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) 5.0 µm, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.408 mins and 3.016 mins. 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 n Tinidazole and Diloxanide furoate was found in concentration range of 50µg-250µg and 5µg-50µg 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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1. Introduction

Tinidazole is a drug used against protozoan infections. Tinidazole may be a therapeutic alternative in the setting of metronidazole intolerance. Tinidazole may also be used to treat a variety of other bacterial infections.
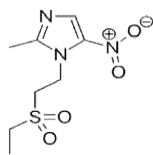

Fig 1: Chemical structure of Tinidazole

Diloxanide is a medication used to treat amoeba infections. In places where infections are not common, it is a second line treatment after paromomycin when a person has no symptoms. For people who are symptomatic, it is used after treatment with metronidazole or tinidazole.
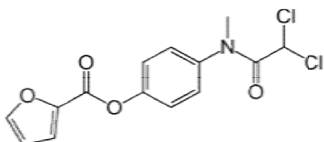

Fig 2: Chemical structure of Diloxanide Furoate

2. Materials and Methods

Instrumentation:

HPLC Auto Sampler : Shimadzu Model number SPD20A, Software LC Solutions, Detector: Photo diode array detector, Thermosil C18 Column (4.0×1.25mm, 5μ), Sonicator: Model number SE60US Enertech , U.V double beam spectrophotometer: PG Instrument Model number T60 Software UV Win5, pH meter: ADWAModel number AD102U, Digital Weighing machine:a Model number ER200A .

Chemicals:

Tinidazole and Diloxanide furoate, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Optimized Chromatographic conditions:

Column	:	Thermosil RP C18
(4.5×100 mm) 5.0 μm		
Mobile phase ratio	:	Sodium acetate buffer:
Methanol (30 : 70% v/v)		
Detection wavelength	:	240nm

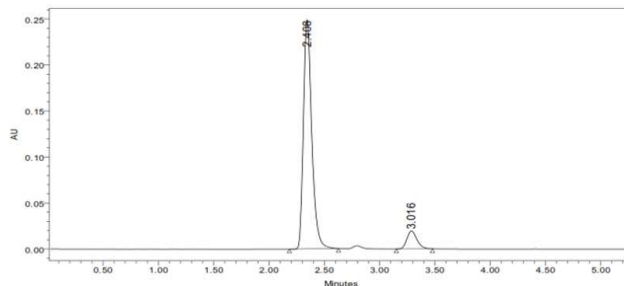|		
Flow rate	:	1.0ml/min
Injection volume	:	20μl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	5min
Retention time	:	2.408 & 3.016 mins

Fig 3: Optimized Chromatogram

Sample solution preparation:

10 mg of Tinidazole and 1 mg Diloxanide furoate tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

10 mg Tinidazole and 1 mg Diloxanide furoate working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

- Linearity
- Accuracy
- Precision
- Intermediate Precision
- Limit of Detection
- Limit of Quantification
- Robustness
- System suitability testing

3. Results and Discussion

Table1: Linearity Results for Tinidazole

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	221543
2	II	100 ppm	426277
3	III	150 ppm	624999
4	IV	200 ppm	826124
5	V	250 ppm	1022139
Correlation Coefficient			0.999

Table 2: Linearity Results for Diloxanide furoate

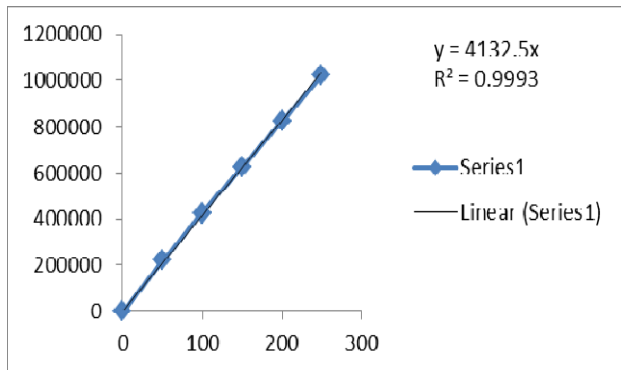| S.No	Linearity Level	Concentration	Area
1	I	5ppm	26472
2	II	10 ppm	50841
3	III	15ppm	78655
4	IV	20ppm	105541
5	V	25ppm	130567
Correlation Coefficient			0.999

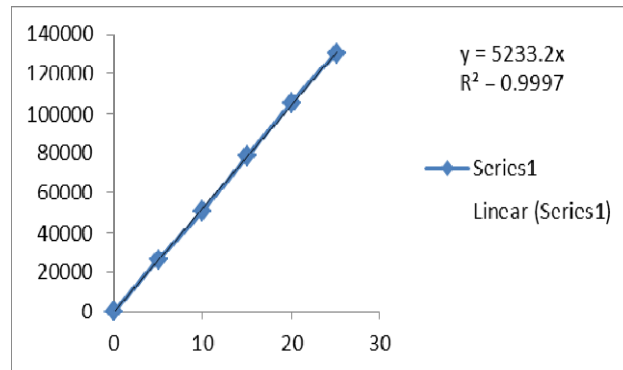**Fig 3:** Calibration graph for Tinidazole

Fig 4: Calibration graph for Diloxanide furoate

Table 3: Showing accuracy results for Tinidazole

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	5265719	5	4.96	99.91%	
100%	6574692	10	9.98	99.18%	100.56%
150%	7828369	15	15.02	99.60%	

Table 4: Showing accuracy results for Diloxanidefuroate

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	499254	0.5	0.99	99.53%	
100%	615492	1.0	1.05	99.38%	101.47%
150%	746684	1.5	1.495	99.52%	

Table 5: Showing% RSD results for Tinidazole

	Name	RT	Area	Height
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			599377	
Std.Dev.			1875.2	
%RSD			0.31	

Table 6: Showing %RSD results for Diloxanide furoate

	Name	RT	Area	Height
1	Diloxanide furoate	3.019	6423669	779071
2	Diloxanide furoate	3.018	6418299	791461
3	Diloxanide furoate	3.017	6435957	781924
4	Diloxanide furoate	3.016	6426016	810297
5	Diloxanide furoate	3.015	6425928	799359
Mean			6425974	

Std.Dev.			6400.9	
%RSD			0.10	

Table 7: Showing results for intermediate precision of Tinidazole

	Name	RT	Area	Height
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 8: Showing results for intermediate precision of Diloxanide furoate

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

Table 9: Showing results for Limit of Detection

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

Table 10: Showing results for Limit of Quantitation

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

Table 11: Showing system suitability results for Tinidazole

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 12: Showing system suitability results for Diloxanide furoate

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 13: Showing system suitability results for Tinidazole

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 14: Showing system suitability results for Diloxanide furoate

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

A simple, accurate, precise, linear and rapid RP-HPLC method was developed for simultaneous quantitative estimation of Diloxanide furoate and Tinidazole in pharmaceutical dosage form and validated as per ICH guidelines.

5. References

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