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A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TINIDAZOLE AND DILOXANIDE FUROATE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of tinidazole and diloxanide furoate. The separation was carried out using a mobile phase consisting of acetonitrile and 0.2 M potassium dihydrogen phosphate (pH 5) in the ratio 45:55% v/v. The column used was Hypersil OSD C18 (250 x 4.6 mm, 5 µ particle size) with a fl ow rate of 1 ml/min and UV detection at 278 nm. The described g/ml for the assay of diloxanide furoate andµg/ml and 25-150 µmethod was linear over a concentration range of 30-180 tinidazole, respectively. The mean recovery was found to be 100-101% for tinidazole and 97-103% for diloxanide furoate when determined at three different levels.

KEYWORDS: Tinidazole, Diloxanide furoate, HPLC, UV detection.

INTRODUCTION

Tinidazole and diloxanide furoate as components of a multi-ingredient formulation is very useful in therapy of diarrhoea due to amoebiasis and associated with mixed infections by bacteria. As found from the literature, diloxanide furoate has been reported to be estimated in combination with other drugs by HPLC1-3 methods. Tinidazole has been reported to be estimated by HPLC4-6 methods. Tinidazole and diloxanide furoate have been simultaneously determined by spectrometric methods7-10. The aim of the present work is to describe a liquid chromatographic procedure for the separation and simultaneous quantification of tinidazole and diloxanide furoate in its formulation. For the proposed method all the chemicals of analytical reagent grade, solvents of HPLC grade and distilled water (Millipore) were used. The LC system m column, Rheodyneµconsisted of LC-10AT pump (Shimadzu), Hypersil OSD C18 (250 x 4.6 mm, 5 µ particle size) sample loop and UVµinjector equipped with a 100 detector (Shimadzu SPD-10A VP) set at 278 nm. The output signal was monitored and integrated using CZ-RA software (Shimadzu). The standard solution of diloxanide furoate andµg/ml and 25-150 µmethod was linear over a concentration range of 30-180 tinidazole were prepared separately by dilution of tinidazole and diloxanide furoaterespectively in mobile phase of acetonitrile and 0.2M potassium dihydrogen phosphate pH 5.0 in the ratio 45:55% v/v. Analysis of marketed sample Amicline Plus (Laboratories Griffon Pvt. Ltd.) of three different batches was carried out. Twenty tablets, each containing 375 mg diloxanide furoate and 300 mg tinidazole were weighed. The tablets were crushed together in a mortar to a fine powder and an amount equivalent to 375 mg diloxanide furoate and 300 mg of tinidazole was transferred into a 100 ml dried volumetric flask. A few drops of acetonitrile were added to dissolve the active solids and then volume made up with the mobile phase. The solution was degassed through membrane fi lter. The standard solution and sample solutions were injected separately into the stabilized liquid chromatographic system. The retention time for tinidazole and diloxanide furoate at a flow rate of 1ml/min were recorded as 2.4 and 3.6 min respectively (fig 1). From the respective peak areas obtained in standard and sample chromatogram. The amount of contents was calculated. The results of.

Chromatographic conditions:

Mobile phase : Phosphate Buffer (KH_2PO_4): Acetoitrile (45:55V/V) Flow rate: 1.0 ml/min
Column : Hypersil OSD C18 (250 x 4.6 mm, 5 µ particle size).Detector wavelength: 278 nm
Injection volume : 10 µl

Run time : 10 min
Temperature : 30^0C

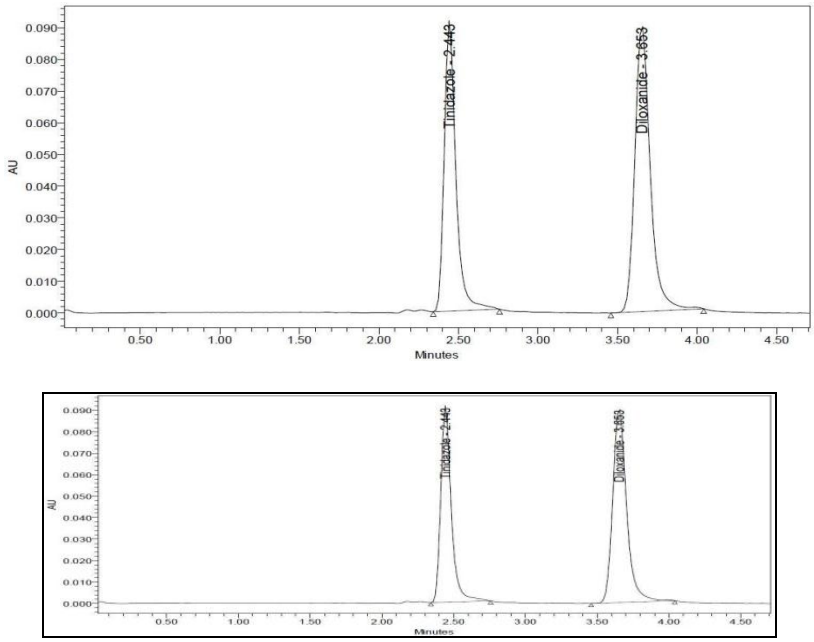

Fig 1: Chromatogram for optimized method.

The linearity of the method was established by analysis of standard solution. The calibration curve was drawn by plotting the peak area versus concentration. The linearity range was found to 25-150 µg/ml for diloxanide furoate and 30-180 µg/ml for tinidazole. The specificity of the method was established by injecting placebo. No interference of the placebo was observed with the principal peaks. Ruggedness of the method was determined by carrying out the experiment on different instruments, by different chemists and on different days. The results showed that the method was rugged as percentage recovery was found to be in the range of 95.1-100.1%. Robustness of the method was determined by making slight changes in the chromatographic conditions. Buffer pH modification by ±5% did not have any significant effect. The effect of organic strength on retention time was studied by small change in percentage polarity of the mobile phase system and it was found that even slight percentage change, up to 10% in ratio of mobile phase did not alter the position.

The system suitability tests were carried out as per USP XXIV requirements. System suitability tests were carried out on freshly prepared standard stock solution of tinidazole and diloxanide furoate and the l0µl injection volume parameters obtained with 100. The number of theoretical plates for tinidazole and diloxanide furoate was calculated as 71790 and 74444, respectively. The symmetry factor for tinidazole and diloxanide furoate peaks was 1.05 and 0.91, respectively. The resolution between the two peaks was 1.92. The obtained results confi rmed that the method is highly suitable for its intended purpose of separation of tinidazole and diloxanide furoate and its simultaneous determination in formulation.

System suitability

System suitability test should be carried out to verify that the analytical system is working properly and can give accurate and precise results. Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters were evaluated from tailing factor, retention times and theoretical plates of standard chromatograms.

Table 1: Linearity studies 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

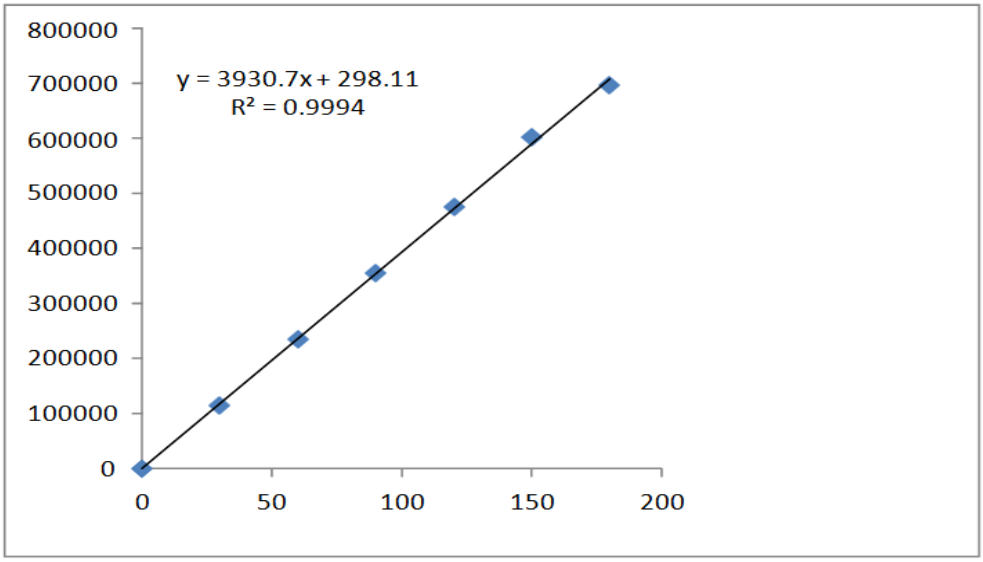

Fig. No 2: Linearity Graph for Tinidazole.

Table.no 2: Linearity of Diloxnide 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

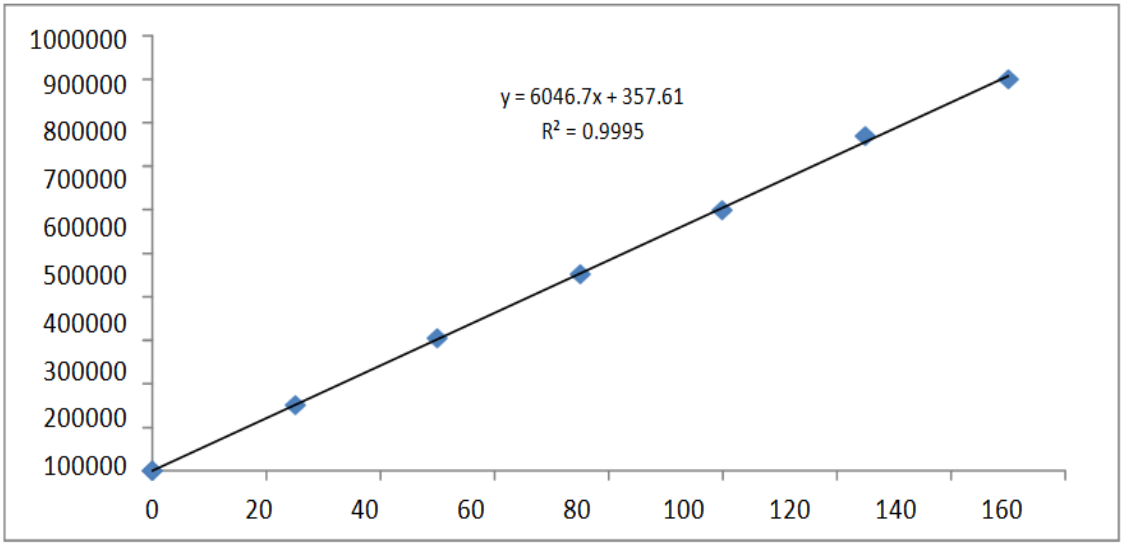

Fig. No.3: Linearity studies for Diloxanide Furoate.

Data interpretation: The correlation coefficient (or) regression coefficient should not be less than 0.999. Calculate the correlation coefficient (r^2), y-intercept and slope. From the statistical treatment of linearity data of Tinidazole and Diloxanide Furoate it was clear that the response is linear between lower levels to higher levels. The correlation coefficient was found to be 0.9.

Table No 4: Acuuracy for Tinidazole and Diloxanide Furoate.

Sample	Accuracy	Peak Area	% Recovery	Mean %Recovery	Overall Mean %Recovery
	50%	225551	99.75	MEAN=99.85	
Tinidazole				S.D = 0.22	MEAN= 99.89
	50%	226374	100.11		
				%RSD = 0.22	S.D = 0.165
	50%	225424	99.69		
					%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				S.D = 0.66	MEAN= 100.11
	50%	297250	99.45		
Furoate				%RSD = 0.65	S.D = 0.095
	50%	300525	100.54		
					%RSD = 0.09
	100%	593292	99.24	MEAN = 100.02	
	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	

Data interpretation

The Mean % Recovery at 50%,100% and 150% at higher levels for Tinidazole and Diloxanide Furoate were found to be between 99.89 % and 100.11% which were within the acceptance criteria limit.The Mean % Recovery for Tinidazole at 50%,100% and 150% levels was found to be 99.85%, 100.08% and 99.76% respectively and are within the limits.The Mean % Recovery for Diloxanide furoate at 50%,100% and 150% levels was found to be 100.21%, 100.02% and 100.12% respectively and are within the limits. The excellent mean recoveries and standard deviation suggested that the good accuracy of the proposed method.

Table No.5: System Precision of 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
	2.441	448218	3.644	592273
Avg		450441		595392
Std Dev		3942.8		3526
RSD		0.9		0.6

Limit of detection and Limit of Quantification

Tinidazole

LOD = 3.3 F/S

= 3.3 ×298.1/3930 = 0.25

LOQ = 10 F/S

=10 × 298.1/3930 = 0.76

Diloxanide Furoate:LOD = 3.3 F/S

= 3.3 ×357.6/6046 = 0.20

LOQ = 10 F/S

Robustness study.

Table. 6: 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- changein mobile phase_1	2.422	466627	1.33	3.541	581740	1.19
5	Robustness-changein mobile phase_2	2.445	449894	1.28	3.666	592388	1.18
6	Robustness-changein temp _1	2.424	466627	1.33	3.541	581740	1.19
7	Robustness-changein temp _ 2	2.208	434640	1.28	3.314	533051	1.16

Table 8: Assay of standard & sample chromatogram.

		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

DEGRADATION STUDIES

Acid Degradation Studies: To 1.0ml of working solution of Tinidazole and Diloxanide, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 120µg/ml&100µg/ml solution and 10µl 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 60^0c. The resultant solution was diluted to obtain 120µg/ml&100µg/ml solution and 10µl 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 60^0c. 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 sampleDry Heat Degradation Studies: The standard drug solution was placed in oven at 105^0c 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/m^2 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/m^2 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.

SUMMARY AND CONCLUSION

Mobile phase	**Potassium dihydrogen phosphate and Acetonitrile(45:55 V/V)**
Stationary phase	Kromasil C18 (150 x 4.6 mm, 5µ)
Wave length	278 nm
Run time	20 min
P.H of mobile phase	3.3
Flow rate	1 ml/min
Injection volume	10µl
Temperature	30^0 c

Table 9: Chromatographic Conditions.

System suitability parameters	RESULTS	
	TINIDAZOLE	DILOXANIDE FUROATE
Retention time	2.438	3.653
Area	464001	605076
Theoretical plates	5630	73622
Tailing factor	1.32	1.24

Table No 8.3 Validation Parameters of Assay.

S.No	Parameter	RESULT					
		Tinidazole			Diloxanide Furoate		
1	Linearity	30-180 µg/ml Correlation coefficient =0.999			25-150 µg/ml Correlation coefficient = 0.999		
2	System precision %RSD	0.9			0.6		
3	Method precision %RSD	0.6			0.6		
4	Intermediate precision %RSD	0.6			0.6		
5	Accuracy	Mean recovery =			Mean recovery =		
	50%	99.85%			100.21%		
	100%	100.08%			100.02%		
	150%	9.76%			100.12%		
6	Robustness	RT	Area	Tailingfactor	RT	Area	Tailingfactor
	Flow-1	2.444	445197	1.25	3.666	586483	1.16
	Flow-2	2.207	434640	1.28	3.309	533051	1.15
	change in mobile phase_1	2.422	466627	1.33	3.541	581740	1.19
	change in mobile phase_2	2.445	449894	1.28	3.666	592388	1.18
	change in temp _1	2.422	466627	1.33	3.541	581740	1.19
	change in temp _ 2	2.208	434640	1.28	3.314	533051	1.16

Table No 8.4 Degradation study results.

S. No	CompoundName	Acid hydrolysis	Alkali hydrolysis	Oxidation	Heat	UV	Neutral hydrolysis
1	Tinidazole	6.74	5.92	5.34	4.40	1.56	0.34
2	DiloxanideFuroate	8.33	6.72	5.91	4.79	1.74	1.28

CONCLUSION

A simple, sensitive, rapid and economical stability indicating RP-HPLC method was developed and validated for the assay of Tinidazole and Diloxanide Furoate in combined tablet formulation. This method yielded high recoveries with good linearity and precision. It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Tinidazole and Diloxanide Furoate combined tablet formulation. From these studies we reported the % of degradation produtcts in various conditions like Acid hydrolysis, Alkali hydrolysis, Oxidation, Heat, UV and Neutral hydrolysis.

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REFERENCES

1. Feng MX, Gao H, Yu FY. Determination of tinidazole and its degradation products in tinidazole injections by reverse-phase HPLC. Fenxi Zazhi, 1997; 17: 247–9.
2. Li SM, Hu FQ, Yu Y. Determination of tinidazole in saliva by HPLC after administration. Fenxi Zazhi, 1997; 17: 307–9.
3. Talwar N, Karajgi JS, Jain NK. Estimation of tinidazole in tablets and plasma by HPLC. Indian Drugs, 1991; 29: 55–6.
4. Prasad CVN, Sripriya S, Shah RN. Simultaneous determination of tinidazole, furazolidone and diloxanide in a combined tablet preparation by second derivative spectrometry. J Pharm Biomed Anal, 1999; 21: 961–8.
5. Tapan D, Dilip H. Simultaneous spectrometric analysis of binary mixtures of metronidazole-diloxanide furoate and tinidazole- diloxanide furoate in pharmaceutical dosage forms. Indian Drugs, 1991; 29: 165–70.
6. Klaus Florey, "Analytical profiles of drug

substances and excipients", ed. Brittain H.G., Academic Press, An Imprint of Elsevier, New Jersey, 27: 67 –112.
7. Martindale, "The Extra Pharmacopoeia", ed. Reynolds, J.E.F., 31st edition, Royal Pharmaceutical Soc, London, 1996; 404-406.
8. Pothana Sadasivudu, Nalini Shastri, Sadanandam A RP-HPLC and an UV spectrophotometric assay method were developed and validated for quantitative determination of fluconazole in pharmaceutical solid dosage form International. Journal of Chem Tech Research, Oct-Dec 2009; 1(4): 1131- 113.
9. Maheshwari RK, Rajput MS, Sinha S A novel, safe and sensitive method of spectrophotometric estimation in the ultraviolet region has been developed using 1M lignocaine hydrochloride(an economic drug) as a hydrotropic solubilizing agent for the quantitative determination of tinidazole. Asian journal of pharmaceutics, 3(4): 319-321.
10. Meshram DB, Bagade SB, Tajne A rapid and sensitive reversed-phase high- performance liquid chromatographic method is developed for simultaneous estimation of fluconazole, an orally active triazole anti-fungal agent, and tinidazole. Journal of chromatographic science2009 nov-dec; vol4: 44-45.
11. Dhananjay B, MeshramShashikant B, Bagade Madhukar, Tajne Use of an internal standard is proposed in this thin-layer chromatographic determination of fluconazole in tablet and capsule formulations. Journal of and nuclear chemistry aug. 1989; 137: 1.
12. Khaja phash, Asgar ali , Shanabhana, Syeda humayir A sample rapid and reproducible high performance reverse phase liquid chromatographic method has been developed for the estimation of tinidazole in bulk drug sample and pharmaceutical dosage form. International journal of pharmacy and pharmaceutical sciences, 2010; 2: 140-150.
13. Ranjit Singh, Mukesh Maithani1, Shailendra K, Sarafb, Shubhini Saraf and Ram .Gupta A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay has been developed for simultaneous estimation of Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole and Ornidazole in tablet formulations. Eurasian J. Anal. Chemistry 2009; 4(2): 161-167.
14. Adel M and S Diana. Stability indicating reversed-phase liquid chromatographic determination of metronidazole benzoate and diloxanide furoate as bulk drug and in suspension dosage form. *J. Pharm. Biomed. Ana.*, 2005; 39: 819-823.