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A VALIDATED RP-HPLC METHOD FOR DETERMINATION OF TERBINAFINE HYDROCHLORIDE IN PHARMACEUTICAL SOLID DOSAGE FORM

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Abstract

A rapid and sensitive reverse phase HPLC method is depicted for the qualitative and quantitative assay of Terbinafine hydrochloride in pharmaceutical dosage form. Terbinafine hydrochloride was chromatographed on a reverse phase C18 column with a mobile phase consisting of methanol: water in the ratio of 80:20% v/v. The mobile phase was pumped at a flow rate of 1ml/min. The detection is carried out at 282 nm. The retention time of the drug was 5.84 min. The linearity curved showed a correlation coefficient (r^2) of 0.9974 with concentration range of 80-160µg/ml. The method was validated with respect to linearity, precision, accuracy and Sensivity (LOQ and LOD).

Key words: Terbinafine Hydrochloride, Reverse Phase HPLC, Tablets, Validation.

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) .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 HPLC method has been reported for the estimation of Terbinafine

Hydrochloride[3,4,5,6,7,8]. The present investigation has been undertaken to develop simple HPTLC in pure form and its formulations.

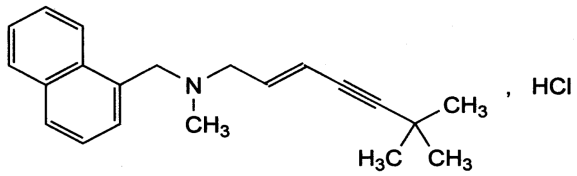

Fig-1: Chemical Structure of Terbinafine hydrochloride

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. Methanol and water were of HPLC grade (E.Merck (India) Ltd., Mumbai). All other reagents in this assay were of analytical grade.

Apparatus

HPLC analysis was performed on Perkin Elmer, U.S.A., Model: series 200, equipped Diode array detector (UV-visible) with gradient pump. The separation was achieved using a RP C18 stationary phase (250mm×4.6mm 5µ particle size). A Sartorius Gottingen AG, Germany**,** Model: BP211D balance was used for weighing standards. All the glass wares were rinse thoroughly with double distilled water and dried in hot air oven.

Chromatographic system and conditions

The composition of the mobile phase is methanol and water in the ratio of 80:20 % v/v. The mobile phase was filtered before use through a 0.45µm membrane filter and degassed for 10 min. The components of the mobile phase were pumped from the solvent reservoir to the column at a flow rate of 1ml/min. The eluents were monitored at 282nm. A model chromatogram was shown in the Fig. 2.

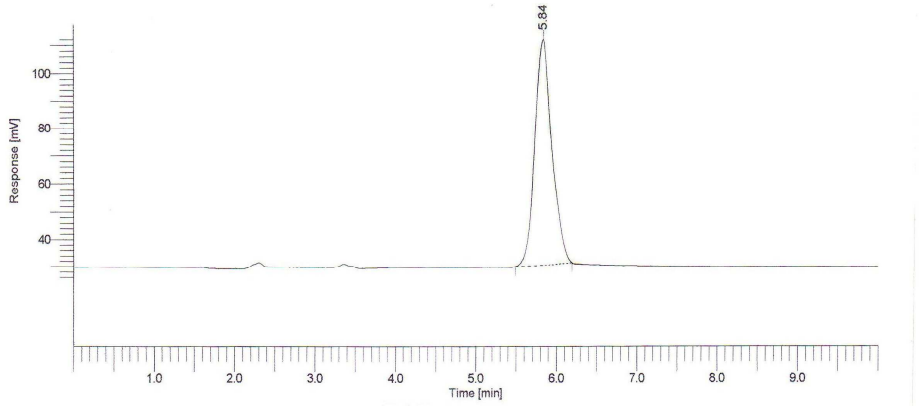

Fig. 2: Chromatogram of standard solution containing 100 µg/ml of Terbinafine Hydrochloride.

Preparation of standard and sample solutions

Terbinafine Hydrochloride standard stock solution: (400µg/ml)

A 10 mg of standard Terbinafine Hydrochloride was weighed and transferred to a 25 ml volumetric flask and dissolved in 15 ml Mobile Phase. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 400µg/ml Terbinafine Hydrochloride.

Calibration curve for the Terbinafine Hydrochloride (80 - 160 µg/ml)

Appropriate volume of aliquots from standard Terbinafine Hydrochloride stock solutions was transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase give a solution containing 80, 100, 120, 140 and 160 µg/ml of Terbinafine Hydrochloride. The mixed standard solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.0 ml/min. The graph is plotted of peak area vs. concentration (Fig.3). Results of calibration are shown in table no.1. Statistical parameters are shown in table no.2. System suitability parameters are shown in table no.3.

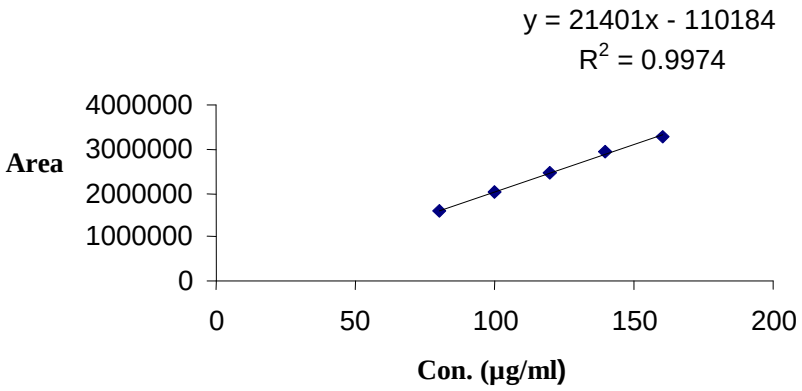

Fig.3: Calibration curve of Terbinafine Hydrochloride.

Table 1: Result of Calibration for Terbinafine Hydrochloride.

Concentrations (µg/ml)	Area at 282 nm. Mean (n=6)
80	1582449
100	2042423
120	2452381
140	2937174
160	3275458

Table 2: Statistical Data for Terbinafine Hydrochloride.

Parameter	Values
Linear Range(µg/ml)	80-160
Slope	21401
Intercept	110184
Standard deviation of slope	625.539
Standard deviation of intercept	77121.62

Table 3: System Suitability Test Parameter.

System Suitability Parameters	Values
Retention times (Rt)	5.84
Theoretical plates(N)	2021.9
Tailing factor (As)	1.4

Sample preparation:

Twenty tablets were weighed; accurately average weight was found and finely powered. A quantity equivalent to 250 mg Terbinafine Hydrochloride was accurately weighed and transferred to volumetric flask of 25 ml capacity. 15 ml of mobile phase was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with mobile phase. The above solution was filtered through whatman filter paper (0.45µ). From this solution 2.5 ml was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark to give a solution containing 100µg/ml. The resulting solution was analyzed by proposed method. Result of assay is shown in table no. 4.

Table 4: Assay Results of Marketed Formulation.

Formulation	Actual concentration(µg/ml)	%Terbinafine Hydrochloride
Tablet	100	100.5

3. 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 99.68%-100.2 % recovery of Terbinafine Hydrochloride[9-12].The results are shown in Table no.5.

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 (0.19-0.85) for intraday (n=3) and CV (0.29-0.66) for interday (n=3) for Terbinafine Hydrochloride[9-12].The results are shown in Table no. 5.

Table 5: Summary of Validation Parameters.

Parameters	Values
Recovery%	99.68-100.2
Precision(CV) Intra-day (n=3) Inter-day (n=3)	 0.19 – 0.85 0.29 – 0.66
Limit of Detection (µg/ml)	0.204
Limit of Quantitation (µg/ml)	0.62

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 s is the slope of the concentration /response graph[9-12].The results are shown in Table no. 5.

Results and Discussion

To achieve precise component peaks with good resolution with mixtures of methanol and water in different combination were tested as mobile phase on a C18 stationary phase. A binary mixture of methanol and water in 80:20%v/v proportions was proved to be the most suitable of all combination since the chromatographic peaks were better defined and resolved and almost tailing with this system. The detection was carried out at 282 nm. The retention time obtained for Terbinafine hydrochloride was 5.84 min. The method was found to be accurate with % recovery 99.68% – 100.2%, precise with coefficient of variation (CV) 0.19-0.85 for intraday (n=3) and CV 0.29-0.66 for interday (n=3), specific as no interference observed when the drugs were estimated in presence of excipients, also rugged as there was no change in area up to 24 hours of preparation of solution in mobile phase for Terbinafine Hydrochloride.

Conclusion

A more accurate, precise and convenient RP-HPLC method was developed and validated in terms of linearity, accuracy, precision and sensitivity. The proposed method is also applicable to the analysis of Terbinafine Hydrochloride in bulk drugs and pharmaceutical formulations.

Acknowledgement

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