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Estimation of terbinafine HCl in tablet dosage form by green gas chromatography

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ABSTRACT

Terbinafine HCl (TFH) is a synthetic antifungal agent used to treat various types of fungal infections. It is estimated in a tablet dosage form using an eco-friendly, gas chromatographic technique with a flame ionization detector. The drug has shown volatile characteristics at its melting point in the range of 204°C–208°C. The TFH was separated in Zebron DB drug column. The study was carried out based on the temperature program by raising the temperature from 230°C to 240°C with a ramp rate of 5°C/minutes and retained for 2 minutes, followed by a raised column temperature to 250°C with a ramp rate of 3°C/minutes which attained a total run time of 10.33 minutes. The drug was eluted with a retention time of 8.5 minutes. The linearity of the method was recorded in the range of 10–60 µg/ml. The results of LOD and LOQ were found to be 0.88 µg/ml and 2.69 µg/ml for TFH, respectively. The method was validated according to the ICH guidelines and values have been found to be in good accordance with the range prescribed. Further stability studies were carried out to evaluate the TFH in tablet dosage forms in the presence of its degradation materials. This new green gas chromatographic method is an easy, rapid, and environmental-friendly way for quantification of TFH in the pure and pharmaceutical dosage form.

INTRODUCTION

Terbinafine HCl (TFH) is a derivative of organic allylamine (Bhadoriya *et al*., 2019; Nussbaumer *et al*., 1995). It is an antifungal agent that works by inhibiting squalene epoxidase which inhibits ergosterol biosynthesis, which leads to the death of fungal cells (Kanakapura and Penmatsa, 2016; Moraes *et al*., 2017). Chemically, it is [(2E)-6-6-dimethyl hept 2-en-4-yn-1-yl] (methyl) (naphthalene-1-yl methyl) amine HCl (Fig. 1) with a MW of 327.9 g/ml (Mrutyunjayarao *et al*., 2012). TFH is freely soluble in methylene chloride and methanol, whereas it is soluble in ethanol and moderately soluble in water. It has a 204°C–208°C melting point. TFH tablets are used to treat fungal infections that damage fingernails and toenails, medically referred to as onychomycosis or Tinea unguium, which is likely caused by *Trichophyton*, *Microsporum canis*, and *Epidermophyton floccosum*. It works by stopping the growth of fungus (Matysova *et al*., 2016; Talaviya and Smita, 2016). Nail fungal infection often triggers an infectious or poor hygiene-related disease. In fact, in Western countries up to 10% of all adults have fungal nail infections. This figure rises to 20% of people 60 years of age or older. Much more common is toenail fungus than fingernail fungus. The FDA-labeled Terbinafine dosage is 250 mg per day which is to be continuously given to treat toenail infections for 12 weeks and for 6 weeks to treat fingernail infections (Amichai

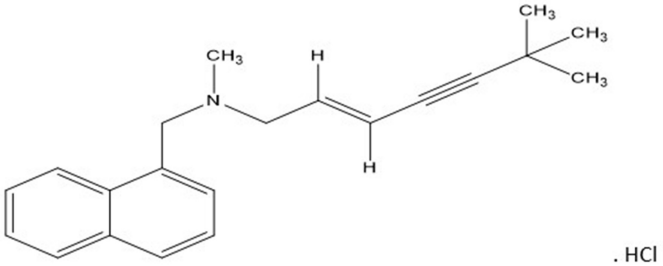

Figure 1. Terbinafine hydrochloride.

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et al., 2010; Arunprasad *et al*., 2010). Studies have shown that toenail treatment results in a mycological cure rate of 71%–82% and a surgical cure rate of 60-70% (Darkes *et al*., 2003).

The literature survey disclosed some of the HPLC methods for the estimation of TFH in which the C18 column, acetonitrile, and methanol (60:40) mobile phase with PDA detector set at 224 nm was used (Kassem and Almardini, 2013). In another Rp-C18, methanol–water (95:5) mobile phase with UV detection at 254 nm was reported (Tagliari *et al*., 2010). ODS column using a mixture of phosphate buffer and acetonitrile (60:40) with UV detection at 283 nm was described (Rani *et al*., 2006). Ion pair reverse-phase liquid phase chromatography was also carried out using sodium-1-heptanesulphonate as the ion-pairing reagent (Florea *et al*., 2009). However, most of the HPLC methods reported involve organic solvents, C18 column with UV detector, but HPLC with other detectors like fluorescence, MS, and electrochemistry are not yet reported (Kanakapura and Penmatsa, 2016). Titrimetric method had been reported in which TFH was estimated using anhydrous acetic acid titrated against perchloric acid with crystal violet indicator (Cardoso and Schapoval, 1999; BP 2012; EP 2011; USP 2012). Several other techniques like UV spectrophotometric method were reported for the assay of TFH in raw materials, tablets, and creams. Using visible spectrophotometry, TFH was found to be measured at 422 nm after forming an ion pair complex with methyl orange (1:1) by using pH 2.6 buffer (Elazazy *et al*., 2008); spectrofluorimetry has been reported based on native fluorescence of TFH in the water at 376 nm (Belal *et al*., 2013); an electrochemical method, such as capillary electrophoresis method, has also been reported for the determination of TFH in pharmaceutical preparation resulted with an RE of 0.64% for commercial tablets (Mikus *et al*., 2005). TFH was determined by the bioanalytical method using GC in cat hair, but all the parameters were not validated according to USFDA guidelines and degradation studies were also not carried out (Kuzner *et al*., 2001). Thus, gas chromatography has an advantage over the reported analytical techniques as minimal quantity of organic solvent and nature friendly gas as mobile phase are being used.

Gas chromatography is a unique and versatile technique which is used to separate and analyze compounds in analytical chemistry that can be vaporized without decomposition. Helium or nitrogen is commonly used as a mobile phase in GC. The typical use of GC involves measuring a particular substance's purity and separating the various components of a mixture using green solvents and easy operation maintenance. Currently, there is no analytical method using gas chromatography reported till now for the estimation of TFH in pharmaceutical formulations. The drug had become volatile at its melting point and was detected using flame ionization detector (FID) and hence does not require any derivatizing reagent for analysis of TFH. This research work is directed toward the development of the GC method for the estimation of TFH in tablet dosage form, followed by degradation studies according to the ICH guidelines.

MATERIALS AND METHODS/EXPERIMENTAL

Materials and reagents

Terbinafine pure drug (API) was procured from RL Fines Chemicals, Bengaluru, India. HPLC analytical grade methanol was procured from specialties private limited, Mumbai, India. The formulation (TFH tablet) was purchased from a local pharmacy shop.

GC (analytical) instrumentation and chromatographic conditions

GC Shimadzu 2014 prototype with FID-operated GC solution software was used for the method development. A 10 µl syringe specimen applicator (Hamilton CO., Reno Nevada, USA) has been used. The product column of Zebron DB (length: 30 m, diameter: 0.25 mm, and film: 0.50 µm) with a −40°C–320°C (Max. 340°C) temperature range was used. The chromatographic conditions used to develop the method are shown in Table 1.

Preparation of TFH standard solutions

The stock solution of 100 µg/ml TFH was prepared using methanol as a diluent. In 100 ml volumetric flask, 10 mg of Terbinafine was weighed, transferred, and made up the volume using methanol to get 100 µg/ml concentration. From the prepared stock solution, 1, 2, 3, 4, 5, and 6 ml was pipetted out to six different 10 ml volumetric flasks and the volume was made up using methanol to get 10, 20, 30, 40, 50, and 60 µg/ml and were sonicated for 5 minutes to remove the entrapped air.

Preparation of sample solution

Ten tablets of TFH were weighed and powdered. The powdered formulation equivalent to 100 mg of Terbinafine was taken and transferred to a 100 ml volumetric flask and made up to the mark using methanol as a diluent (1,000 µg/ml). A stock solution was prepared by taking 10 ml which was pipetted into a 100 ml volumetric flask and made up with methanol to get 100 µg/ml concentration.

Force degradation studies

Force degradation studies was carried out by treating the drug samples of same concentrations (40 µg/ml) to various stress conditions like acidic (0.1 M hydrochloric acid for 30 minutes at 60°C and later neutralized with the same amount of base), basic (0.1 M sodium hydroxide for 30 minutes at 60° and neutralized using the same amount of acid), oxidation (treating the sample

Table 1. Chromatographic conditions.

Parameter	Values
Column temperature	230°C (ZB-Drug-1 column)
Detector temperature	250°C
Sampling time	1 minute
Stop time	10.33 minute
Flow control mode	Linear velocity
Pressure	125.3 kPa
Total flow	12.7 ml/minutes
Linear velocity	29.8 cm/seconds
Purge flow	3.0 ml/minutes
Split ratio	1:10
Carrier gas	Nitrogen
Detector	Flame ionization detector (FID)
Injection volume	1 µl

solution with 3% 2 ml of H_2O_2) and photolysis (keeping the sample in a UV chamber for 1 hours).

RESULTS AND DISCUSSION

Method development

This study aimed to develop a simple, robust, and derivatization-free analytical technique to evaluate the TFH in tablet dosage forms in the presence of its degradation materials that is cost-effective. The developed GC-FID method could support the proposed results. The quality of the method was proved by satisfactory validation results within a good manufacturing environment (GMP) as per the ICH guidelines and the method was in accordance with the pharmacopoeial requirements. Various method development parameters were optimized during the initial screening of the technique.

Sample solvent selection

The sample solvent selection was based on the solubility of the drug in the chosen diluent. Initial trials were conducted in ethanol, but the drug was not effectively soluble in ethanol. Furthermore, the polarity of the diluent was reduced by using methanol as the diluent. Good peak shape was obtained using methanol for the sample preparation.

Selection of sample concentration

Experimental trials were conducted using 1 µg/ml. This injection showed a very low peak intensity. The sample concentration of 10 µg/ml could demonstrate effective results, thus being the initial concentration for the linearity curve. The sample with a concentration of 100 µg/ml showed good response, whereas 200 µg/ml resulted in carryover.

Column selection

The polarity of the column affects the peak shape and resolution. So, for effective separation, the column polarity should match the polarity of the chosen drug selected for the analysis. The different trials were conducted using nonpolar columns, i.e., the polydimethylsiloxane (OV-1), ZB drug-1 column and Zebron DB column. In the effective separation, without tailing in the chromatogram, the drug column did not provide a clear separation of TFH, whereas Zebron DB column was found to serve the purpose.

Temperature programming

TFH has a melting point of 204°C–208°C and the drug produced a peak without initial derivatization. The preliminary study was carried out by raising the temperature from 230°C to 240°C with a ramp rate of 5°C/minutes and retained for 2 minutes. Then, subsequently, the column temperature was raised to 250°C with a ramp rate of 3°C/minutes and retained the same temperature for 3 minute. The drug was eluted at 8.5 minutes and attained a total run time of 10.33 minutes.

Method validation

System suitability

After the method's conditions were established as described, the method was validated as per the ICH guidelines for accuracy, precision, linearity, limit of detection, and limit of quantitation. The precision of the method was established by injecting 10 µg, 30 µg, and 60 µg concentrations six times intraday and inter-day. Accuracy, in terms of percentage recovery was established by spiking the formulation at 50%, 100%, and 150% (n = 3). Linearity was established by injecting a series of dilutions in increasing concentrations (n = 5). LOD and LOQ were established using the standard deviation and slope.

Based on the comparison of retention time of standard with formulation, the selectivity of the method has been evaluated, and quantitative analysis of TFH was carried out under the established conditions. The results of the system's suitability are shown in Table 2.

Linearity

Linearity is the ability to view "reports directly proportional to the sample analyte concentration." For the analysis of the analyte's concentration within a given range, linearity is significant for confirming the sensitivity of the system. Linearity was established by preparing a series of dilutions ranging from 10 to 60 µg/ml and each dilution was injected into the GC. The mean peak areas of each dilution were recorded and the calibration curve was constructed by plotting concentration on the x-axis and peak area on y-axis (Fig. 2). The linear regression equation for TFH is as follows: y = 407.27x – 2,671.5 (R^2 = 0.9805). The results of the linearity curve are shown in Table 3. Figures 3 and 4 show the blank and TFH overlay chromatograms, respectively.

Precision

The precision of a measurement system, related to reproducibility and repeatability, is the degree to which repeated measurements under unchanged conditions show the same results. Precision was established by injecting 10, 30 and 60 µg

Table 2. GC validation report for determination of TFH.

Parameter	Value
Linearity	10–60 µg/ ml
Limit of detection (LOD)	0.88 µg/ ml
Limit of quantification (LOQ)	2.69 µg/ ml
Recovery (%)	100.45%
Regression coefficient	0.995
Retention time	8.5 minutes

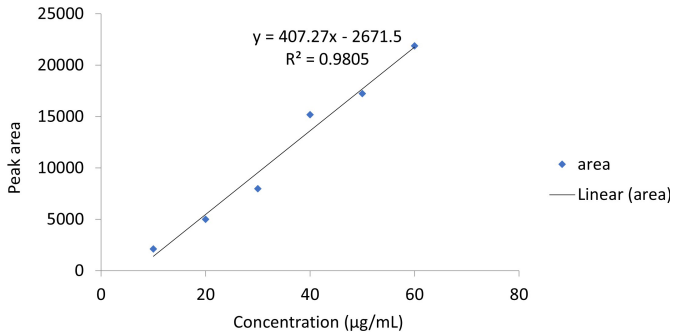

Figure 2. Linearity curve for terbinafine HCl.

concentrations six times into the GC on the intra-day and inter-day and %RSD was calculated. The results of the precision are shown in Tables 4 and 5, which are found to be highly precise and within the limits according to the ICH guidelines.

Accuracy

Accuracy indicates the closeness of the measured value to the true value. Accuracy was determined in terms of percentage recovery, which involves spiking of a known portion of stock solution to test samples and determining percentage recovery at three different concentration levels. The recovery study was carried out by preparing the standard drug solution and formulation solution at three different levels of concentrations, i.e., 50%, 100%, and 150% and then percentage recovery was calculated and found to be an accurate method (Table 6).

LOD and LOQ

LOD is the lowest concentration of an analyte that can be reliably detected but not generally quantified. The signal to noise ratio should be at least 3:1 for the limit of detection.

Table 3. Linearity of TFH.

Concentration (µg/ml)	Peak area
10	2,189
20	5,914
30	9,876
40	15,221
50	18,042
60	22,145

LOQ is the lowest quantity of an analyte that can be quantitatively determined under the specified experimental conditions, with defined precision. The signal to noise ratio should be at least 10:1 for the limit of quantitation.

The equation for LOD and LOQ used is as follows: LOD = 3.3 µ/s and LOQ = 10 σ /s, where σ is the standard deviation and s is a slope. The results of the LOD and LOQ were found to be 0.88 µg/ml and 2.69 µg/ml for TFH, respectively.

Robustness

Robustness is the measure of an analytical process wherein the results obtained are found to be reliable or within the method's specified tolerance limits, even after making deliberate changes. Robustness was carried out by slightly changing the parameters like split ratio, column flow, split ratio, and column temperature. The statistical data given in Table 7 show no

Table 4. Intra-day precision of TFH.

S. No	10 µg	30 µg	60 µg
1	2,189	8,023	22,145
2	2,119	8,327	22,012
3	2,126	8,215	22,189
4	2,085	8,022	22,031
5	2,112	8,219	22,078
6	2,118	8,132	21,344
Average	2,124.83	8,156.33	21,966.5
Standard deviation	31.492	110.235	285.054
% RSD	1.482	1.351	1.297

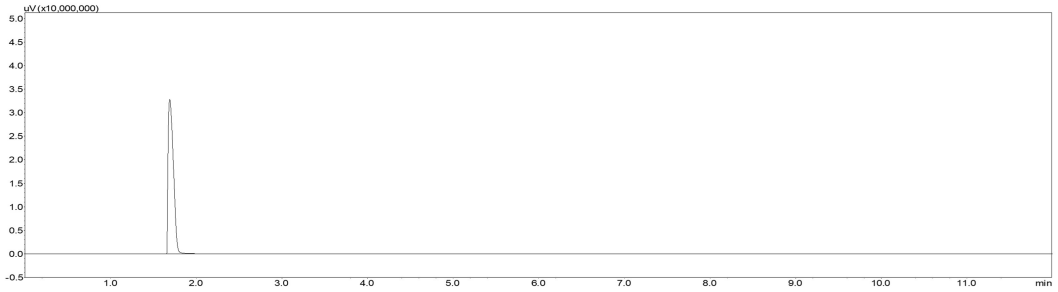

Figure 3. Chromatograms for blank (Methanol).

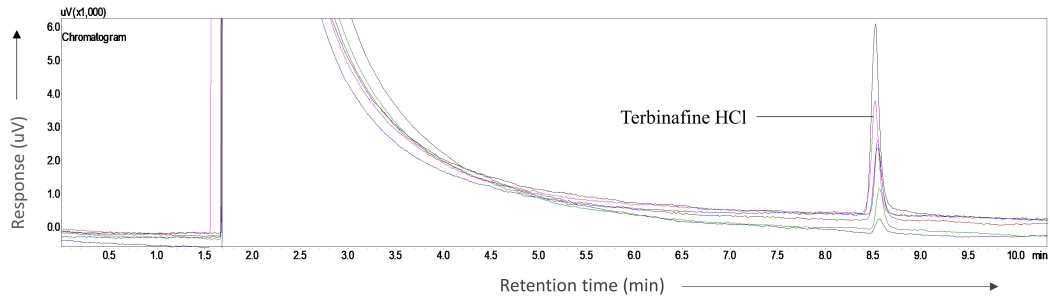

Figure 4. Overlay chromatogram for terbinafine HCl.

Table 5. Inter-day precision of TFH.

S. No	10 µg	30 µg	60 µg
1	2,212	8,317	22,192
2	2,198	8,316	22,014
3	2,237	8,311	22,865
4	2,215	8,012	22,167
5	2,196	8,379	22,563
6	2,123	8,367	22,765
Average	2,196.83	8,283.66	22,427.66
Standard deviation	35.653	124.324	320.929
% RSD	1.622	1.5	1.43

Table 6. Accuracy of TFH.

Level of recovery	Amount of formulation (µg/ml)	Amount of pure drug (µg/ml)	Total amount of drug (µg/ml)	Peak area	Diff.	% recovery	Mean
50%	30	10	40	10,029	7,840	97.71	
	30	10	40	10,178	7,989	99.57	99.57%
	30	10	40	10,287	8,098	100.93	
100%	30	20	50	13,837	7,923	98.75	
	30	20	50	13,909	7,995	99.65	99.65%
	30	20	50	14,134	8,220	102.45	
150%	30	30	60	16,026	8,003	99.75	
	30	30	60	16,114	8,091	100.84	100.84%
	30	30	60	16,241	8,218	102.43	

Table 7. Robustness result for TFH.

Condition		Tailing	% RSD	Theoretical plates	% RSD
Split ratio	10:01	1.384	1.378	202,172.8	1.311
	9:09	1.335		129,233.1	
	10:02	1.318		169,411.2	
Column Flow	0.9 ml/minutes	1.38	1.608	145,763.2	1.5896
	1.0 ml/minutes	1.36		168,522.1	
	1.1 ml/minutes	1.34		154,654.2	
Column Temperature	199°C	1.32	1.4612	153,246.3	1.4801
	200°C	1.333		178,654.2	
	210°C	1.37		156,489.6	

Table 8. Recovery studies results for terbinafine hydrochloride after the stress conditions (% recovery of drug).

Degradation condition	Concentration and volume of a reagent	Stress condition	Percentage of degradation
Acid	0.1 M, HCl (1 ml)	Reflex for 1 hour	25%
Base	0.1 M, NaOH (1 ml)	Reflex for 1 hour	35%
Oxidation	3% H_2O_2 (2 ml)	Reflex for 1 hour	65%
Photolysis	Purified water (1 ml)	Under UV light at 254 and 366 nm	22%

significant changes in the given parameters and thus represents that the method is robust. The results of robustness are shown in Table 7.

Forced degradation studies

Forced degradation studies were conducted using the GC method. The procedure and results obtained are described briefly in Table 8. The results showed that TFH has undergone more degradation, i.e., 65%, on oxidation with 2 ml of 3% hydrogen peroxide, as it showed a decline in the peak area in comparison to the standard drug peak area. However, 25% degradation occurred when reflexed with 1 ml of 0.1 M HCl for 1 hour , while 35% was degraded after reflexation with 1 ml of 0.1 M NaOH, whereas TFH exposure to UV light for 6 hours underwent lesser degradation

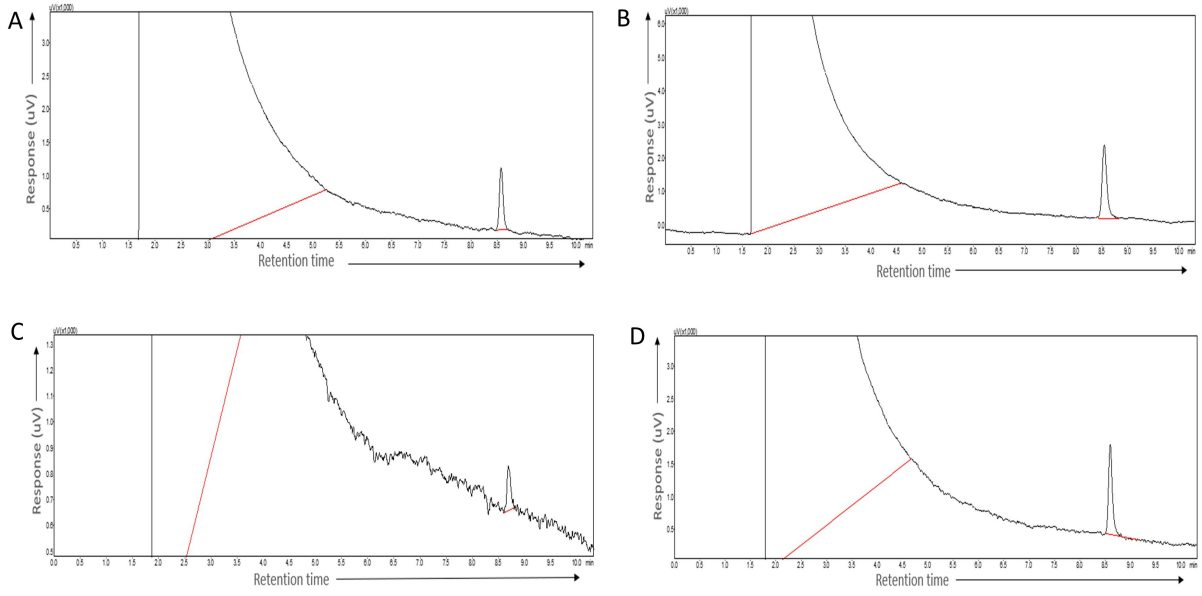

Figure 5. Force degradation chromatograms of terbinafine HCl (A) Acid stress; (B) Basic stress; (c) Peroxide stress; (D) Photolytic stress.

(22%) compared to the other three stress conditions. Moreover, no additional peaks were found in any of the four stress conditions (Fig. 5).

CONCLUSION

For estimating TFH in tablet dosage form validated in compliance with ICH guidelines, the proposed green GC analytical method has been established. This method follows all validation requirements such as reliability, accuracy, device suitability and precision, linearity, and robustness. The method simultaneously meets the study of forced degradation requirements. Therefore, the established method can be used in drug quality control laboratories for regular consistency assessment.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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