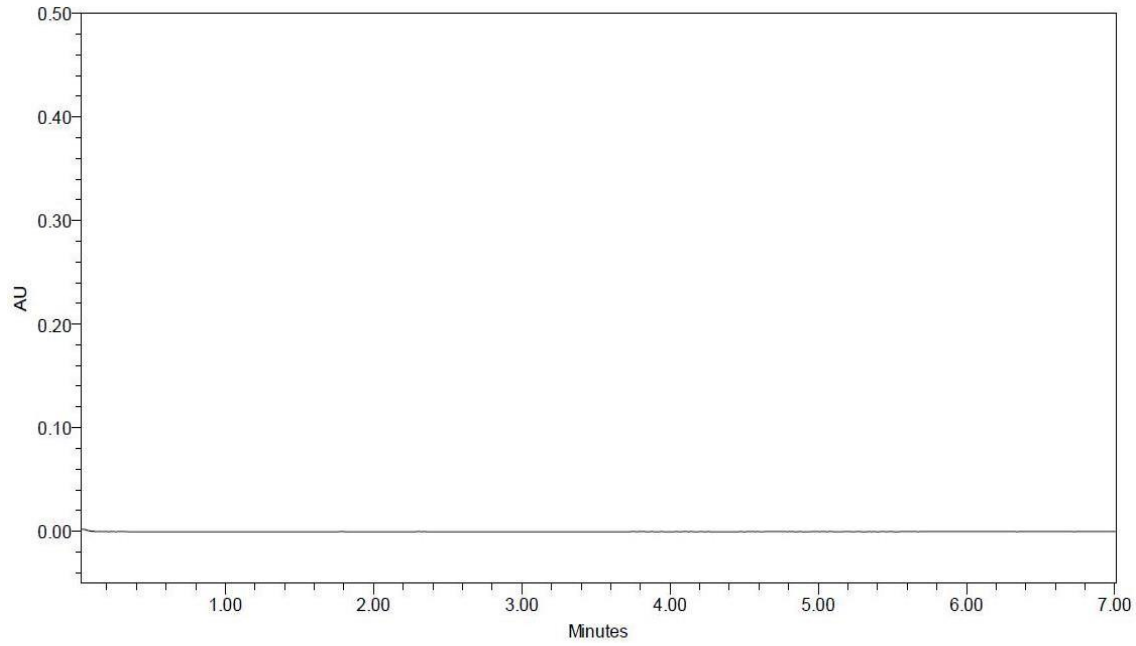

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 30 ppm to 180 ppm and 25 ppm to 150 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3,4.

Table 3: Linearity results of Tinidazole

S.NO	Concentration (µg/ml)	Peak Area
1	30	115108
2	60	235766
3	90	355006
4	120	474538
5	150	600806
6	180	697209
Correlation coefficient(r^2)		0.999

Slope (m)	**3930**
Intercept	**298.1**

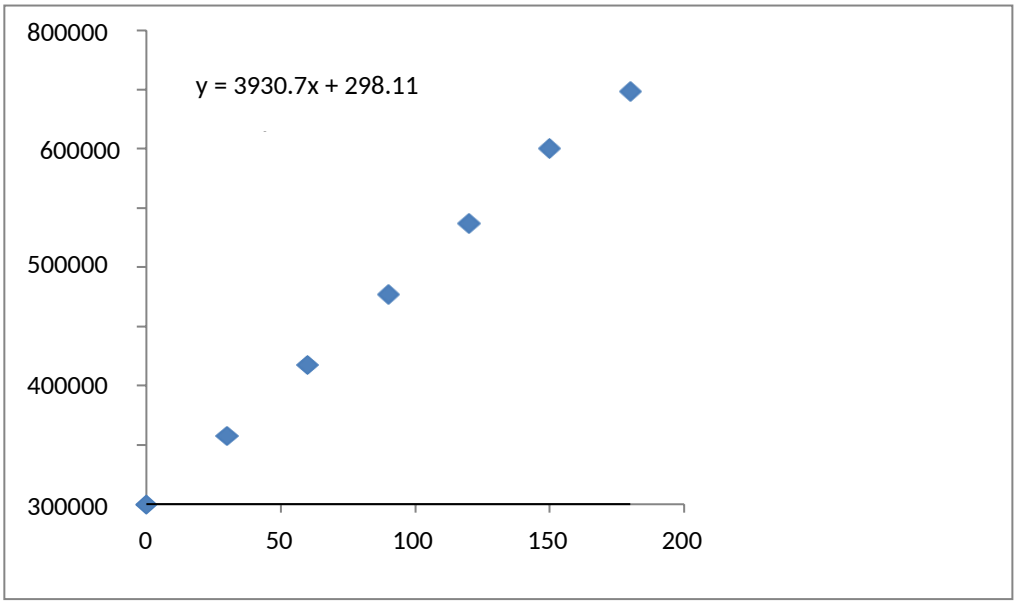

Figure 6: Linearity graph for Tinidazole

Table 4: Linearity results of Diloxanide Furoate

S.NO	**Concentration (µg/ml)**	**Peak Area**
1	**25**	**149734**
2	**50**	**305874**
3	**75**	**452938**
4	**100**	**598634**
5	**125**	**770138**
6	**150**	**899711**
Correlation coefficient(r^2)		**0.999**
Slope (m)		**6046**
Intercept		**357.6**

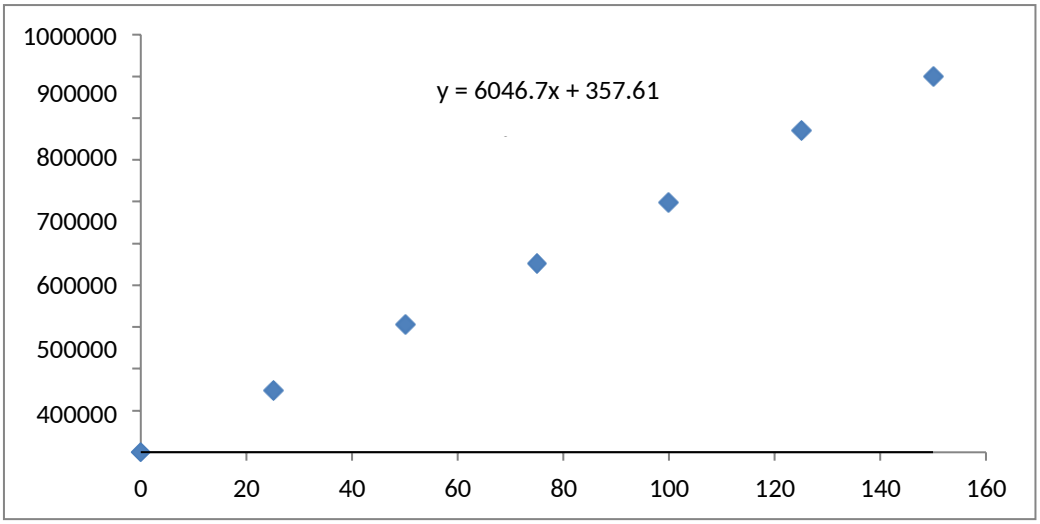

Figure 6: Linearity graph for Diloxanide Furoate

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Tinidazole and Diloxanide Furoate and calculate the individual recovery and mean recovery values. The results are shown in table 5.

Table 5: Showing accuracy results for Tinidazole and Diloxanide Furoate

Sample	Accuracy	Peak Area	% Recovery	Mean % Recovery	Overall Mean %Recovery
Tinidazole	50%	225551	99.75	MEAN=99.85	MEAN= 99.89
	50%	226374	100.11	S.D = 0.22	S.D = 0.165
	50%	225424	99.69	%RSD = 0.22	%RSD = 0.165
	100%	454713	100.55	MEAN=100.08	
	100%	451882	99.92	S.D = 0.40	
	100%	451282	99.79	%RSD = 0.39	
	150%	674477	99.43	MEAN=99.76	
	150%	677286	99.84	S.D = 0.30	
	150%	678582	100.03	%RSD = 0.30	
	50%	300874	100.66	MEAN=100.21	

Diloxanide Furoate	50%	297250	99.45	S.D = 0.66	MEAN= 100.11
	50%	300525	100.54	%RSD = 0.65	S.D = 0.095
	100%	593292	99.24	MEAN = 100.02	%RSD = 0.09
	100%	600461	100.44	S.D = 0.67	
	100%	600065	100.38	%RSD = 0.66	
	150%	899800	100.34	MEAN=100.12	
	150%	899510	100.31	S.D = 0.34	
	150%	894341	99.73	%RSD = 0.33	

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 6.

Table 6: Precision results for Tinidazole and Diloxanide Furoate

	Tinidazole		**Diloxanide Furoate**	
S No	**RT**	**Area**	**RT**	**Area**
1	2.438	453961	3.640	593318
2	2.439	450640	3.640	600062
3	2.440	455090	3.642	594119
4	2.440	450337	3.642	599686
5	2.441	444217	3.642	592891
6	2.441	448218	3.644	592273
Avg		450441		595392
Std Dev		3942.8		3526
RSD		0.9		0.6

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 7.

Table 7: Ruggedness results of Tinidazole and Diloxanide Furoate

S No	Tinidazole		Diloxanide Furoate	
	RT	Area	RT	Area
1	2.438	448247	3.640	592102
2	2.439	442242	3.640	593877
3	2.440	449008	3.642	589066
4	2.440	444679	3.642	592811
5	2.441	444217	3.642	586570
Avg		445116		590302
Std Dev		2902.1		3042.6
% RSD		0.7		0.5

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The resulte are shown in table 8.

Table 8: Robustness of Tinidazole and Diloxanide Furoate

		Tinidazole			Diloxanide Furoate		
S No	Parameter	RT	Area	Tailing factor	RT	Area	Tailing factor
1	Standard						
2	Robustness-Flow-1	2.444	445197	1.25	3.666	586483	1.16
3	Robustness-Flow-2	2.207	434640	1.28	3.309	533051	1.15
4	Robustness- change in mobile phase_1	2.422	466627	1.33	3.541	581740	1.19
5	Robustness-change in mobile phase_2	2.445	449894	1.28	3.666	592388	1.18
6	Robustness-change in temp _1	2.424	466627	1.33	3.541	581740	1.19
7	Robustness-change in temp _ 2	2.208	434640	1.28	3.314	533051	1.16

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 9.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 9: LOD, LOQ of Tinidazole and Diloxanide Furoate

Drug	LOD	LOQ
Tinidazole	0.25	0.76
Diloxanide Furoate	0.20	0.59

DEGRADATION STUDIES:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Tinidazole and Diloxanide Furoate using the proposed method. The results are shown in table 10,11.

Acid Degradation Studies:

To 1 . 0 ml of working solution of Tinidazole and Diloxanide, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution was diluted to obtain 120µg/ml&100µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1.0 ml of working solution of Tinidazole and Diloxanide, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 120µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidation:

To 1.0 ml of working solution of Tinidazole and Diloxanide, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 120µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 1050c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 120µg/ml&100µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 120µg/ml&100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

The photochemical stability of the drug was also studied by exposing the solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 120µg/ml&100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Table 10: Degradation results for Tinidazole

S.No	Name	Peak Area	Degradation Assay	% Net Degradation
1	Acid Hydrolysis	415006	92.90	6.74
2	Base Hydrolysis	418710	93.72	5.92
3	Heat Exposure	425486	95.24	4.40
4	Oxidation (peroxide)	421280	94.30	5.34
5	UV Exposure	438180	98.08	1.56
6	Neutral	443619	99.30	0.34

Table 11: Degradation results for Diloxanide Furoate

S.No	Name	Peak Area	Degradation Assay	% Net Degradation
1	Acid Hydrolysis	5467557	92.12	8.33
2	Base Hydrolysis	556333	93.73	6.72

3	Heat Exposure	567746	95.66	4.79
4	Oxidation (peroxide)	561104	94.54	5.91
5	UV Exposure	585874	98.71	1.74
6	Neutral	588589	99.17	1.28

CONCLUSION:

The validated HPLC method developed for the quantitative quality control determination of Tinidazole and Diloxanide Furoate in combination was evaluated for system suitability, specificity, sensitivity, linearity, range, accuracy (recovery), precision (repeatability and intermediate precision), and robustness. All the validation results were within the allowed specifications of ICH guidelines. The developed method has proven to be rapid, accurate, and stability-indicating for the simultaneous determination of combined Tinidazole and Diloxanide Furoate in tablet dosage form in the presence of excipients and the degradation products. There was always a complete separation of both ingredients from their degradation products and from the placebo. As a result, the proposed HPLC method could be adopted for the quantitative quality control and routine analysis of the tablet dosage form.

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