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Research Article

STABILITY-INDICATING RP-HPLC METHOD FOR ANALYSIS OF TERBINAFINE HYDROCHLORIDE IN BULK AND IN TABLET DOSAGE FORM

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ABSTRACT

Terbinafine Hydrochloride (TH) is a new potent antifungal agent. Several HPTLC, non-aqueous voltametric, spectrometric methods, ion-pair RP chromatography and Stability-indicating HPTLC methods have been published till now. The aim of the present study is to develop and validate simple, precise, specific and sensitive stability indicating reversed-phase HPLC (RP-HPLC) method for analysis of Terbinafine Hydrochloride in bulk and in tablet dosage form. Terbinafine hydrochloride was analysed on a Neosphere C18 (250 x 4.6 mm, 5µm) with a mobile phase comprising of methanol: 0.5% Triethanolamine. 0.5% Triethanolamine was added to pure methanol to reduce tailing problem. Wavelength of detection was 250 nm. Linear regression study revealed a good linear relationship (R2 = 0.997) between peak area and concentration in the range of 2-12 µg/mL. The method was validated for precision, accuracy, specificity and sensitivity (LOD and LOQ). TH was subjected to acidic, basic and neutral (with water) hydrolysis, oxidative, thermal and photodegradation and it was found that drug was degraded in acidic and photolytic condition. Statistical analysis proved that the developed method was accurate, precise, reproducible, specific and sensitive and can be used for routine analysis.

Keywords: Forced degradation, Methanol, RP-HPLC, Stability-indicating, Terbinafine Hydrochloride, Validation.

INTRODUCTION

Terbinafine Hydrochloride (TH) is a new potent antifungal agent. It belongs to an allyl amine class and has broad-spectrum activity against yeasts, dimorphic fungi, molds, and dermatophytes. 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. It is highly lipophilic base and it is used both orally and as a topical application for cutaneous mycoses, depending on the severity and specific nature of the mycoses. Molecular structure of TH is shown in fig-1. Chemically TH is 1-naphthalenemethanamine, n-(6, 6-dimethyl-2-hepten-4-ynyl)-n methyl-, (E)-, hydrochloride, having molecular formula $C_{21}H_{25}N.HCl$ and molecular weight 293. TH is very slightly or slightly soluble in water, freely soluble in anhydrous ethanol, methanol and in methylene chloride, slightly soluble in acetone [1-3].

The stability-indicating assay is a validated quantitative method that can detect the changes with respect to time by analyzing property of drug substances and drug product and helps in analysis of stability of samples in pharmaceutical industry [4-5]. The purpose of a stability-indicating assay method is to accurately quantitate the intact drug or drugs in the presence of decomposition products and other components/excipients as stated by ICH. It is best that all components in the formulation be present to confirm there is not any peak overlap between the excipients, degradants and the active drug [6,7-9, 30].

Literature survey shows several HPTLC [16-18], non-aqueous voltametric [10-11], spectrometric methods [12-15] and ion-pair RP chromatography [28] have been used for assay of TH in raw material and dosage forms and only stability-indicating HPTLC [15-16] method is reported till now. These methods are simple and rapid but due to their low sensitivity, their use is limited. Reported spectrophotometric [12] and chromatographic [19, 29] methods estimates TH in presence of its degradant or metabolites. Also TH has been determined in biological fluids (plasma, urine) tissues, nails and cat hair by HPLC [17-22] and in tablets and creams by HPLC [26-29]. The present investigation has been undertaken to develop stability-indicating RP-HPLC method to determine TH in bulk and tablet dosage form using methanol: 0.5% Triethanolamine where 0.5% Triethanolamine was added to pure methanol to reduce tailing problem solvent.

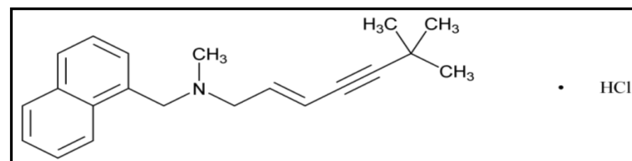

Fig. 1: Chemical Structure of Terbinafine hydrochloride

MATERIALS AND METHOD

TH pure drug was obtained as a gift sample from Cipla Ltd. Maharashtra India. Fintrix film coated tablets (250 mg) were purchased from local medical shop. Methanol was of HPLC grade (Merck, Germany). Reagents and chemicals used for this assay were of analytical grade.

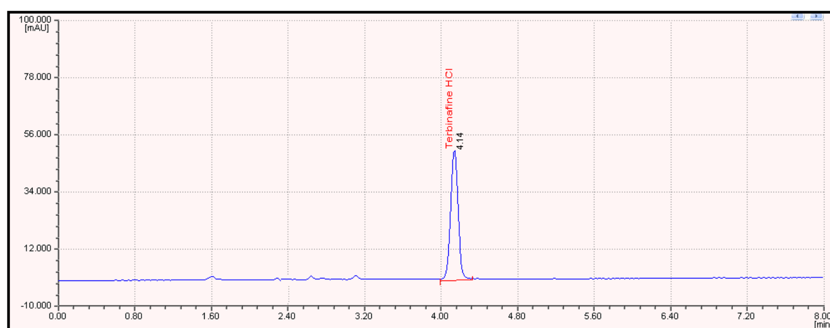

Fig. 2: Chromatogram of standard solution containing 20µg/ml of TH

Apparatus

HPLC analysis was performed on Cyberlab™, Model: LC-100 B binary gradient HPLC system, equipped with UV-100, UV-VIS detector. The separation was achieved using a Neosphere C18 (250 x 4.6 mm, 5µm, Hexon laboratories Pvt. Ltd) column and injector was Rheodyne. A Shimadzu Corporation, U.S.A. and Model: AUX-220 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: 0.5% Triethanolamine (v/v). The mobile phase was filtered through a 0.45µm membrane filter before use 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 1.2 ml/min with a run time of 8min. The eluents were monitored with UV detector at a wavelength of 250nm. A model chromatogram was shown in the Fig. 2.

Preparation of standard and sample solutions

5 mg of TH was weighed and transferred to 25 ml volumetric flask. Then it was dissolved in small amount of methanol and then volume was adjusted to 25 ml to make final concentration of 200µg/ml.

For analysis of tablet formulation, 14 tablets were weighed and powdered. The amount of powdered drug equivalent to 5 mg of TH was weighed accurately and transferred into a suitable flask. The tablet powder was dissolved in small amount of methanol and sonicated for 15 min. The flask was shaken and volume was made up to the mark with methanol to give 200µg/ml. The resultant solution was then filtered through a Whatman filter paper (0.45µ). From this filtrate 0.5 ml of solution was transfer to 10 ml capacity volumetric flask. The volume was made up to the mark with methanol to give a solution of 10µg/ml. The peak areas were measured and the drug content of the sample was calculated using a linear regression equation obtained from calibration curve.

Procedure for forced degradation study of standard TH

TH was subjected to acidic, basic and neutral (with water) hydrolysis, oxidative, thermal and photolytic degradation. Thermal and photodegradation of drug was carried out in solid state. For degradation studies 1000 µg/ml of pure TH was prepared with methanol and exposed to degradation in different conditions. After degradation stock solution were prepared by dissolving in methanol.

Degradation procedure for neutral hydrolysis

5 ml aliquot from stock solution was taken in round bottom flask; 5 ml of distilled water was added to it and this solution was refluxed at 85ºC for 2hr. Then for analysis 20µg/ml solution was prepared.

Degradation procedure for acidic hydrolysis

5 ml aliquot from stock solution was taken in round bottom flask and 5 ml of 1N HCl was added to it and this solution was refluxed at 85ºC for 2hr. Then the solution was allowed to cool and it was neutralized by 1N NaOH. For analysis 20µg/ml solution was prepared. Similar procedure is followed using 0.1N HCl.

Degradation procedure for basic hydrolysis

Similar procedure as acidic hydrolysis was followed with 1N NaOH and 0.1 N NaOH and the solution was neutralised by 1N HCl.

Degradation procedure for oxidative degradation

Similar procedure as neutral hydrolysis was followed with 6% H_2O_2 and 3% H_2O_2.

Degradation procedure for thermal degradation

TH (approximate amount) was kept in crucible and exposed to dry heat at 85° C for 2 hrs. 20µg/ml of solution was prepared for analysis.

Degradation procedure for photodegradation

TH (approximate amount) was exposed to U.V light (in U.V chamber) at wavelengths 254nm and 365nm for 2 hrs. 10µg/ml of solution was prepared for analysis.

Analytical method validation

Proposed method was validated for different parameters like linearity, precision, accuracy, specificity, sensitivity and system suitability. Linearity was checked by calculating co-efficient of regression. For accuracy of method, recovery studies were carried out by applying a known amount of standard TH at a level of 80%, 100% and 120% to the sample solution (standard addition method). Precision of the proposed method was determined by estimating the corresponding responses three times on the same day (intraday) and on three different days (interday) over a period of one week and results are reported in terms of percentage relative standard deviation and repeatability of sample was assessed using six replicates of the same concentrations. Sensitivity is determined in terms of detection limit and quantitation limit (LOD and LOQ).

RESULTS AND DISCUSSION

Method validation

Proposed method was found to be simple, rapid, precise, accurate and sensitive as indicated by results shown in table 1-5. It was found that AUC was linearly correlated with concentration in the range 2-12 µg/mL with R^2=0.997 indicating good linearity (Fig 2). From results it is observed that method was accurate with % RSD of less than 2% (Table 2). The proposed method was found to be precise as indicated by percent RSD not more than 2% showing good repeatability of the method (Table no 3 and 4). LOD and LOQ were found to be 0.22 µg/ml and 0.66 µg/ml respectively. The specificity of the method was checked for the interference of impurities and excipients in the analysis of drug solution under optimized chromatographic condition. The proposed method was found to be specific as the retention time of sample was similar (RT 4.13) to that of standard drug as shown in fig 4 and no interference was observed during analysis between drugs and excipients in tablet [30-31].

Table 1: Result of Calibration curve of Terbinafine Hydrochloride

Conc. (µg/ml)	AUC (n=3)
2	3092.5
4	5479.4
6	7966.2
8	10325.8
10	12940.9
12	16041.7

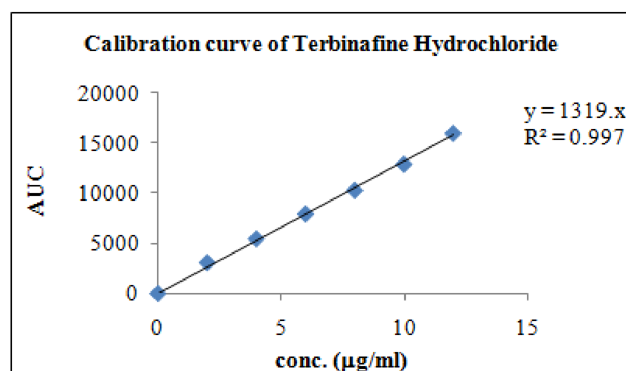

Fig. 3: Calibration curve of Terbinafine Hydrochloride

Table 2: Accuracy result of Terbinafine Hydrochloride

Amount of sample (µg/ml)	Amount of drug added(µg/ml)	Amount of drug recovered (µg/ml)	% Recovery	% RSD
10	8 (80%)	17.8	98.88	0.3495
10	10 (100%)	20.12	100.6	0.8699
10	12 (120%)	21.7	98.63	0.5285

Table 3: Precision result of Terbinafine Hydrochloride

Conc. (µg/ml)	Intraday precision			Interday precision		
	AUC*	% of TH*	%RSD	AUC*	% of TH*	%RSD
10	13108.96	99.38	1.163	13274.57	100.6	0.949

*Average of three determination

Table 4: Repeatability result of Terbinafine Hydrochloride

Conc. (µg/ml)	AUC*	SD	RSD	%RSD
10	13080.93	123.8528	0.009468	0.94682
12	16016.13	22.17123	0.001384	0.138431

*Average of six determination

Table 4: System suitability parameters

Parameters	Stastical data
Theoretical plate	3772-4580
Tailing factor	0.9-1.4

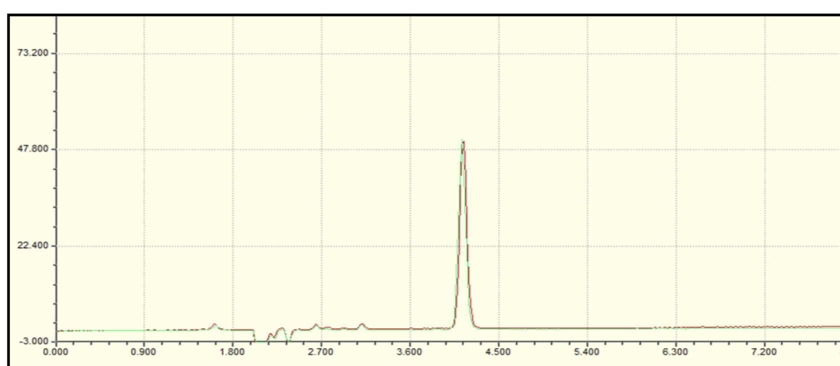

Fig. 4: Overlay spectra of standard and sample TH showing same RT

Forced degradation studies

TH was subjected to acidic, basic and neutral (with water) hydrolysis, oxidative, thermal and photodegradation. Results are shown in Table 6 and chromatograms of degraded sample are shown in Fig 5 and 6. [32]

Table 6: Results of Forced degradation studies

Degradation condition	% degradation
Hydrolytic degradation (neutral)	No degradation
Acidic hydrolysis	17% degradation with 1 N HCl
Basic hydrolysis	No degradation
Oxidative degradation	No degradation
Thermal degradation	No degradation
Photodegradation	5 to 7 % degradation when exposed to both wavelengths.

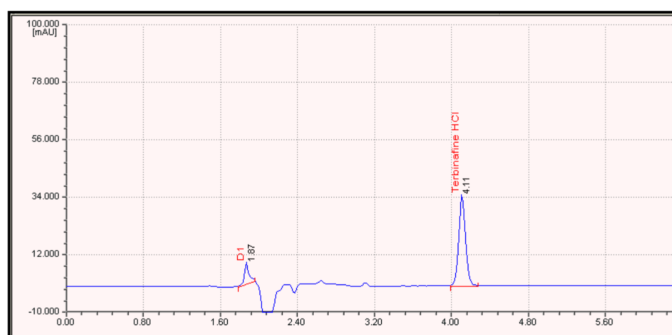

Fig. 5: Chromatogram of TH subjected to acidic hydrolysis in 1N HCl

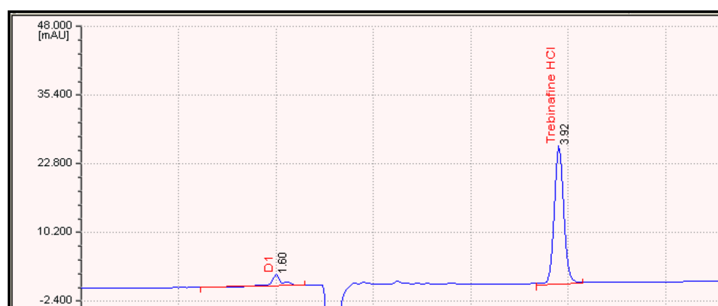

Fig. 6: Chromatogram of TH subjected to photolysis at 365 nm

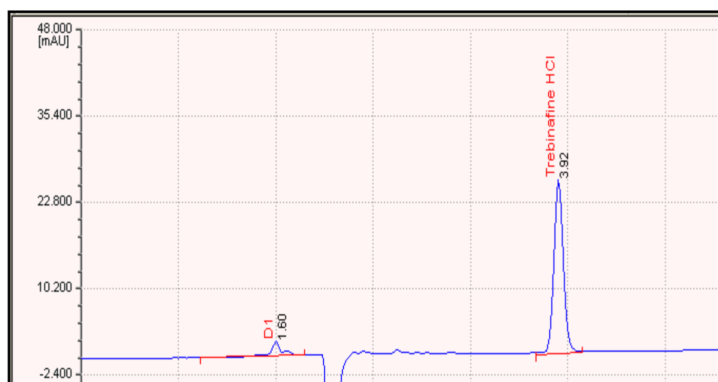

Fig. 7: Chromatogram of TH subjected to photolysis at 254 nm

CONCLUSION

The validated HPLC method employed proved to be simple, specific, accurate, precise, and stability indicating. The developed method was 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 without 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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