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Simultaneous RP-HPLC determination of tinidazole and diloxanide furoate in pharmaceutical preparations

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ABSTRACT

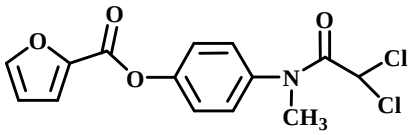
A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of diloxanide furoate and tinidazole using metronidazole as an internal standard. Chromatographic separation of the two drugs was performed on a inertsil C_{18} column (250mm×4.6 mm, 5μm) as stationary phase with a mobile phase comprising of 0.1% ortho phosphoric acid: acetonitrile (40:60 v/v), at a flow rate of 0.8mL min^{-1} and UV detection at 215nm. The proposed method was validated for linearity, accuracy, precision,LOD, LOQ. Linearity, accuracy and precision were found to be acceptable over the ranges of 150-600μg mL^{-1} for tinidazole, 125-500μg mL^{-1} for diloxanide furoate. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

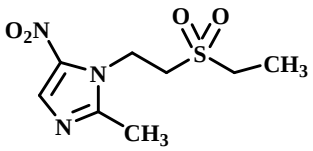
ICH Guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Diloxanide furoate;
Tinidazole;
Metronidazole.

INTRODUCTION

Tinidazole (1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole) is a drug having antimicrobial action and helps to fight infections in body. It is also used for treating certain intestinal infections, liver infections and sexually transmitted diseases[1]. Diloxanide furoate (2-2-dichloro-4'-hydroxy-N-methyl acetanilide 2-furoate) is an anti-protozoal drug used in the treatment of entamoeba histolytica and some other protozoal infections[2]. The structures of these two drugs are shown in figure 1. One such combination contains 300mg of tinidazole and 250mg of diloxanide furoate. It is widely used as anti-protozoal and anti-amoebic. The literature revealed no method was available for simultaneous determination of these two drugs in such pharmaceutical preparations by HPLC. Therefore an HPLC method was developed



Diloxanide furoate ($C_{14}H_{11}Cl_2NO_4$)



Tinidazole ($C_8H_{13}N_3O_4S$)

Figure 1: Structures of diloxanide furoate and tinidazole

for determination of diloxanide furoate and tinidazole from their combined dosage form[5-12]. The method described is simple, fast, precise and accurate for simultaneous determination of diloxanide furoate and tinidazole from pharmaceutical preparation.

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Chemicals and reagents

Standards were supplied from J.B. Chemicals and Pharmaceutical Ltd., Mumbai, India. Wotinex tablets manufactured by Wockhardt, India was procured from the market. Acetonitrile and orthophosphoric acid were from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

EXPERIMENTAL

Method development and optimization of chromatographic conditions

To develop a suitable LC method for the analysis of tinidazole and diloxanide furoate in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involve, time required for the analysis, better separation of drugs. Chromatographic separation was preformed with Agilent 1100 series high performance liquid chromatography having HPLC isocratic pump, equipped with auto sampler and a photo-diode array detector. The UV spectrum of all the three drugs were scanned on photo diode array detector for selecting the working wavelength. Peak purity of all the three drugs were checked using photo diode array detector. Chromatograms and data were recorded by means of chemstation software. An Inertsil C_{18} column (250mm×4.6 mm, 5µm particle) was used for the analysis. The mobile phase comprising of 0.1% ortho phosphoric acid: acetonitrile in the ratio (40:60) v/v. The system was run at a flow rate of 0.8mL min^{-1}, 10µL of sample was injected in the chromatographic system and detection wavelength was set at 215nm for simultaneous determination of the two drugs. A typical HPLC chromatogram for simultaneous determination of diloxanide furoate and tinidazole from pharmaceutical formulation is shown in figures 2 and 3.

Preparation of standard stock solutions

The stock solution of tinidazole (3000µg mL^{-1}) was prepared by dissolving 149.91 mg of tinidazole (99.8 %) in mobile phase in a standard 50mL volumetric flask (solution A). The stock solution of diloxanide furoate (2500µg mL^{-1}) was prepared by dissolving 125.21 mg

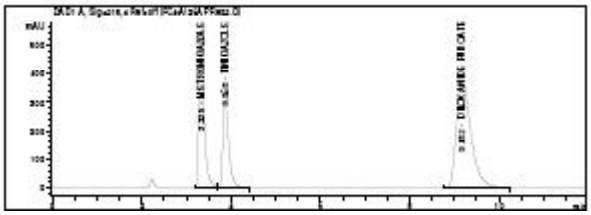

Figure 2 : Chromatogram of tinidazole and diloxanide furoate with metronidazole(internal standard) in standard preparation

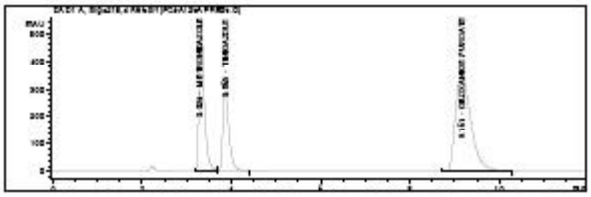

Figure 3: Chromatogram of tinidazole and diloxanide furoate with metronidazole(internal standard) in sample preparation

of diloxanide furoate (99.9 %) in mobile phase in a standard 50mL volumetric flask (solution B). Internal standard (Metronidazole) stock solution (3000µg mL^{-1}) was prepared by dissolving 149.80 mg of metronidazole in mobile phase in a 50mL standard volumetric flask.

Working standard solution

Transferred 10.0 mL of each stock solutions A, B and C to a 100 mL volumetric flask and diluted up to the mark with mobile phase.

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed into a homogeneous powder and a quantity equivalent to one tablet was transferred in a 100mL volumetric flask, dissolved in mobile phase, and filtered through Whatman no. 41 filter paper. The filtrate (10mL) was quantitatively transferred to a 100mL volumetric flask, 10mL of internal standard solution was added to it, and solution was diluted up to the mark with mobile phase.

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out. System suitability tests were

performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 10µL standard solutions of diloxanide furoate and tinidazole of strengths 250µg mL^{-1} and 300µg mL^{-1} respectively using metronidazole as an internal standard. This was repeated five times. The RSD values of diloxanide furoate and tinidazole were 0.38 and 0.28 respectively. The RSD values were found to be satisfactory and meeting the requirements of USP 31 (RSD less than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

Linearity

Linearity was evaluated by analysis of working standard solutions of diloxanide furoate and tinidazole of seven different concentrations[2,3]. The range of linearity was from 125-500µg mL^{-1} for diloxanide furoate and 150-600µg mL^{-1} for tinidazole. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the two pharmaceuticals are represented in TABLE 2. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration of each drug.

TABLE 1: Result of system suitability

Parameters	Metronidazole (IS)	Tinidazole	Diloxanide furoate
Resolution	-	2.57	12.95
Tailing factor	1.61	1.61	1.51
Theoretical plates	4634	4619	3829

TABLE 2 : Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (r^2) (n=7)
Diloxanide furoate	0.0076	0.147	0.9993
Tinidazole	0.0014	-0.010	0.9999

TABLE 3 : Results of assay experiment

	Diloxanide furoate	Tinidazole
Drug found in mg/ TABLEt (mean)	249.91	299.18
Mean %	99.96	99.73
RSD	0.34	0.23

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively[2,3]. The LOD and LOQ of diloxanide furoate and tinidazole were experimentally determined by six injections of each drug. The LOD of diloxanide furoate and tinidazole were found to be 0.2µg mL^{-1} and 0.4µg mL^{-1} respectively. The LOQ of diloxanide furoate and tinidazole were found to be 0.6µg mL^{-1} and 1.0µg mL^{-1} respectively.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[3]. The relative standard deviations were less than 2% for the two drugs. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of diloxanide furoate and tinidazole in the product. The RSD were 0.54 and 0.56 respectively. Refer TABLE 3.

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of each drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of each drug was added, to determine if there are positive or negative interferences from excipients present in the formulation[4]. Each set of addition was repeated three times .The accuracy was expressed as the percentage of analytes recovered by the assay.

TABLE 4: Accuracy of the method

Analyte	Initial conc. (mg)	Conc. added(mg)	Total conc.(mg)	Conc. ound(mg)	RSD (%) n= 3	Recovery(%)	%Bias
Diloxanide furoate	250	0	250	249.54	0.26	99.82	+0.18
	250	25	275	273.16	0.14	99.33	+0.67
	250	50	300	297.24	0.09	99.08	+0.92
	250	75	325	320.12	0.18	98.50	+0.50
Tinidazole	300	0	300	299.20	0.16	99.73	+0.27
	300	30	330	328.24	0.24	99.47	+0.53
	300	60	360	362.09	0.09	100.58	-0.58
	300	90	390	391.98	0.21	100.51	-0.51

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TABLE 4 lists the recoveries of the drugs from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of the three drugs.

DISCUSSION AND CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried but good peak shape and good resolution between diloxanide furoate, metronidazole and tinidazole was observed using the mobile phase mentioned in chromatographic conditions. The method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of %RSD for method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate, easily reproducible and can be used for simultaneous determination of tinidazole and diloxanide furoate from pharmaceutical preparations.

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