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A VALIDATED HPTLC METHOD FOR DETERMINATION OF TERBINAFINE HYDROCHLORIDE IN PHARMACEUTICAL SOLID DOSAGE FORM

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ABSTRACT

An HPTLC method for estimation of Terbinafine hydrochloride in its tablet formulation has been developed. It employs aluminium backed silica gel 60 F254 TLC plates,(100×50 mm, thickness of layer 0.2 mm), prewashed with methanol and dried at room temperature)and mobile phase comprising of Acetonitrile: 1, 4 dioxan: Hexane: Acetic acid (1:1:8:0.1) (v/v/v/v). The developing solvent was run upto 70 mm in Camag chamber. Densitometic scanning was then performed with Camag TLC scanner-3 at λmax 282 nm. The R_f value was found to be 0.45. The linearity and range for Terbinafine Hydrochloride was found to be 500-4500ng/spot. The method was validated for precision, accuracy and sensitivity.

INTRODUCTION: Terbinafine Hydrochloride is an allylamine antifungal agent and acts by inhibiting squalene epoxidase, thus blocking the biosynthesis of ergosterol, an essential component of fungal cell membranes. Chemically it is (2E)-N, 6, 6-trimethyl-N-(naphthalene-1-ylmethyl)hept-2-en-4-yn-1-amine hydrochloride. The empirical formula of Terbinafine Hydrochloride is $C_{21}H_{25}N.HCl$ and its molecular weight is 327.92, CAS Number: 78628-80-5., Brands; TEBIF (250mg), structural formula (**Fig. 1**).

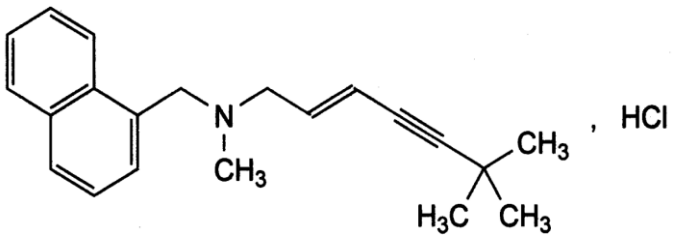

FIG. 1: STRUCTURAL FORMULA OF TERBINAFINE HYDROCHLORIDE

Terbinafine Hydrochloride is White or almost white crystal powder. It is slightly soluble in water and acetone, freely soluble in anhydrous ethanol and methanol [1, 2].

Survey of literature shows several HPLC determination spectrometric determinations in presence of its photodegradation products. Literature survey revealed that no HPTLC method has been reported for the estimation of Terbinafine Hydrochloride. The present investigation has been undertaken to develop simple HPTLC in pure form and its formulations [3, 4, 5, 6, 7].

MATERIALS AND METHODS: Terbinafine Hydrochloride pure drug was obtained as a gift sample from Systopic Laboratories Pvt. Ltd. New Delhi, India. TEBIF (250mg) tablets were purchased from the local market. Reagents in this assay were of analytical grade.

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Apparatus: The instrument used for the estimation, was Camag Linomate V semi-automatic sample applicator, Camag TLC scanner 3, Camag reprostur 3 with digital camera, Camag UV cabinet with dual wavelength UV lamp, Hamilton 100 µl HPTLC syringe and Camag twin trough glass chamber (10×10 and 20×10).

Preparation of Mobile Phase: A mixture of Acetonitrile: 1, 4 dioxan: Hexane: Acetic acid (1:1:8:0.1, v/v) previously filtered through 0.45 µm filter paper in a flask was used as a mobile phase.

Preparation of Standard Stock Solution (500µg/ml): Standard Terbinafine Hydrochloride 5.0 mg was weighed and transferred to a 10 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 500ng/µl Terbinafine Hydrochloride

Calibration Curve for Terbinafine Hydrochloride (500 – 4500ng/µl): Semi-automatic spotter was used containing a syringe having capacity of 100 µl/ml. Mixed stock solution having concentration of 500 µg/ml of Terbinafine Hydrochloride was filled in the syringe and under nitrogen stream, it was apply in form of band of desired concentration range for each of drug on a single plate having concentration of 500 to 4500ng/spot each of Terbinafine Hydrochloride. Plate was developed using Acetonitrile: 1, 4 dioxan: Hexane: Acetic acid (1:1:8:0.1) (v/v) at 25±1°C and dried in air.

Developed plates were subjected to densitometic measurements in absorbance mode at wavelength 282 nm using Camag TLC Scanner 3. A plot of peak area vs. concentration for drug was obtained. A spectrum of drug was recorded in the range of 500-4500ng/spot and purity of chromatographic peak was checked by scanning individual peak at 3 different positions (peak start, peak apex and peak end). Calibration curve is shown in **Fig. 2**.

TABLE 1: RESULT OF CALIBRATION READINGS FOR TERBINAFINE HYDROCHLORIDE BY HPTLC METHOD

Concentrations (ng/spot)	Area (Mean (n=5))
500	4080.66
1500	7607.58
2500	11790.78
3500	14684.4
4500	18311.58

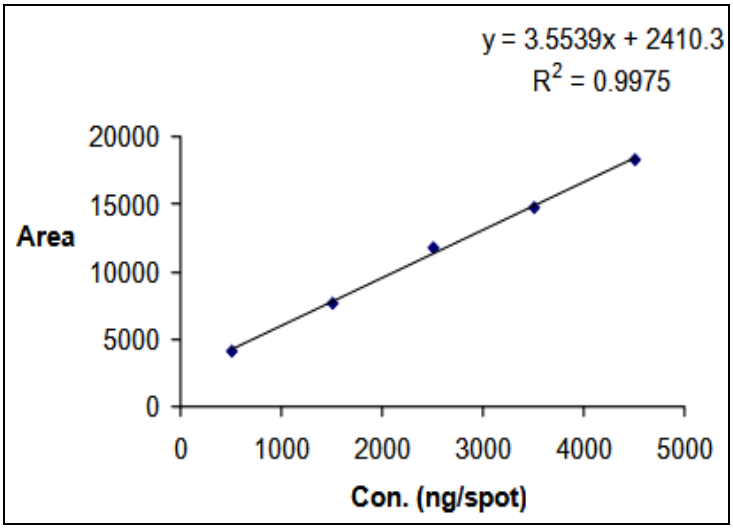

FIG. 2: CALIBRATION CURVE OF TERBINAFINE HYDROCHLORIDE

TABLE 2: STATISTICAL DATA FOR TERBINAFINE HYDROCHLORIDE BY HPTLC METHOD

Parameter	Values
Linear Range (ng/spot)	500-4500
Slope	3.5539
Intercept	2410.3
Standard deviation of slope	0.10712
Standard deviation of intercept	292.1443

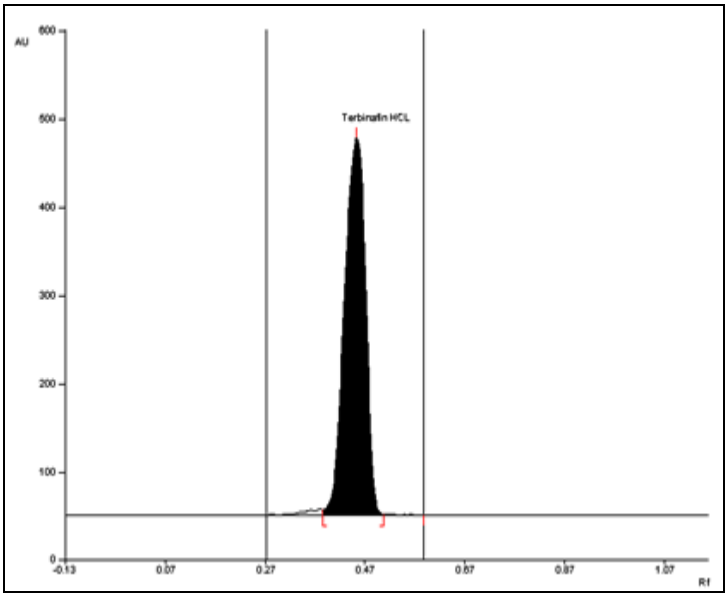

FIG. 3: CHROMATOGRAM OF STANDARD SOLUTION OF TERBINAFINE HYDROCHLORIDE (2500 ng/spot)

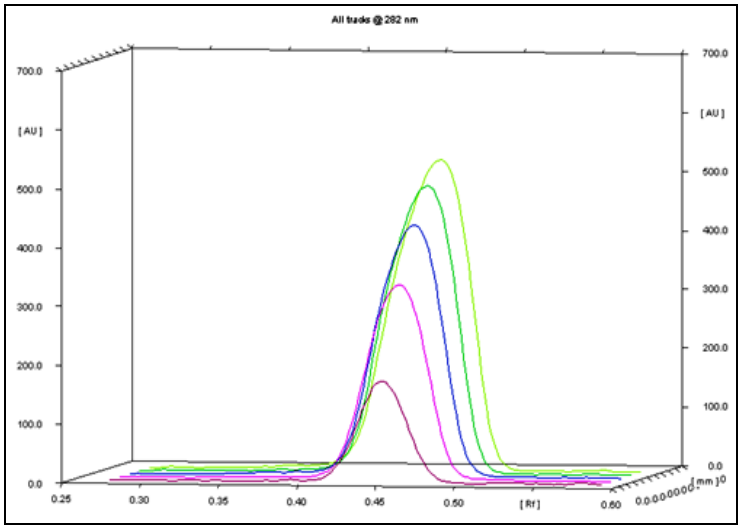

FIG. 4: 3D VIEWS OF ALL TRACKS OF TERBINAFINE HYDROCHLORIDE AT 282nm

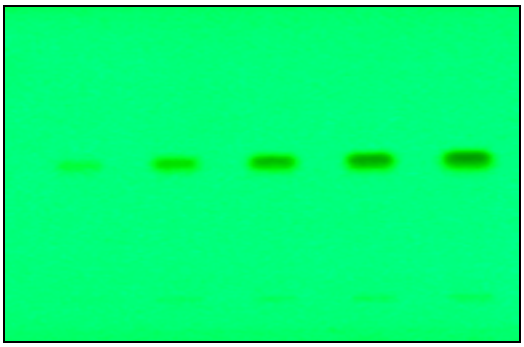

FIG. 5: PHOTOGRAPH OF DEVELOPED HPTLC PLATE OF TERBINAFINE HYDROCHLORIDE

Determination of Terbinafine Hydrochloride from Pharmaceutical Dosage Form (Tablet):

Sample Preparation: Twenty tablets were weighed and finely powdered. The powder equivalent to 250 mg Terbinafine Hydrochloride was accurately weighed and transferred to volumetric flask of 10 ml capacity. 5 ml methanol was transferred to volumetric flask and sonicated for 10 minutes. The flask was shaken and volume was made sup to the mark with methanol. The above solution was filtered through Whatman filter paper (0.45µ). Volume was made up to the mark with methanol to give a solution containing 500 µg/ ml Terbinafine Hydrochloride (Solution A). This solution was used for the estimation of Terbinafine Hydrochloride.

Estimation of Terbinafine Hydrochloride in Pharmaceutical Dosage Form: 5µl of the prepared sample solution A was applied on pre-washed TLC plate, developed in the above mobile phase, dried in air and photometrically analyzed as described above. From the peak area obtained in the chromatogram, the amount of the drug was calculated.

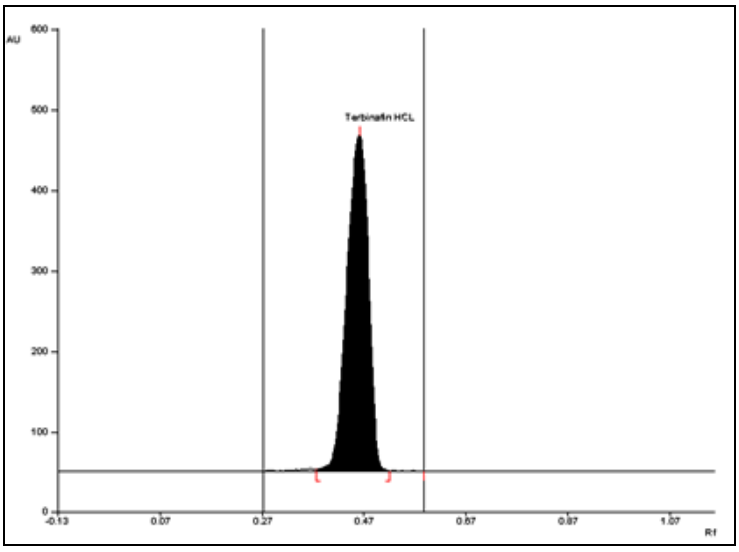

FIG. 6: CHROMATOGRAM OF TABLET SOLUTION OF TERBINAFINE HYDROCHLORIDE (2500 ng/spot)

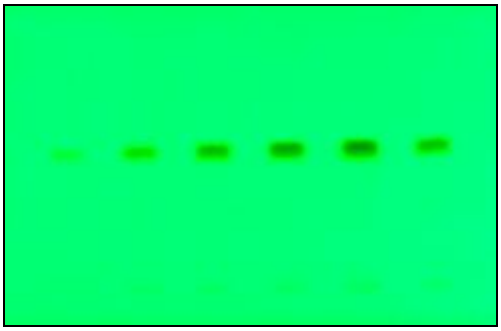

FIG. 7: PHOTOGRAPH OF DEVELOPED HPTLC PLATE FOR ASSAY OF TERBINAFINE HYDROCHLORIDE

TABLE 3: ASSAY RESULTS OF MARKETED FORMULATION.

Formulation	Actual concentration (ng/spot)	%Terbinafine Hydrochloride
Tablet	2500	99.95

Validation of the Method:

Accuracy: Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples. Accuracy was determined by calculating the recovery. The method was found to be accurate with 99.79% - 99.94% recovery of Terbinafine Hydrochloride [8, 9, 10]. The results are shown in **Table 3**.

Precision: Variation of results within the same day (intraday), variation of results between days (interday) was analyzed. Intraday precision was determined by analyzing Terbinafine Hydrochloride for three times in the same day at 282 nm. Inter day precision was determined by analyzing Terbinafine Hydrochloride daily for three days at 282 nm. Precision was calculated as repeatability and intra and inter day variation for the drug. The method was found to be precise with Coefficient of variation (0.55-0.97) for intraday (n=3) and CV (0.33-0.84) for interday (n=3) for Terbinafine Hydrochloride [8, 9, 10]. The results are shown in **Table 4**.

Sensitivity: Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined by kSD/s, where k is a constant (3.3 for LOD and 10 for LOQ), SD is the standard deviation of the analytical signal, and is the slope of the concentration /response graph [8, 9, 10]. The results are shown in **Table 4**.

TABLE 4: SUMMARY OF VALIDATION PARAMETERS OF HPTLC

Parameters	Values
Recovery %	99.79-99.94
Precision(CV)	
Intra-day (n=3)	0.55-0.97
Inter-day (n=3)	0.33-0.84
Limit of Detection (ng/spot)	298.62
Limit of Quantitation (ng/spot)	385.9

RESULTS AND DISCUSSION: TLC plates were prewashed with methanol and activated prior to use. The chromatographic conditions maintained were, Precoated Silica gel 60 F254 (20×10 cm) aluminium sheets as stationary phase. Acetonitrile: 1, 4 dioxan: Hexane: Acetic acid (1:1:8:0.1) (v/v) as mobile phase for Terbinafine Hydrochloride. Samples were applied using Camag Linomate V semiautomatic sample applicator. Developed plates were subjected to densitometeric measurements in absorbance mode at wavelength 282 nm using Camag TLC Scanner 3. A plot of peak area vs. concentration for drug was obtained. A spectrum of drug was recorded in the range of 500-4500 ng and purity of chromatographic peak was checked by scanning individual peak at 3 different positions (peak start, peak apex and peak end). Calibration graph was plotted with peak area vs. concentration. Rf value of Terbinafine Hydrochloride was found to be 0.45. Detection was carried out at 282nm. The proposed method has been validated for assay of Terbinafine Hydrochloride in bulk and tablet dosage forms using following parameters. Linear calibration plots were obtained over the calibration ranges tested, i.e., 500 to 4500 ng/spot. The corresponding linear regression equation was y = 3.5539x + 2410.3

CONCLUSION The developed HPTLC technique is simple, precise, specific and accurate. The result of analysis clearly indicates absence of interference from the excipients in the formulation. The statistical analysis proves that method is reproducible and selective for the analysis of Terbinafine Hydrochloride in bulk and tablet formulation.

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