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RP-HPLC method development and validation for the estimation of antifungal drug terbinafine HCL in bulk and pharmaceutical dosage form

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ABSTRACT

In the present work RP-HPLC method has been developed for the quantitative estimation of Terbinafine hydrochloride in bulk drug and pharmaceutical formulations.A rapid and sensitive RP-HPLC Method with PDA detection (220 nm) for routine analysis of in Bulk drug and Pharmaceutical formulation was developed. Chromatography was performed with mobile phase containing a mixture of Potassium dihydrogen phosphate and Acetonitrile (65:35 v/v) with flow rate 1.5 ml/min. The linearity was found to be in the range of 50-150 µg/ml with (r2=0.999). The proposed method was validated by determining sensitivity, accuracy, precision, LOD, LOQ and system suitability parameters according to ICH guidrelines.

Keywords: Reverse phase high pressure liquid chromatography (RP-HPLC); Terbinafine Hydrochloride; International Conference on Harmonisation(ICH); Photodiode array(PDA) detection.

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INTRODUCTION

Terbinafine hydrochloride (Lamisil)[1] is a synthetic allylamine derivative and 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[2]. The resultant high concentration of squalene and decreased amount of ergosterol are both thought to contribute to terbinafine's antifungal activity. Terbinafine is active against dermatophytes. Chemically it is[(2E)-6,6-dimethylhept-2-en-4-yn-1-yl](methyl)(naphthalen-1-ylmethyl)amine hydrochloride having molecular formula $C_{21}H_{26}CIN$ HCl and molecular weight 326.9299. It is white to off-white fine crystalline powder It is freely soluble in methanol and dichloromethane, freely soluble in anhydrous ethanol, soluble in ethanol, slightly and very slightly soluble in water, and slightly soluble in acetone. Literature survey[3-5] reveals that Terbinafine hydrochloride can be estimated by spectrophotometrically by HPTLC and by HPLC individually with other drugs in bulk drugs and in human plasma. Investigations of some new instrumental methods are in need for the quantitative estimation of Terbinafine Hydrochloride in bulk drug and its pharmaceutical dosage forms with high sensitivity, accuracy, precision and economic too.

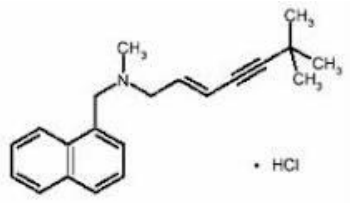

Figure 1: Structure of Terbinafine hydrochloride

MATERIALS AND METHODS

Chemicals and reagents: Acetonitrile, Ortho phosphoric acid, Potassium dihydrogenphospate, Milli-Q water All the chemicals used were of HPLC grade procured from qualigens Mumbai, S.D. Fine Chem Ltd, Merck and Spectrochem, Mumbai, India. Methanol was used for making the solutions.

Instruments: A gradient high pressure liquid chromatograph (Waters – Separation Module e 2695) with one LC-10 AT VP pumps, PDA detector 2998, Reverse Phase C-18 Column (50 mm x 4.6 i.d., particle size 5 µm) was used The HPLC system was equipped with the EMPOWER 2 software.

Drug sample: Standard Terbinafine hydrochloride was obtained as a gift sample from Hetero Pvt. Ltd., Hyderabad, India.

Marketed formulations: (Lamisil-150 mg tablets) from Cipla Pvt. Ltd., Delhi, India was collected from local market.

Chromatographic Conditions

Column : INERTSIL ODS 50×4.6mm5µm

Wavelength : 220nm (UV detector)

Temperature : 250c

Flow rate : 1.5ml/min

Injection volume : 10µl

Mobile phase : Potassium dihydrogenphosphate: acetonitrile (65:35)

Run time : 10 minutes

Prior to injection of the drug solution the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The datas were acquired, stored and analyzed with the software class EMPOWER2 (WATERS-2695).

The chromatogram obtained through the injection of the placebo solution did not contain any peak at the retention time of Terbinafine hydrochloride. The chromatogram peak purity tools show that the peak was 100%.

Preparation of Standard Stock Solution of Terbinafine hydrochloride: 50 mg of Terbinafine hydrochloride standard was accurately weighed and transferred to a 100 ml volumetric flask, dissolved in 35 ml of mobile phase and diluted to 100ml with mobile phase then sonicated for 10 min. From this a standard solution[6] 100 µg/ml was prepared. 10 µl of standard solution was injected into the column with a flow rate of 1.5 ml/min.

Preparation of Standard working solutions (100% solution): From the above each stock solution[7], 1 ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Preparation of Sample stock solutions: 20 tablets were weighed, crushed, powdered and then the tablet powder weight equivalent to 10mg of Terbinafine was transferred into a 100ml volumetric flask, 75ml of diluent added and sonicate for 30 min, further the volume made up with diluent and filtered through 0.45 µm nylon membrane.

Preparation of Sample working solutions (100% solution): From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Buffer Preparation: Transfer 1.35gms of Potassium dihydrogen phosphate into 1000ml of beaker and dissolve with water. Make up the volume with water.Adjust pH 3 with Ortho phosphoric acid.

Mobile phase preparation: Transfer buffer solution 650ml and 350ml of acetonitrile. Mix well and sonicate for 30minutes.

RESULTS

After several trials with different combinations and ratio of solvents, the mobile phase potassium dihydrogenphosphate : acetonitrile (65:35) was selected, because it was found that it ideally resolve the peaks with retention time [8] (Rt) of Terbinafine Hcl is 6.2mins and the same is shown in Figure 2. Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200nm to 400 nm.

METHOD VALIDATION

Validation[9] of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended for analytical application.The present study was carried method was validated based on ICH (Q2B) parameters [10,11].

The following parameters were validated for the proposed method.

Specificity

The specificity of the method was evaluated with regard to interference due to presence of any other excipients. The figure shows that drug was clearly separated from its excipients. Thus, the HPLC method presented in this study is selective.

Determination: Volume of 10µl of working placebo sample solution was injected into the chromatograph and the chromatogram was recorded and presented in the Figure 3.

Linearity and range

The calibration curve was plotted with five concentrations of the standard drug solution 50-150 µg/ml and chromatography was repeated six times for each dilution. The linearity was evaluated by

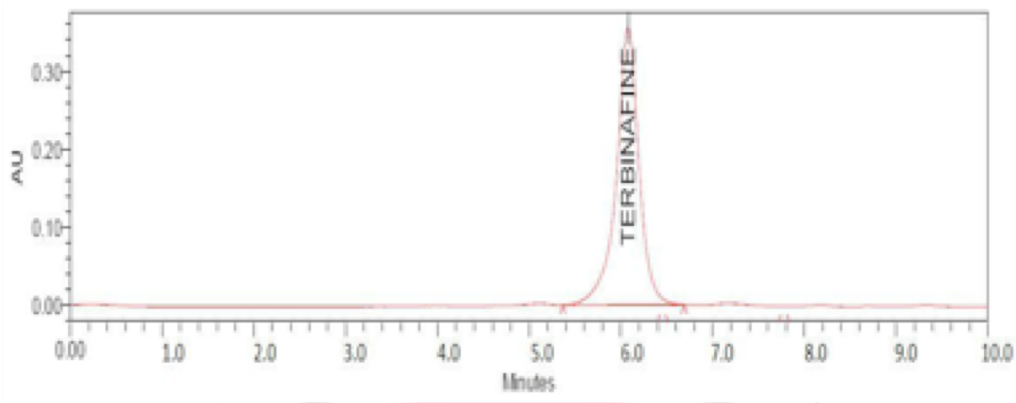

Figure 2: Chromatogram of Terbinafine Hcl

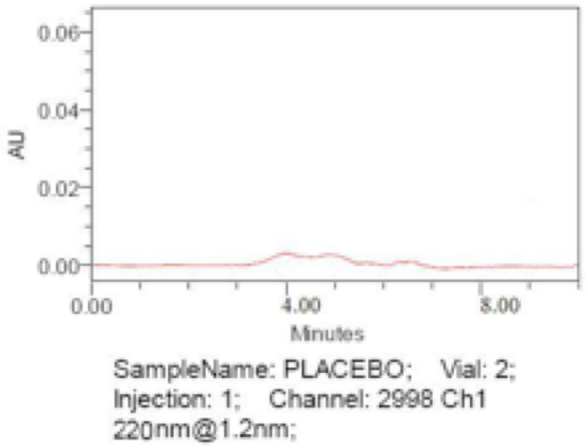

Figure 3: Chromatogram of placebo

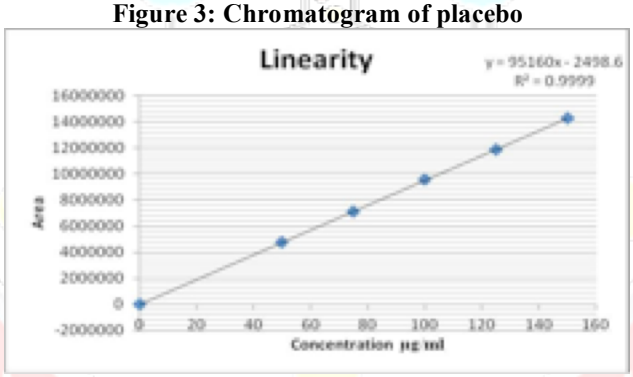

Figure 4: Chromatogram of Sample solution

Table 1: Linearity data for Terbinafine hydrochloride

Sl.No	Concentration(µg/ml)	Area
1	0	0
2	50	4749950
3	75	7107990
4	100	9578950
5	125	11848700
6	150	14279650

linear regression analysis. Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Five determinations were carried out for each solution, peak areas were recorded for all the solutions. The correlation graph was constructed by plotting the peak areas obtained at the optimum wavelength of detection v/s the injected amounts of the respective concentrations.

By using the working standard, aliquots of 50, 75, 100, 125 and 150 µg/ml were prepared with acetonitrile six dilutions of each of the above mentioned concentrations were prepared separately and from these six dilutions, 10 µl of each concentration was injected into the HPLC system and their chromatograms were recorded. Peak areas were recorded for all the peaks and a standard

Table 2: Statistical Data for Precision

Components	Precision	Mean	Standard Deviation	Co-efficient of Variation
TERBINAFINE HCL	Intra-day	99.68%	0.01527	0.01532
	Inter-day	99.61%	0.03605	0.03619

Table 3: Repeatability Data for Terbinafine hydrochloride

Conc.(µg/ml)	0	50	75	100	125	150
Area 1	0	4749000	7110122	9480788	11849885	14336769
Area 2	0	4750425	7108701	9482684	11853439	14308209
Area 3	0	4752325	7107279	9488372	11848700	14279650
Area 4	0	4749475	7107990	9489320	11846330	14275366
Area 5	0	4751375	7105858	948800	11845145	14278222
Mean	0	4750520	7107990	9487993	11848700	14295643
Std.Dev	0	1360.193	1589.174	7049.704	3244.891	26550.31
%RSD	0	0.028633	0.022358	0.074301	0.02738	0.18572

Table 4: Determination of Recovery studies of Terbinafine hydrochloride

Level of % recovery	Amount present	Amount of standard drug	Total amount recovered	% Recovery
50%	50	25	74.955	99.94
	50	25	75.097	100.13
	50	25	75.112	100.15
100%	50	50	100.45	100.45
	50	50	100.09	100.09
	50	50	100.04	100.04
150%	50	75	124.75	99.8
	50	75	125.62	100.5
	50	75	125.87	99.9

Table 5: Statistical Data for Recovery studies

Level of %recovery	Mean	Standard Deviation	Co-efficient of Variation
50%	100.106%	0.1866	0.1864
100%	100.193%	0.2236	0.2232
150%	100.093%	0.2459	0.2456

Table 6: LOD and LOQ of Terbinafine Hcl

S. No.	Results observed (*SD)	Concentration
1	LOD(µg $mL^{-1)}$	0.0585
2	LOQ(µg $mL^{-1)}$	0.1950

Table 7: Results of system suitability parameters

Parameters	Data obtained for Terbinafine
Theoretical plates per column	4530
Symmetry factor/ Tailing factor	0.8

Table 8: Assay Results of Tablet Formulation

Sl.No	Amount present in (mg/tab)	Amount obtained in (mg/tab)	Area	% Obtained
1	75	75.15	7124100	100.2
2	75	75.3	7138320	100.4
3	75	75.225	7131210	100.3
4	75	75.15	7124100	100.2
5	75	75.075	7116990	100.1
6	75	75	7109880	100

Table 9: Statistical Data for Tablet Formulation

Components	Mean	StandardDeviation	%RSD
Terbinafine	100.2	0.141421	0.001411

calibration curve of peak area against concentration was plotted.

Precision

The precision of the assay was determined in terms of intra and inter-day variation in the peak area for a

set of drug solution (100 µg/ml) assayed five times on the same day and on three different days.The intra and inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of standard deviation to the mean with 100(CV=SD/MEAN X 100)

Accuracy

The procedure for the preparation of solutions for Accuracy determination at 50%, 100% and 150% level were prepared . The solutions were filtered through 0.4 µm membrane filter paper and then they were subjected to analysis by RP-HPLC method under the same chromatographic conditions as described above. At each level, three determinations were performed. The results obtained were compared with expected results and were statistically validated.

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

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in Table 6.

System Suitability

All the system suitability parameters are within range and satisfactory as per ICH guidelines.

DISCUSSION

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were within the limits.

Assay of Terbinafine hydrochloride in Tablets

Ten tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 100 mg of Terbinafine hydrochloride was transferred to 100 ml volumetric flask containing 40 ml of mobile phase and the contents of the flask were sonicated for 15 min, to ensure the complete solubility of the drug, then the mixture was made up to 100 ml with mobile phase.The resulting solution was thoroughly mixed and filtered through a 0.45 µm membrane filter. From this a working standard solution of 100 µg/ml of strength was prepared. concentration of 10µg/ml were prepared in 100 ml volumetric flask & diluted with acetonitrile. This concentration (10 µl) was injected six times into the column. The mean values of peak areas were calculated and the drug content in the tablets was quantified.

CONCLUSION

In the present investigation, we have developed a simple, sensitive, precise, accurate, rapid and economical RP-HPLC method for the quantitative estimation of Terbinafine Hcl in bulk drug and pharmaceutical formulations. After development of the method, it was validated for system suitability, specificity and linearity, limit of detection and limit of quantification, precision and accuracyThe results obtained was found to be within limits. Hence, this method can be used for the routine determination of Terbinafine Hcl in bulk drug and in pharmaceutical formulations.

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