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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TERBINAFINE & ITRACONAZOLE BY RP-HPLC METHOD

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Terbinafine and Itraconazole in tablet dosage form. Chromatogram was run through Agilent C18 150 x 4.6mm, 5.0µ. Mobile phase containing Buffer 0.01N KH_2PO_4 (4.8pH) : Acetonitrile taken in the ratio 60:40v/v was pumped through column at a flow rate of 0,8 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 270nm. Retention time of Terbinafine and Itraconazolewere found to be 2.340 min and 2.940min. %RSD of the Terbinafine and Itraconazole were and found to be 1.2 and 1.2 respectively. %Recovery was obtained as 99.56% and 100.16% for Terbinafine and Itraconazole respectively. LOD, LOQ values obtained from regression equations of Terbinafine and Itraconazole were 0.77, 2.34 and 1.33, 3.42 respectively. Regression equation of Terbinafine is y = 1316.x + 560.8 and y = 2912x + 3286 of Itraconazole. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Terbinafine, Itraconazole, RP-HPLC.

INTRODUCTION

Chemically Terbinafine (TRB) is hypothesized to act by inhibiting squalene monooxygenase, thus blocking the biosynthesis of ergosterol, an essential component of fungal cell membranes. This inhibition also results in an accumulation of squalene, which is a substrate catalyzed to 2,3-oxydo squalene by squalene monooxygenase. The resultant high concentration of squalene and decreased amount of ergosterol are both thought to contribute to terbinafine's antifungal activity. Structure of the TRB was shown in figure 1 (A).[1]

Chemically Itraconazole (ITC) interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Itraconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis. Structure of the ITC was shown in figure 1 (B).[2]

Literature survey reveals there are several methods to estimated these drugs in single or in combination of two or three drugs.[5-9] But there is only very few HPLC methods are available for simultaneous estimation of TRB and ITC, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

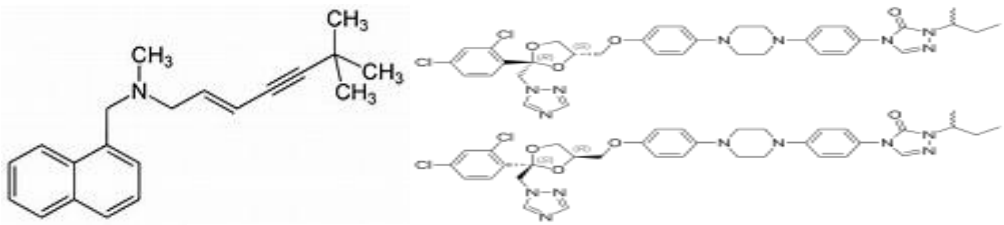

Figure 1: Structure of (A) Terbinafine (B) Itraconazole.

MATERIALS AND METHODS

Reagents and Chemicals: Dapagliflozin Terbinafine and Itraconazole pure drugs (API), Combination Terbinafine and Itraconazole tablets (IGFORCE FT), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Agilent C18 150 x 4.6mm, 5.0µ. As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The Agilent C18 150 x 4.6mm, 5.0µ column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of Potassium dihydrogen phophate and Acetonitrile was taken in the ratio of (60:40%v/v) mobile phase for the investigation with a flow rate of a 0.8 ml/min. The temperature was maintained at 30^0C. The injection volume was 10µl and the UV detection was achieved at 270nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

Preparation of mobile phase
Buffer: Water - in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Preparation of mixture Standard stock solution: Accurately weighed 62.5 mg of Terbinafine, 25 mg of Itraconazole and transferred to 50 ml volumetric flasks separately. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1250µg/ml of Terbinafine and 500µg/ml of Itraconazole)

Preparation of Sample (Tablet) stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 25ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2500µg/ml of Terbinafine and 1000µg/ml of Itraconazole).

Optimized chromatographic conditions
Column Used: Agilent C18 (4.6 x 150mm, 5µm)
Mobile phase: 0.01N KH_2 PO_4: Aceonitrile (60:40 v/v)
Flow rate: 0.8ml/min
Wavelength: 270.0 nm
Temperature: 30°C
Injection Volume: 10.0µl

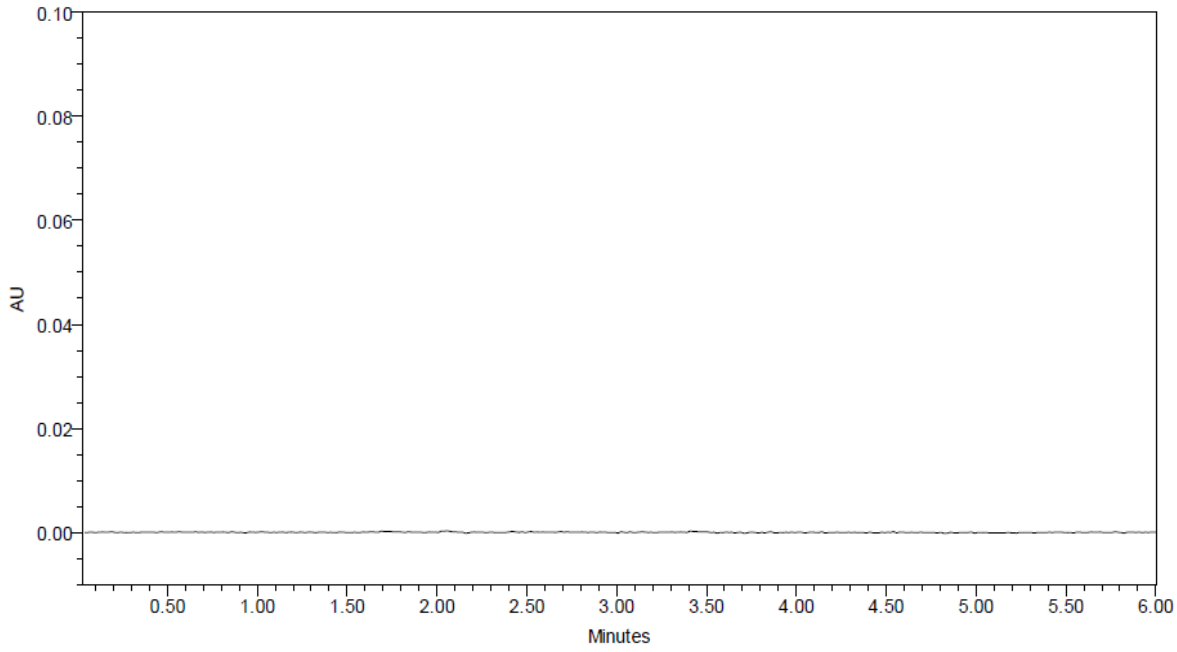

Figure 2: Blank chromatogram.

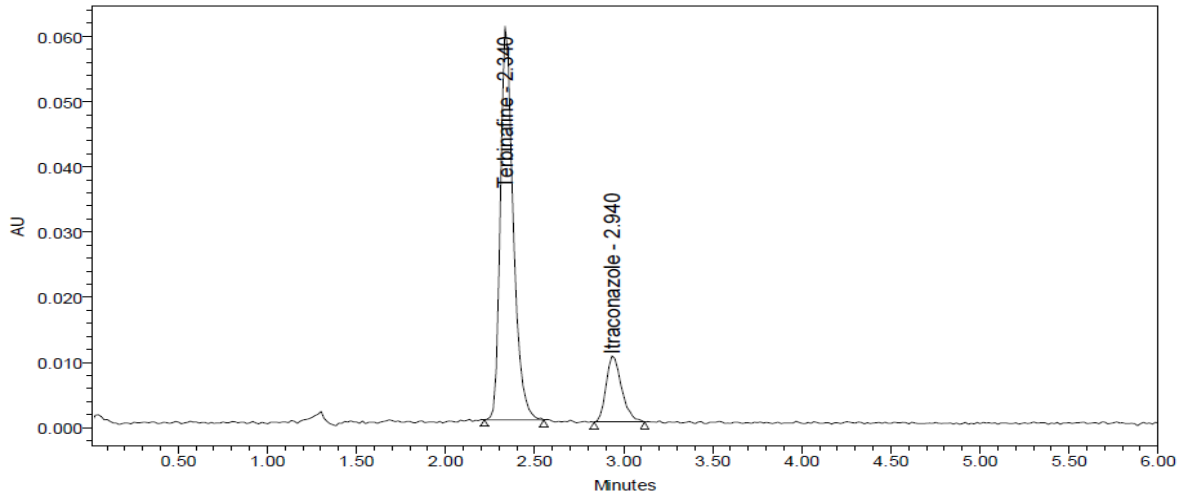

Figure 3: Chromatogram of standard mixture of TRB & ITC.

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Terbinafine	2.340	379188	1.33	4.5	3442
2	Itraconazole	2.940	65643	1.34	5.5	5666

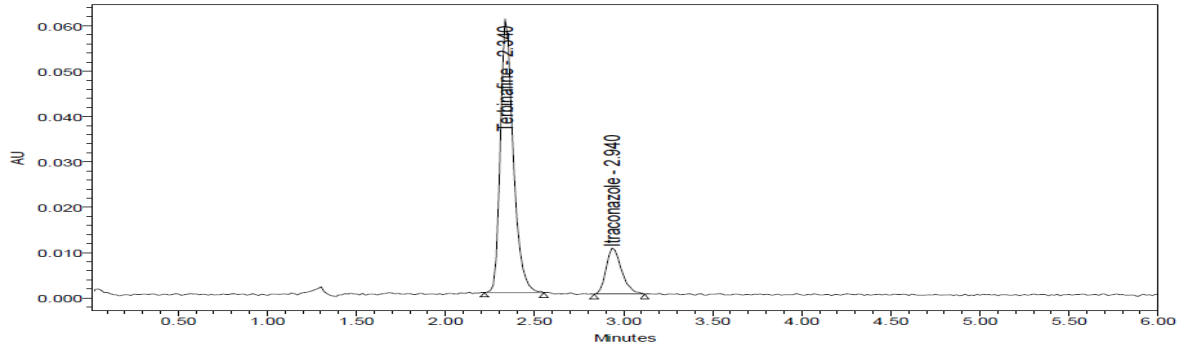

Figure 4: Chromatogram of sample mixture of TRB & ITC.

Validation

The above optimized chromatographic method has been validated for the assay of TRB & ITC using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Terbinafine and Itraconazole (TRB & ITC) drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Terbinafine (62.5mg) + Itraconazole (25mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of Terbinafine (62.5mg) + Itraconazole (25mg) respectively. The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of Terbinafine and Itraconazole. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

Table 1: Linearity table for TRB & ITC.

Terbinafine		Itraconazole	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
31.25	95222	12.5	17745
62.5	188082	25	32779
93.75	274199	37.5	50158
125	373968	50	67216
156.25	455003	62.5	82948
187.5	547507	75	98629

Table 2: System precision table of TRB & ITC.

S. No	Area of Terbinafine	Area of Itraconazole
1.	376448	64962
2.	378255	66388
3.	382089	66726
4.	375198	64919
5.	376023	65071
6.	387114	65791
Mean	379188	65643
S.D	4594.6	782.9
%RSD	1.2	1.2

Table 3: Summary of Validation data of TRB & ITC.

Parameters		**Terbinafine**	**Itraconazole**	**LIMIT**
Linearity Range (µg/ml)		31.25-187.5µg/ml	12.5-75 µg/ml	R< 1
Regression coefficient		0.9991	0.9991	
Slope(m)		2912	1316	
Intercept(c)		3286	560.8	
Regression equation (Y=mx+c)		y = 2912x + 3286.	y = 1316.x + 560.8	
Assay (% mean assay)		99.68%	99.95%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		1.2	1.2	NMT 2.0%
Method precision %RSD		0.6	0.9	NMT 2.0%
Accuracy %recovery		99.56%	100.16%	98-102%
LOD		0.77	1.13	NMT 3
LOQ		2.34	3,42	NMT 10
Robustness	**FM**	0.5	0.4	%RSD NMT 2.0
	FP	1.2	1.6	
	MM	1.4	1.8	
	MP	0.9	0.7	
	TM	1.o	1.8	
	TP	1.6	0.8	

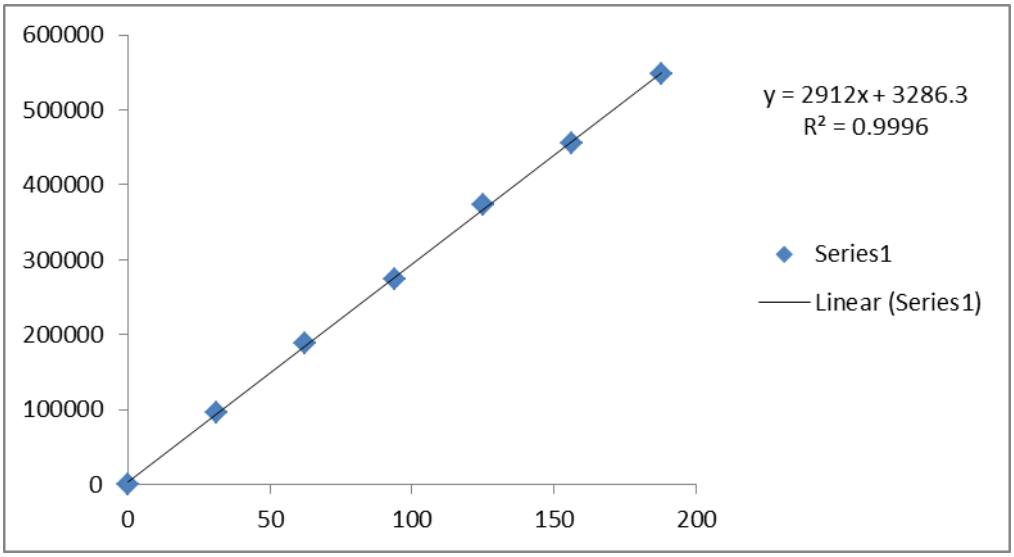

Fig. 7: Linearity curve of Terbinafine.

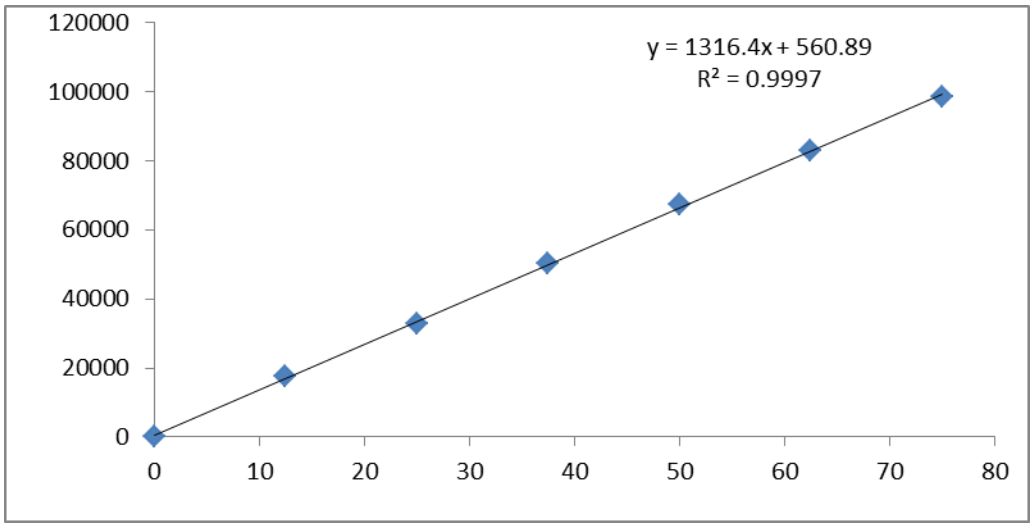

Fig. 8: Linearity curve of Itraconazole.

Table 4: Degradation data of TRB & ITC.

Type of degradation	Terbinafine			Itraconazole		
	Area	%Recovered	% Degraded	AREA	%Recovered	% Degraded
Acid	366436	96.25	3.75	366436	96.22	3.78
Base	376611	98.92	1.08	376611	96.50	3.50
Peroxide	376040	98.77	1.23	376040	97.50	2.50
Thermal	371960	97.70	2.30	371960	97.91	2.09
Uv	374215	98.29	1.71	374215	98.90	1.10
Water	371294	97.53	2.47	371294	97.96	2.04

RESULTS AND DISCUSSION

The regression equation for TRB was found to be y = 2912x + 3286 (slope, intercept and correlation coefficient were found to be 2912, 3286 and 0.999 respectively) and linear over beer's range of 31.25-187.5µg/ml. The regression equation for ITC was found to be y = 1316.x + 560.8 (slope, intercept and correlation coefficient were found to be 1316, 560.8 and 0.999 respectively) and linear over beer's range of 12.5-75 µg/ml. Linearity graph of TRB & ITC were shown in Figure 5 & 6 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for Terbinafine and Itraconazole were and found to be 1.2 and 1.2 respectively. %RSD of method precision for Terbinafine and Itraconazole were and found to be 0.6 and 0.9 respectively. % recovery was obtained as 99.56% and 100.16% for Terbinafine and Itraconazole respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table

2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug TRB & ITC and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 99.68% and 99.95% w/w for 50%, 100% and 150% respectively. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for TRB & ITC was found to be 0.77µg/ml and 1.13µg/ml respectively. LOQ for TRB & ITC was found to be 2.34µg/ml and 3.42µg/ml respectively. Summary of all the validation parameter shown in table 3.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Terbinafine and Itraconazole in Tablet dosage form was developed and the proposed method as suitable for routine analysis of TRB & ITC.

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