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HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ITRACONAZOLE AND TERBINAFINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:
A simple, Accurate, precise method was developed for the simultaneous estimation of the Terbinafine and Itraconazole in bulk and pharmaceutical dosage form. Chromatogram was run through phenomenex C18 150 x 4.6mm, 5.0µ. Mobile phase containing Buffer 0.1%OPA: Methanol taken in the ratio 60:40v/v was pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 245 nm. Retention time of Terbinafine and Itraconazole were found to be 2.331 min and 2.934 min. %RSD of the Terbinafine and Itraconazole were and found to be 0.4 and 0.9 respectively. %Recovery was obtained as 99.95% and 100.98% for Terbinafine and Itraconazole respectively. LOD, LOQ values obtained from regression equations of Terbinafine and Itraconazole were 0.94, 2.86 and 0.70, 2.13 respectively. Regression equation of Terbinafine is y = 6951.x + 2494., and y = 7657x + 2075. 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.

Key Words: *Terbinafine, Itraconazole, RP-HPLC*

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INTRODUCTION:

Terbinafine N-methyl-1-naphthalenemethylamine in which the amino hydrogen is replaced by a 3-(tertbutylethynyl)allyl group Terbinafine hydrochloride (Lamisil) is a synthetic allylamine antifungal. It is highly lipophilic in nature and tends to accumulate in skin, nails, and fatty tissues. Like other allylamines, terbinafine inhibits ergosterol synthesis by inhibiting the fungal squalene monooxygenase (squalene 2,3-epoxidase), an enzyme that is part of the fungal cell wall synthesis pathway.**Itraconazole**2-butan-2-yl-4-[4-[4-[4-[[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-1,2,4-triazol-3-one, and its chemical formula, $C_{35}H_{38}Cl_2N_8O_4$, gives a molecular mass of 705.6334 g/mol One of the triazole antifungal agents that inhibits cytochrome P-450-dependent enzymes resulting in impairment of ergosterol synthesis. It has been used against histoplasmosis, blastomycosis, cryptococcal meningitis & aspergillosis**. Terbinafine Itraconazole** were introduced into the market in combined dosage form (**Itrogen -TR**) it is a fixed-dose combination antiretroviral medication used to **treat fungal infections** It is highly lipophilic in nature and tends to accumulate in skin, nails, and fatty tissues.[1] Like other allylamines, terbinafine inhibits ergosterol synthesis by inhibiting the fungal squalene monooxygenase (also called squalene epoxidase), an enzyme that is part of the fungal cell wall synthesis pathway Itraconazole is a **highly selective inhibitor** of fungal cytochrome P-450 sterol C-14 α-demethylation via the inhibition of the enzyme cytochrome P450 14α-demethylase. This enzyme converts lanosterol to ergosterol, and is required in fungal cell wall synthesis. toothers The literature review reveals that few analytical methods have been reported for the simultaneous estimation **Terbinafine Itraconazole** of in bulk, pharmaceutical dosage forms and in biological samples. They are UV Spectrophotometric, HPLC and LC-MS/MS methods.Fewanalytical methods are reported for the **Terbinafine Itraconazole** in bulk and pharmaceutical formulations. They are UV Spectrophotometric, HPLC and UPLC methods. simultaneous estimation **Terbinafine Itraconazole** of Hence an attempt has been made to develop a simple ,precise, accurate, sensitive, reliable and cost effective stabilityindicating RP- HPLC method for the simultaneous estimation of **Terbinafine, Itraconazole** in bulk and pharmaceutical dosage form.

Chromatographic conditions

Phenomenex Luna C18 (250 x 4.6mm, 5µ) was the column used for separation. Mobile phase consisting of a mixture of Acetonitrile and Buffer (7.8 gm of sodium dihydrogen orthophosphate and 1.8 gm of hexane sulfonic acid in 1000 ml of water and pH was adjusted to 4) in the ratio 50:50 v/v was delivered at a flow rate of 1.0 ml/min with detection at 210 nm. The mobile phase was filtered through a 0.45µ nylon filter and sonicated for 15 min. Analysis was performed at ambient temperature

Pharmaceutical formulation

The branded formulations (tablets) (Lopimune tablets containing 200 mg of Lopinavir and 50 mg of Ritonavir) were procured from the local market

Preparation of Standard stock solutions:

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)

From above solution 1ml stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (125µg/ml Terbinafine of and 50µg/ml of Itraconazole)

Procedure for analysis of tablets

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)

Method Validation

Linearity

By appropriate aliquots of the standard Terbinafine and Itraconazole prepared six working solutions ranging between 31.25-187.5µg/mL& 12.5-75µg/. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficient on curves for Terbinafine and Itraconazole.

Precision

The repeatability of the method was verified by calculating the % RSD of six replicate injections of 100% concentration (125µg/ml of Terbinafine and 50µg/ml of Itraconazole) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days.

Accuracy

Accuracy was carried out by % recovery studies of Terbinafine and Itraconazole at three different concentration levels (50%, 100%, and 150%). Percentage recovery was calculated from the amount added and the amount recovered. The percentage recovery was within the acceptance criteria, this indicates the accuracy of the method. (Acceptance criteria: % recovery between 98 to 102).

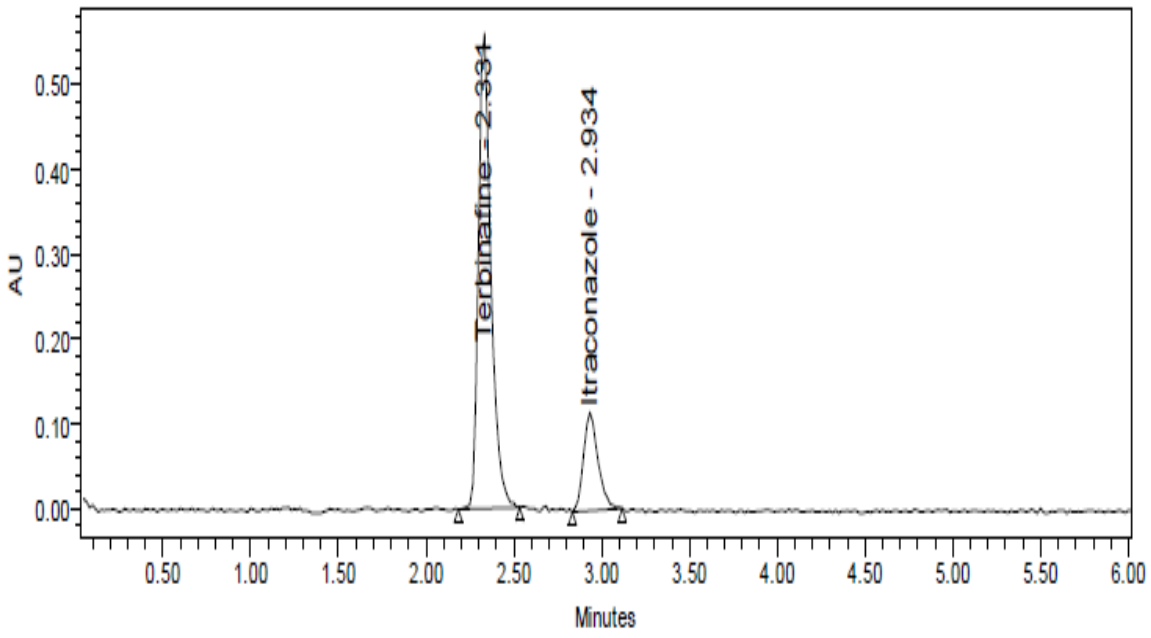
Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s.

Robustness

Robustness of the method were verified by altering the chromatographic conditions like flow rate, mobile phase ratio and temperature are made, but there were no recognized change in the result and all are within range as per ICH guidelines. Robustness conditions like flow minus (0.7 ml/min), flow plus (0.9 ml/min), 65:35mobile phase minus 55:45 mobile phase plus, temperature minus (25°C) and temperature plus (35°C) were maintained and samples were injected in duplicate manner. System suitability parameter was passed. % RSD was within the limit.

Typical Chromatogram of ITR and TER



Summary of validation parameters

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)		6951	7657	
Intercept(c)		2494	2075	
Regression equation (Y=mx+c)		y = 6951.x + 2494.	y = 7657x + 2075	
Assay (% mean assay)		100.95%	100.55%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.4	0.9	NMT 2.0%
Method precision %RSD		0.7	0.8	NMT 2.0%
Accuracy %recovery		99.95%	100.98%	98-102%
LOD		0.94	0.70	NMT 3
LOQ		2.86	2.13	NMT 10
Robustness	**FM**	0.2	1.1	%RSD NMT 2.0
	FP	0.3	0.8	
	MM	0.5	1.2	
	MP	0.2	1.1	
	TM	0.5	0.5	
	TP	0.3	0.9	

CONCLUSION:

Retention time of Terbinafine and Itraconazole were found to be 2.331 min and 2.934 min. %RSD of the Terbinafine and Itraconazole were and found to be 0.4 and 0.9 respectively. %Recovery was obtained as 99.95% and 100.98% for Terbinafine and Itraconazole respectively. LOD, LOQ values obtained from regression equations of Terbinafine and Itraconazole were 0.94, 2.86 and 0.70, 2.13 respectively. Regression equation of Terbinafineis y = 6951.x + 2494., and y = 7657x + 2075. 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.

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