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Stability-Indicating High-Performance Thin-Layer Chromatographic Method for Analysis of Terbinafine in Pharmaceutical Formulations

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Summary. A new, simple, selective, precise, robust and stability-indicating high-performance thin-layer chromatographic (HPTLC) method has been established for analysis of terbinafine hydrochloride (TH) in the bulk drug and in pharmaceutical formulations. Separation was achieved on aluminium plates precoated with silica gel $60F_{254}$, with toluene–ethyl acetate–formic acid 4.5:5.5:0.1 (*v*/*v*) as mobile phase. Densitometric analysis was performed at 284 nm. Compact bands of TH were obtained at R_F 0.31 ± 0.02. Linearity (r^2 = 0.9985), limit of quantification (35 ng per band), recovery (97.6–101.6%), and precision (≤2.19) were satisfactory. The method was applicable for routine analysis and accelerated stability testing of TH in pharmaceutical drug-delivery systems. Because the method can effectively separate the drug from its degradation products, it can be used as a stability-indicating method.

Key Words: terbinafine, high-performance thin-layer chromatography, degradation, stability

Introduction

Terbinafine hydrochloride (TH; (*E*)-*N*-(6,6-dimethyl-2-hepten-4-ynyl)-*N*-methyl-1-naphthalenemethanamine hydrochloride; *Fig. 1*) is a potent antifungal agent of the allylamine class that is used for the treatment of mycoses [1].

TH is used orally in the form of tablets for treatment of dermatophyte infections and is also applied to the skin as creams, on occurrence of superficial fungal infections [2]. For quality control and stability testing of TH in pharmaceutical formulations, limited reports have been published, because the drug is not yet official in any pharmacopoeia. UV derivative spectro-

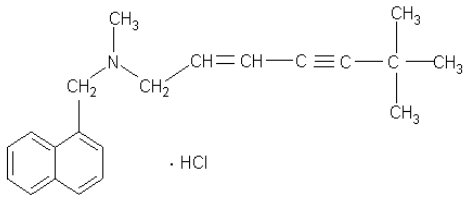

Fig. 1. The chemical structure of TH

metry [3], non-aqueous volumetry [4] and HPLC [5] have been used for assay of TH in raw material and dosage forms. These methods are simple and rapid but their low sensitivity limits their use [3–5]. Microbiological assay, using *Aspergillus flavus* ATCC 15546 as the test organism, described in the literature for quantification of TH, is time consuming and inconvenient for routine analysis [6]. Reversed-phase HPLC with UV detection is the most sensitive method reported for analysis of TH and its degradation product [7]. Chromatographic separation in this method was performed on a Nucleosil 100- 5-CN column using an elaborate mobile phase, however, and has the disadvantage of being time-consuming [7]. All these studies have further emphasized the need to perform rapid and sensitive quality-control analysis of pharmaceutical formulations containing TH. As far as we are aware, however, an HPTLC method for analysis of TH in pharmaceutical formulations has not been reported. Because of their rapidity, selectivity, sensitivity, and overall versatility, development of precise and validated HPTLC methods for quality control of drugs has received much attention [8, 9]. The objective of this study was to develop and validate a rapid, simple, sensitive and reproducible HPTLC method for quantification of TH as the bulk drug and in tablet and cream formulations that would be applicable to stability studies.

Experimental

Chemical, Reagents, and Solutions

TH of assigned purity of 99.6% was received as gift from Jubilant Organosys (Noida, India). TH tablets, claimed to contain 125 mg (as base) of the drug and TH cream 1% (as the hydrochloride) were obtained commercially. Toluene, ethyl acetate and formic acid were purchased from Merck (Mumbai, India). All chemicals and solvents were of analytical grade.

A stock solution of TH (100 mg mL^{-1}) was prepared in methanol. By appropriate dilution, standard solutions were prepared in the concentration range 0.2–1.0 mg mL^{-1}.

Sample Preparation

Tablets

The tablets (label claim 125 mg per tablet) were powdered and powder equivalent to 50.0 mg TH was transferred to a 100 mL volumetric flask with 50 mL methanol. The mixture was sonicated for 20 min then diluted to volume with methanol (concentration 500 μg mL^{-1}). The resulting solution was centrifuged at 1500 rpm for 5 min and filtered, and 1 μL (500 ng per band) of the solution was applied to a plate for analysis.

Creams

A quantity of cream (1%) containing 50.0 mg TH was extracted by warming with 60 mL methanol in a water-bath at 50°C for 10 min with occasional shaking. The mixture was sonicated for 20 min then the volume was diluted to 100 mL with methanol (concentration 500 μg mL^{-1}). The resulting solution was centrifuged at 1500 rpm for 5 min and filtered, and 1 μL (500 ng per band) of the solution was applied to a plate for analysis.

Chromatography

Chromatography was performed, as described previously [8], on 20 cm × 10 cm aluminum HPTLC plates precoated with 200-μm layers of silica gel $60F_{254}$ (E. Merck, Darmstadt, Germany). Samples were applied as bands 6 mm wide and 10 mm apart by means of Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100-μL syringe. The constant application rate was 160 nL s^{-1}. Linear ascending development with toluene–ethyl acetate–formic acid 4.5:5.5:0.1 (v/v) as mobile phase was performed in a 20 cm × 10 cm twin-trough glass chamber (Camag) previously saturated with mobile phase for 15 min at room temperature (25 ± 2°C) and relative humidity 60 ± 5%. The development distance was 8 cm (development time 10 min) and 20 mL mobile phase was used. Densitometric analysis was performed at 284 nm with a Camag TLC scanner III operated by WinCATS software (Version 1.2.0). The slit dimensions were 5 mm × 0.45 mm and the scanning speed of 20 mm s^{-1}.

For calibration, each standard solution (1 μL) was applied to a TLC plate to furnish amounts in the range 200–1000 ng per band. Each amount was applied six times. Peak area and amounts applied were treated by lin-

ear least-squares regression. QC samples were applied at of 500 and 1000 ng per band.

Method Validation

The method was validated by determining linearity, range, precision, accuracy, limits of detection (LOD) and quantification (LOQ), and recovery. To estimate LOD and LOQ, blank methanol was applied six times and the standard deviation (σ) of the analytical response was determined. LOD was expressed as $(3.3 \times \sigma)/(\text{slope of calibration plot})$ and LOQ was expressed as $(10 \times \sigma)/(\text{slope of calibration plot})$. Intra-day precision and accuracy of the assay were evaluated by performing replicate analyses ($n = 6$) of QC samples (500 and 1000 ng per band). Inter-day precision and accuracy were determined by repeating the intra-day assay on three different days. Precision was expressed as the coefficient of variation (CV, %) of measured concentrations for each calibration level whereas accuracy was expressed as percentage recovery [(TH found/TH applied) × 100]. Recovery was studied by applying the method to drug samples to which known amounts of TH, corresponding to 50, 100, and 150% of the TH label claim, had been added. For each amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations. To measure stability, bulk TH, aqueous TH solution, and tablet and cream formulations of TH were stored under conditions likely to accelerate decomposition (40 ± 1°C, 75 ± 5% RH) and the drug content was determined after 0, 3, and 6 months. The possibility of interference by excipients was also studied.

Results

Under the chromatographic conditions described, TH was well resolved at R_F 0.31 ± 0.02.

Method Validation

Linear regression data for calibration plots for TH ($n = 6$) were indicative of a good linear relationship (correlation coefficient, $r^2 = 0.9985 \pm 0.0013$) between peak area and amount in the range 200–1000 ng per band. The coefficient of variation of the slope was <8%, indicative of the high precision of the assay. LOD and LOQ were 10.5 and 35.0 ng per band, respectively, indicating adequate assay sensitivity. Intra-day and inter-day precision, as coefficient of variation (CV, %) and the accuracy of the assay, determined for TH concentrations of 500 and 1000 ng per band, are summarized in *Table I*.

Intra-day precision (n = 6) was ≤1.80% and inter-day precision was ≤2.19%. Intra-day and inter-day accuracy were 99.1–101.8 and 98.6–99.36%, respectively. When the repeatability of the method was studied by assaying six samples of tablets and creams, at same concentration under the same experimental conditions, the coefficient of variation was 0.95 and 1.06% for tablets and creams, respectively. These values are within the acceptable range, so the method was accurate, reliable, and reproducible. When the method was used for analysis of TH after spiking with 50, 100, and 150% additional drug, recovery ranged from 98.9–101.6% (tablets) and 97.6–98.9% (creams) as indicated in *Table II*. The RSD of recovery for tablets and creams were 1.45–1.94 and 1.08–2.12%, respectively. The absence of interference peaks from degradation products, impurities and excipients indicate the specificity of the method. *Table III* shows results from assessment of the stability bulk TH, aqueous TH solution, and tablet and cream formulations stored at 40 ± 1°C and 75 ± 5% RH. After storage of the aqueous TH solution under these conditions for six months, the chromatogram contained four additional peaks at R_F 0.17, 0.23, 0.46, and 0.52. Student's t-test showed there was a significant difference ($P < 0.05$) between the drug content of the solution exposed to accelerated storage conditions compared with the initial drug content (*Table III*). In contrast, a single band at R_F 0.31 was observed in chromatograms obtained from bulk TH and from TH extracted from tablets and creams. The drug content of bulk TH, tablets, and creams varied from 97.8 to 100.5% (*Table III*). It may therefore be inferred that degradation of TH had not occurred in the tablet and cream formulations of the bulk drug, after accelerated storage.

Table I. Precision and accuracy of the method

Nominal concentration (ng per band)	Concentration found[a] (ng per band)	Precision[b] (CV, %)	Accuracy[c] (%)
Intra-day			
500.0	495.5	1.80	99.1
1000.0	1018.0	1.76	101.8
Inter-day			
500.0	493.0	2.19	98.6
1000.0	993.6	1.83	99.36

[a]Mean from six determinations (n = 6)
[b]Precision as coefficient of variation (CV, %) = [(standard deviation)/(concentration found)] × 100
[c]Accuracy = [concentration found)/(nominal concentration)] × 100

Table II. Results from studies of the recovery of TH from samples of known concentration

Formulation	Theoretical (mg)[a]	Added (mg)	Detected[b] (mg)	Recovery[b] (%)	RSD (%)
Tablets					
50%	125	62.5	185.44	98.9	1.94
100%		125	54.0	101.6	1.45
150%		250	377.62	100.7	1.60
Creams					
50%	10	5.0	14.64	97.6	2.12
100%		10	19.78	98.9	1.76
150%		15	24.57	98.3	1.08

[a]Units: mg per tablet, mg per g cream
[b]Mean from six determinations (n = 6)

Table III. Results from studies of the stability of TH under storage conditions (40°C/75% RH) likely to lead to accelerated decomposition

Formulation	Degradation products R_F	Amount (%) of drug recovered[a] after storage for:			
		Initial	1 month	3 months	6 months
Bulk TH	Not detected	100.5	100.3[b]	100.2[b]	100.1[b]
TH solution	0.17, 0.23, 0.46, 0.52	99.9	87.6[c]	73.4[c]	59.8[c]
Tablets	Not detected	99.7	99.6[b]	99.7[b]	99.5[b]
Cream	Not detected	100.3	99.8[b]	99.8[b]	97.8[b]

[a] Mean from six determinations (n = 6) at 1000 ng per band
[b] $P > 0.05$ compared with initial, Student's t-test
[c] $P < 0.05$ compared with initial, Student's t-test

Discussion

There have been no reported HPTLC methods for analysis of TH in dosage forms. The objective of our work was to develop simple, rapid, and sensitive HPTLC method for quantification of TH in raw materials and pharmaceutical formulations. The main criteria for development of successful analytical method for determination of TH are that the method should be free from interference from excipients and simple enough for routine use in

quality control. Initially, the pure drug was applied to TLC plates and chromatographed with different mobile phases. When used alone, toluene and ethyl acetate were able to chromatograph the drug on the TLC plate but the bands were highly diffused. Thereafter, toluene and ethyl acetate in different ratios was tried. When the amounts of toluene and ethyl acetate were approximately equal, tailing was significantly reduced but the typical peak shape was still missing. For a good peak shape for TH, it was necessary to add formic acid to the mobile phase. Addition of a small amount reduced the tailing and improved the peak characteristics. Finally, the optimum mobile phase, toluene–ethyl acetate–formic acid 4.5:5.5:0.1 (*v*/*v*) resulted in a sharp, well-defined symmetrical peak for TH at R_F 0.31 ± 0.02, as shown in *Fig. 2a*.

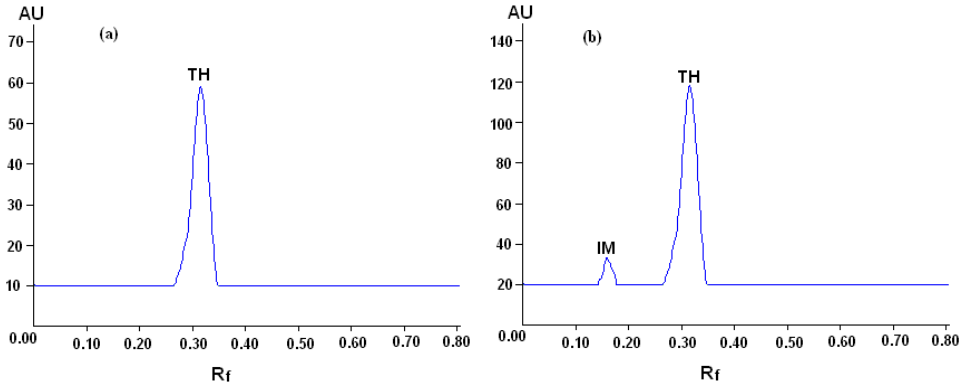

Fig. 2. Chromatograms obtained from reference TH: (a) 1000 ng per band (b) 2000 ng per band. Chromatographic conditions: mobile phase, toluene–ethyl acetate–formic acid 4.5:5.5:0.1 (*v*/*v*); UV detection at 284 nm; TH peak R_F 0.31; impurity (IM) peak R_F 0.17

The UV spectrum of TH shows the compound absorbs intensely at 224 nm, but detection was performed at 284 nm because selectivity was better. Densitometric analysis at 284 nm also improved the detection sensitivity and minimized interference. Chromatograms obtained from bulk TH (*Fig. 3a*) were compared with chromatograms obtained from formulations (tablets, *Fig. 3c*, and creams, *Fig. 3d*) to assess the specificity and selectivity of the procedure. No peaks were observed at or near the R_F of TH, indicating the high selectivity of the HPTLC method. It should be noted that when high concentrations of the drug were applied to TLC plates, a band at R_F 0.17 also appeared (*Fig. 2b*). The area of this band was much less than that of TH. This band could be assumed to be of impurity, perhaps a residue from the manufacturing process.

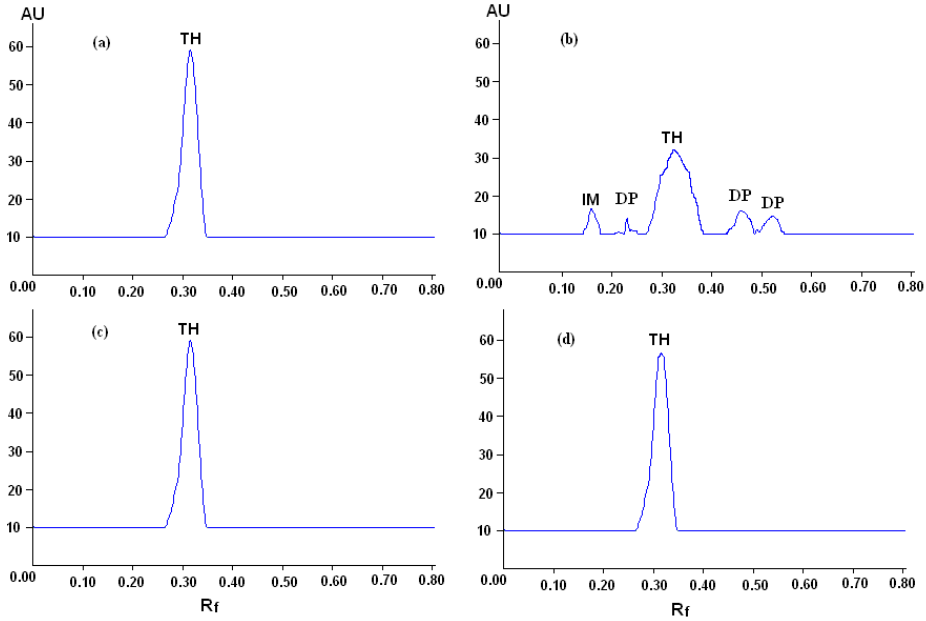

Fig. 3. Chromatograms obtained from samples stored for six months at 40°C/75% RH (1000 ng per band) (a) bulk TH; (b) aqueous TH solution; (c) tablets; and (d) creams. Chromatographic conditions: mobile phase, toluene–ethyl acetate–formic acid 4.5:5.5:0.1 (*v*/*v*); UV detection at 284 nm; TH peak R_F 0.31; impurity (IM) peak R_F, 0.17; degradation product (DP) peaks R_F 0.21, 0.42, 0.50

HPLC has previously been used for assay of TH in pharmaceutical formulations [5]; the reported LOQ was 2.5 μg mL^{-1}. The LOQ (35.0 ng per band) for TH in this HPTLC assay was better. The total analytical run-time using this method was also much shorter than the reported run-time of 32 min in a previous study [7]. This HPTLC assay also has the advantage of simplicity and convenience. The chromatograms (*Fig. 3a, 3c, 3d*) and drug recovery (97.8–100.5%, *Table III*) indicated that degradation of TH had not occurred in the tablet and cream formulations or in the bulk drug, after accelerated storage for six months at 40 ± 1°C and 75 ± 5% RH. The drug content of TH solution stored under these conditions was, however, significantly different ($P < 0.05$) from the initial drug content (*Table III*), indicating hydrolytic degradation of drug. As a result of hydrolytic decomposition of TH, the chromatogram of TH solution (*Fig. 3b*) contained four additional peaks (degradation product peaks) with the TH peak. The method also seems to be stability-indicating, because the degradation product peaks (R_F 0.17, 0.23, 0.46, and 0.52) were well resolved from the drug peak (R_F 0.31) with significantly different R_F values. These results indicated that this

HPTLC method is suitable for routine analysis and accelerated stability testing of TH in pharmaceutical drug-delivery systems.

Conclusion

The HPTLC method developed in this study has the advantage of simplicity, precision, accuracy, and convenience. It is of potential value for analysis of TH as the bulk drug and in commercial formulations. The method also seemed to be stability-indicating.

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