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HPTLC Method for determination of Terbinafine in the Bulk drug and Tablet dosage form

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Abstract: A simple, selective, precise and stability indicating high performance thin layer chromatographic method for analysis of Terbinafine, both as the bulk drug and in a tablet formulation has been developed and validated. In this, method pre coated silica gel 60F $_{254}$ aluminum foil TLC plates was used as stationary phase and chromatogram was developed using of n hexane: acetone: glacial acetic acid in the ratio of 8:2:0.1v/v as mobile phase. A compact band with R_f 0.42 was obtained for Terbinafine. Densitometric analysis was performed in absorbance mode at 223nm using Camag TLC scanner. Linear regression analysis revealed a good linear relationship (r^2 =0.9997) between peak area and concentration in the range of 200-1000ng/spot.The method was validated for precision, recovery and robustness. The limit of detection and quantification was 1.204 ng/ spot and 3.648 ng /spot respectively. Terbinafine was subjected to acid, alkaline hydrolysis, oxidative, photochemical and thermal degradation and compound underwent degradation only under photo chemical condition when the standard drug was exposed to UV light. This method under statistical analysis proved a selective, repeatable and accurate analysis of the drug. This method can be used for quantitative analysis of Terbinafine in the bulk drug and in tablet formulation.

Key words: Terbinafine, HPTLC, validation, Forced stress degradation.

Introduction and Experimental

Terbinafine Hydrochloride (E) – N (6, 6, dimethyl-2-hepten-4-ynyl) –N-Methyl-naphthalene ethanamine hydrochloride [1] (Fig.1), is an allyl amine derivative with antifungal activity. The drug has been found to be a potent inhibitor of squalene epoxidase which is an enzyme present in fungal and mammalian cell systems important in ergo sterol biosynthesis.[2]

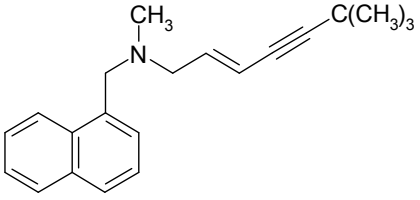


Fig. 1 Structure of Terbinafine

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influences of a variety of environmental factors such as temperature, humidity and light and enables recommendation of storage conditions, resets periods and shelf lives to be established. The two main aspects of drug products that play an important role in shelf life determination are assay of active drug and degradation products generated during the stability study. [3] The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference On Harmonization (ICH) guideline.[4]. The objective of this work was to develop a simple, precise and rapid HPTLC procedure which would serve as stability indicating assay method for Terbinafine hydrochloride.

Terbinafine hydrochloride is official in British pharmacopoeia [1]. The literature survey reveals that the drug has been determined in biological fluids (plasma, urine) tissues, nails and hair by HPLC [5,6,7,8,9,10,11,12], in tablets and creams by HPLC [13], ,UV-Spectrophotometry, Spectrodensitometry,[14,15,16], colorimetric method [17], extraction Spectrophotometric method [18,19], and Stability indicating HPTLC [20]

This paper describes the forced degradation of drug under stress conditions that is thermal, acid/base hydrolytic, photochemical, oxidative and stressed samples were analyzed by the proposed method. The method was validated as per ICH guide line for linearity, accuracy and precision [21]

Experimental data

Chemicals and Reagents:
Terbinafine hydrochloride working standard was a generous gift from Novarits (India) Ltd, and was used. All the other reagents used were of analytical grade n-Hexane (AR grade), Acetone (AR grade), Acetic acid (AR grade) were purchased from Merck Ltd.

Instrumentation:
Chromatographic separation of drug was performed on Merck TLC plates pre coated with silica gel $60F_{254}$ (10cmx10 cm) with 250 µm layer thickness from E Merck, Germany. The samples were applied on to plates as a band with 5mm width with slit dimension of 6.00x0.3mm micro using Camag 100 µl sample syringe (Hamilton, Switzerland) with the Linomat 5 applicator (Camag Switzerland) Linear ascending development was carried out in a twin through glass chamber (10 cm x 10cm) previously saturated with the mobile phase at room temperature, using 30 minutes of chamber saturation .The development distance was approximately 90mm. Densitometry scanning was performed using Camag TLC Scanner 3 in the range of 200-400nm and operated by Win cats software (V1.43, Camag) using deuterium lamp as source of radiation.

Selection of detection wavelength:
Terbinafine hydrochloride shows good absorbance at 223nm .So it was selected as wave length of detection.

Method development:
Chromatographic separation of active and its degraded product was achieved using mobile phase of n hexane: acetone: glacial acetic acid in the ratio of 8:2:0.1v/v.

Standard and sample preparation:
The standard stock solution 1mg/ml was prepared by dissolving working standard in methanol.

For the preparation of sample solution, 20 tablets were weighed individually; average weight was calculated and finally powdered. Appropriate portion of this powder equivalent to 50mg of Terbinafine hydrochloride was weighed and transferred to 50ml volumetric flask dissolved in little amount of methanol, sonicated for 15 minutes, filtered through Whatmann filter paper No.41, then volume was made up to 50ml with methanol and the same concentration as per standard solution was used.

Procedure for forced degradation study of standard Terbinafine
Forced degradation of drug product was carried out under thermolytic, acid/base hydrolytic and oxidative stress condition. Thermal degradation of drug was carried out in solid state. After degradation stock solution were prepared by dissolving in methanol. From this solution aliquots were diluted with methanol to get concentration of (600ng) and spotted on the TLC plates in triplicate and chromatographed as described above

Stress degradation of Terbinafine
Degradation under base catalyzed hydrolytic condition:

Accurately 25mg of standard drug was weighed and dissolved in 0.1 N methanolic sodium hydroxide in a 25 ml volumetric flask, refluxed for 6hrs at 60°C from this 1 ml solution taken diluted to 10 ml with methanol, 600ng/spot of sample was spotted at time interval of 0, 1hr, 8hr, 12 hr and 24 hr, with a blank reagent and appropriately diluted standard solution spotted in adjoining track.

Degradation under acid catalyzed hydrolytic condition:

Accurately 25mg of standard drug was weighed and dissolved in 1 N methanolic hydrochloric acid solution in a 25 ml volumetric flask, refluxed for

6hrs at 60°C from this 1 ml solution taken diluted to 10 ml with methanol, 600ng/spot of sample was spotted at time interval of 0, 1hr, 8hr, 12 hr and 24 hr, with a blank reagent and appropriately diluted standard solution spotted in adjoining track.

Oxidative degradation:

Accurately 25mg of standard drug was weighed and dissolved in25mlof methanolic solution of hydrogen peroxide (30%v/v) in a 25 ml of volumetric flask, refluxed 6hrs at 60 °C, from this 1ml solution was taken and final volume was made up to 10ml with methanol, 600ng/spot of sample was spotted at time interval of 1hr, 8hr, 12 hr and 24 hr, with a blank reagent and appropriately diluted standard solution spotted in adjoining track.

Degradation under dry heat:

Accurately 25mg of standard drug was weighed and transferred to a Petri dish and was kept in oven at 100°c, for 7days , then sample solution of 600ng/spot of sample in methanol was spotted, with a blank reagent and appropriately diluted standard solution spotted in adjoining track.

Photo degradation:

Accurately 25mg of standard drug was weighed and transferred to a Petri dish and was kept in an UV cabinet and exposed to UV rays for 0-36hrs , then sample solution of 600ng/spot in methanol was spotted, with a blank reagent and appropriately diluted standard solution spotted in adjoining track.

Method Validation:

As per the ICH guide lines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantification, robustness and specificity.

Preparation of calibration curve:

A stock solution containing 1000 µg/ml Terbinafine hydrochloride was prepared in methanol. Upon appropriate dilution a concentration of 200 ng-1000 ng/spot was applied on the plate and chromatogram was carried out as described above. Peak areas were plotted against corresponding concentration to get the calibration curve and least square regression analysis was performed to generate the calibration equation for Terbinafine hydrochloride.

Accuracy:

For accuracy of method, recovery studies were carried out by applying a known amount of standard Terbinafine hydrochloride at a level of 80,100,120 % to the sample solution (standard addition method). Three determinations were performed at each level and the results obtained were compared with the expected results.

Precision:

Repeatability of sample application and measurement of peak were assessed by chromatography of six replicates of the same concentrations (600 ng/spot).The intraday and interday precision of the proposed method was determined by estimating the corresponding responses three times on the same day and on three different days over a period of one week for three different concentration (400, 600, 800 ng/spot) and results are reported in terms of percentage relative standard deviation.

Limit of Detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ were calculated using following equations as per International conference on Harmonization guide line

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ is standard deviation of the response and S is the standard deviation of y intercept of regression lines.

Robustness:

Robustness was checked by making a slight deliberate change in the experimental procedure. Mobile phase having different composition like n hexane: acetone: glacial acetic acid (7.8:2:0.1v/v) and n hexane: acetone: glacial acetic acid (8:1.8:0.1v/v) was tried and chromatogram was run. Robustness of the method was checked at three different concentration levels 400, 600, 800 ng/spot.

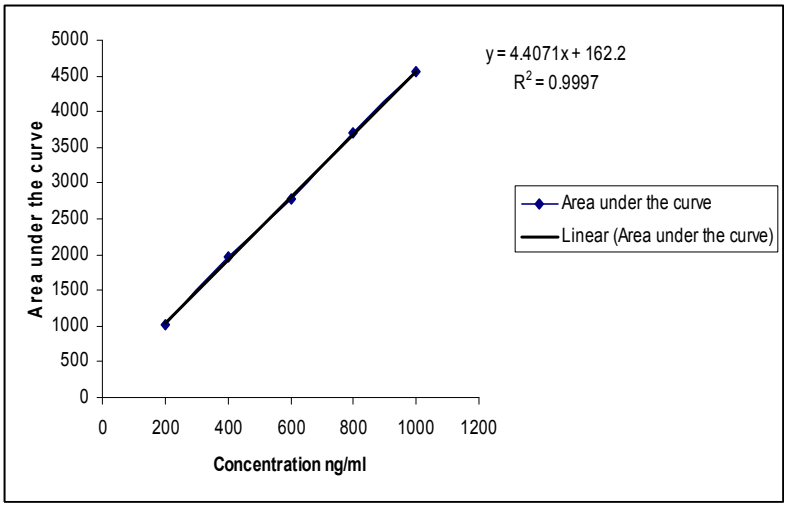
Specificity:

The specificity of the method was determined by analysis of drug standard and samples. The band for Terbinafine hydrochloride in the sample was identified by comparing the R_f value and the spectrum of the band with those of the band from a standard.

Table 1: Method Validation Parameters

Parameters	Results
Linearity range	200-1000 ng
Correlation Coefficient(r^2) *	0.9997
LOD (ng/spot)	1.204ng
LOQ (ng/spot)	3.648 ng
Accuracy (%)	99.74 ± 0.2627
Precision (%RSD)	
Intra-day*	0.3067-0.8104
Inter-day*	0.07320-0.6233
%Recovery studies#	
80%	99.62± 0.1447
100%	98.81±0.24876
120%	100.85± 1.666

* indicates mean of six observation, ± indicates standard deviation.
# Average of three trials



$y = 4.4071x + 162.2$ $R^2 = 0.9997$

Fig 2 Representative linearity of Terbinafine (average of six readings)

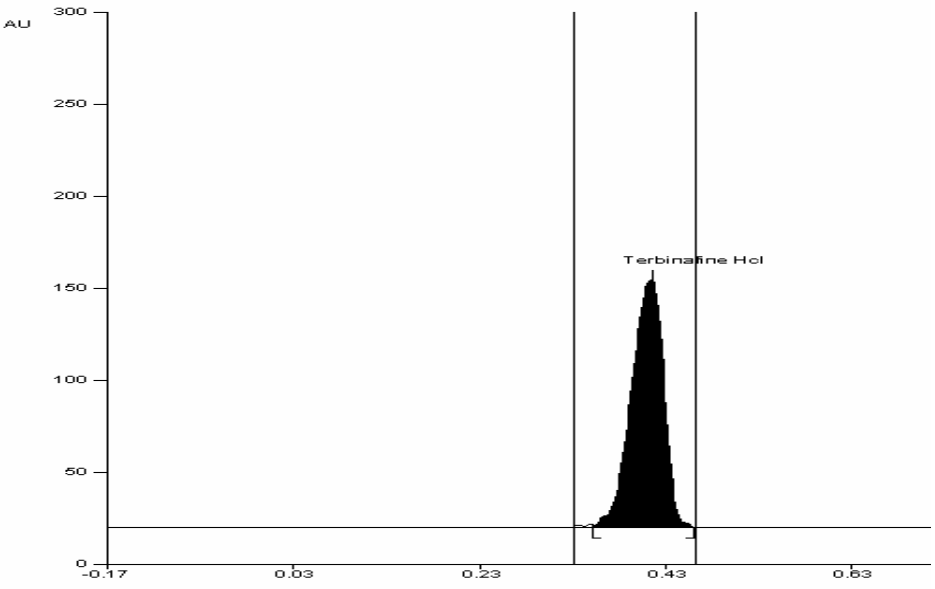


Fig 3 Representative chromatogram of Terbinafine

Results

HPTLC method optimization and Validation:
To optimize the HPTLC parameters, several mobile phase compositions were tried. Satisfactory separations of Terbinafine hydrochloride and its degraded products were obtained with mobile phase consisting of n hexane: acetone: glacial acetic acid (8:2:0.1v/v), the mobile phase enabled good resolution, a sharp and symmetrical peak of R_f 0.43. (Fig 3) from a compact and non diffuse band

Validation:

Linearity and range:
The linear regression data revealed a good linear relationship over the concentration range of 200-1000ng /spot with correlation coefficient (r^2 =0.9997). The results are showed in Table 1 and Fig 2

Analysis of tablet formulation:
By the proposed method assay values of commercial formulations was found to be 99.74%± 0.2621

Precision:
The proposed method was found to be precise as indicated by percent RSD not more than 2%.

Accuracy:
The proposed method when used for the estimation of Terbinafine hydrochloride from pharmaceutical dosage form after spiking with the standard, afforded recovery of 99.62-100.85% at different levels was found.

Limit of Detection (LOD) and Limit of quantification (LOQ):
The limit of detection and limit of quantification was 1.204 ng/ spot and 3.648ng /spot respectively.

Robustness:
Robustness was checked by making a slight deliberate change in the experimental procedure. Mobile phase having different composition like n hexane: acetone: glacial acetic acid (7.8:2:0.1v/v) and n hexane: acetone: glacial acetic acid (8:1.8:0.1v/v) was tried and chromatogram was run. Robustness of the method was checked at three different concentration levels 400, 600, 800 ng/spot. The method was found to be robust since the peak area values were not significantly affected.

Specificity:
The method was found to be specific since no interferences spots were seen when carried out in presence of additives, as active constituent is soluble in methanol where as additives are insoluble in methanol. (Fig 4)

Forced degradation:
There was no significant degradation was observed under acid, base, hydrogen peroxide as chromato graphed as described earlier, however Terbinafine hydrochloride was found to be degraded when exposed to UV light at 36 hr. as shown on the **Fig 5,** an addition three peaks were found at R_f 0.18., R_f 0.25, R_f 0.52 The spot of the degradation product was well resolved from that of the drug.

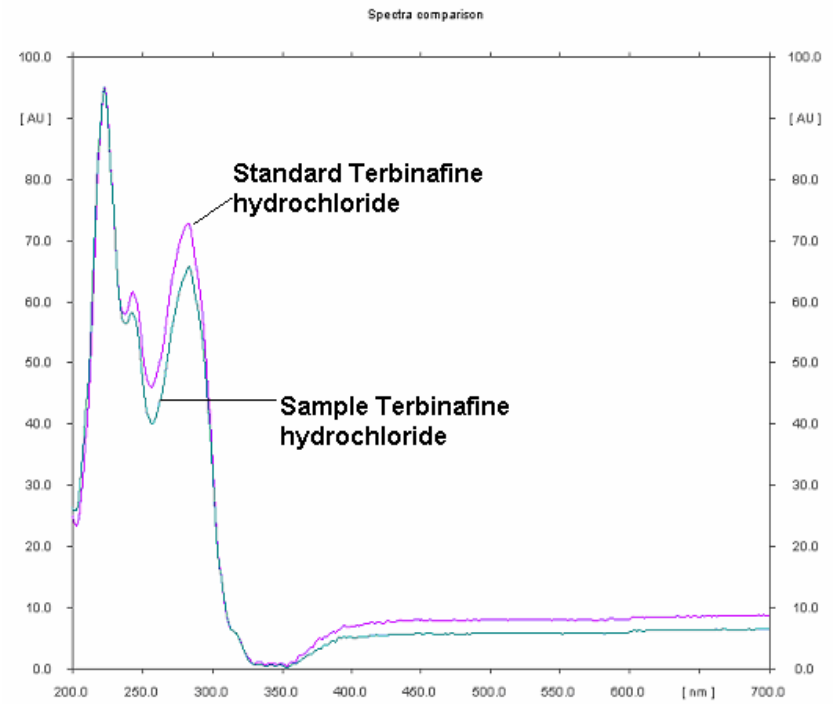


Fig 4 Spectrum of Standard and sample Terbinafine hydrochloride

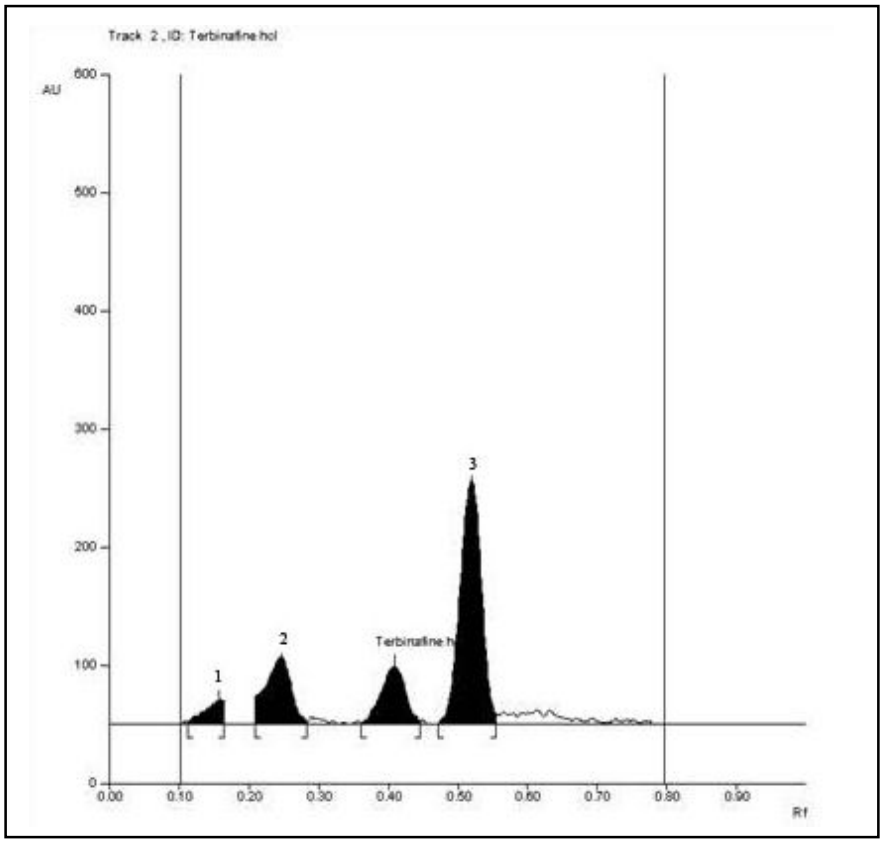


Fig 5 Representative chromatogram of Terbinafine degraded under UV radiation

Discussion

The validated HPTLC method employed here proved to be simple, specific, accurate, precise, sensitive, robust and stability indicating. The developed method is able to discriminate between Terbinafine hydrochloride and its possible degradation products. Statistical analysis proves that the method is suitable for the analysis of Terbinafine hydrochloride as bulk drug and in pharmaceutical formulation with out any interference from the excipients. Hence, this proposed method can be used for the routine analysis of Terbinafine hydrochloride in pure, tablet form and in its degraded products.

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