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Simultaneous RP HPLC determination of clotrimazole and tinidazole in pharmaceutical preparations

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ABSTRACT

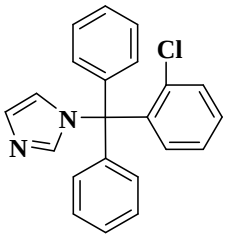
A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of clotrimazole and tinidazole using metronidazole as an internal standard. Chromatographic separation of the two drugs was performed on a inertsil C_{18} column (250mm×4.6 mm, 5µm) as stationary phase with a mobile phase comprising of 0.1% ortho phosphoric acid: acetonitrile (50:50 v/v), at a flow rate of 0.7mL min^{-1} and UV detection at 215nm. The proposed method was validated for linearity, accuracy, precision and limit of quantitation. Linearity, accuracy and precision were found to be acceptable over the ranges of 100-300µg mL^{-1} for clotrimazole, 250-750µg mL^{-1} for tinidazole. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

ICH guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Clotrimazole;
Tinidazole.

INTRODUCTION

Clotrimazole (1-[(2-chlorophenyl)-diphenyl-methyl]) imidazole is an anti fungal medication used for treating fungal infections of both humans and animals such as vaginal yeast infections and ringworm. 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]. The structures of these two drugs are shown in figure 1. One such combination contains 200mg of clotrimazole and 500mg of tinidazole. It is widely used as broad spectrum anti-fungal. 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 for determination of clotrimazole and tinidazole from their combined dosage form[4-10]. The method described is simple, fast, precise and accurate for simultaneous determination of clotrimazole and tinidazole from pharmaceutical preparation.



Clotrimazole ($C_{22}H_{17}ClN_2$)

Figure 1: Structures of clotrimazole and tinidazole

Chemicals and reagents

Standards were supplied from J.B. Chemicals and

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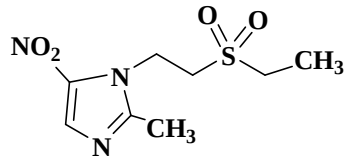
pharmaceutical Ltd., Mumbai, India. Vagi tablets manufactured by Rekvina pharma, India was procured from the market. Acetonitrile and orthophosphoric acid were from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.



Tinidazole ($C_8H_{13}N_3O_4S$)

Figure 2: Structures of tinidazole

EXPERIMENTAL

To develop a suitable LC method for the analysis of Clotrimazole and Tinidazole 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 UV-Visible variable wavelength detector. Chromatograms and data were recorded by means of chemstation software. An Inertsil C_{18} column (250mm×4.6 mm, 5 μm particle) was used for the analysis. The mobile phase comprising of 0.1% ortho phosphoric acid: acetonitrile in the ratio (50:50) v/v. The system was run at a flow rate of 0.7mL 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 clotrimazole and tinidazole from pharmaceutical formulation is shown in figure 3 and figure 4.

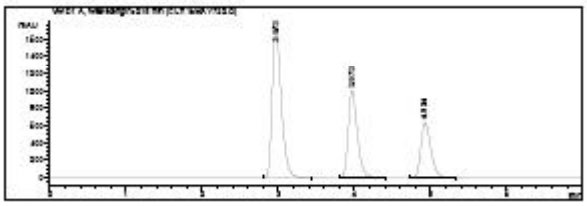


Figure 3: Chromatogram of clotrimazole and tinidazole with metronidazole (as an internal standard) in standard preparation

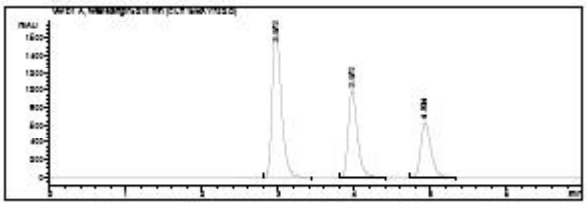


Figure 4: Chromatogram of clotrimazole and tinidazole with mmetronidazole (as an internal standard) in sample preparation

Preparation of stock and working dtandard dolutions

The stock solution of clotrimazole (1000μg mL^{-1}) was prepared by dissolving 49.75 mg of clotrimazole (99.8 %) in mobile phase in a standard 50mL volumetric flask (solution A). The stock solution of tinidazole (1000μg mL^{-1}) was prepared by dissolving 49.80 mg of tinidazole (99.9 %) in mobile phase in a standard 50mL volumetric flask (solution B). Internal standard (Metronidazole) stock solution (1000μg mL^{-1}) was prepared by dissolving 49.80 mg of metronidazole in mobile phase in a 50mL standard volumetric flask.

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 (1000 mg) was transferred in a 100mL volumetric flask, dissolved in mobile phase, and filtered through Whatman no. 41 filter paper. The filtrate (1mL) was quantitatively transferred to a 10mL volumetric flask, 1mL 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 30 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 10μL standard solutions of clotrimazole and tinidazole of strengths 200μg mL^{-1} and 500μg

TABLE 1: Result of system suitability

Parameters	Clotrimazole	Metronidazole (IS)	Tinidazole
Resolution	-	6.5	5.31
Tailing factor	1.34	1.36	1.29
Theoretical plates	3028	5341	5930

TABLE 2 : Results of linearity

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

TABLE 3 : Results of assay experiment

	Clotrimazole	Tinidazole
Drug found in mg/tablet (mean)	198.31	495.66
Mean %	99.15	99.13
RSD	0.54	0.56

TABLE 4: Accuracy of the method

Analyte	Initial conc. (mg)	Conc. added (mg)	Total conc. (mg)	Conc. found (mg)	RSD (%) n= 3	Recovery (%)
Clotrimazole	200	0	200	198.45	0.06	99.23
	200	20	220	219.86	0.04	99.94
	200	40	240	240.11	0.02	100.04
	200	60	260	259.46	0.14	99.79
Tinidazole	500	0	500	449.17	0.01	99.83
	500	50	550	549.48	0.02	99.91
	500	100	600	600.65	0.09	100.11
	500	150	650	650.19	0.03	100.03

mL^{-1} respectively using metronidazole as an internal standard. This was repeated five times. The RSD values of clotrimazole and tinidazole were 0.38 and 0.28 respectively. The RSD values were found to be satisfactory and meeting the requirements of USP 30 (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 clotrimazole and tinidazole of seven different concentrations[2,3]. The range of linearity was from 100-300 μg mL^{-1} for clotrimazole and 250-750μg 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.

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 clotrimazole and tinidazole were experimentally determined by six injections of each drug. The LOD of clotrimazole and tinidazole were found to be 0.2μg mL^{-1} and 0.4μg mL^{-1} respectively. The LOQ of clotrimazole and tinidazole were found to be 0.7μg 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 clotrimazole 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[3]. Each set of addition was repeated three times .The accuracy was expressed as the percentage of analytes recovered by the assay. 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-methanol-acetonitrile in different ratios were tried but good resolution between Clotrimazole, Metronidazole and Tinidazole was observed using the mobile phase mentioned in chromatographic conditions. The method after being completely validated showed satisfactory

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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 clotrimazole and tinidazole from pharmaceutical preparations.

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