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

PUSHPA D GOSWAMI*  
Department of Quality Assurance, P. E. S’s Modern College of Pharmacy (for ladies), Moshi, Pune, Maharashtra, India.  
Email: pushpa.dsg21@gmail.com  
Received: 07 Jun 2013, Revised and Accepted: 29 Jun 2013

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 ergosterol 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 C21H25N.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 quantify 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.

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. 1: Chemical Structure of Terbinafine hydrochloride](image1)

![Fig. 2: Chromatogram of standard solution containing 20μg/ml of TH](image2)
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 NovaSphere C18 (250 x 4.6 mm, 5 μm) 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 8 min. 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 into 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 200 μg/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 200 μg/ml. The resultant solution was then filtered through a Whatman filter paper (0.45 μm). From this filtrate 0.5 ml of solution was transfer to 10 ml capacity volumetric flask and this solution was adjusted to 25 ml with methanol to give 200 μg/ml solution. Then for analysis 20 μg/ml solution was prepared.

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 85°C for 2 hr. Then for analysis 20 μg/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 1 N HCl was added to it and this solution was refluxed at 85°C for 2 hr. Then the solution was allowed to cool and it was neutralized by 1 N NaOH. For analysis 20 μg/ml solution was prepared. Similar procedure is followed using 0.1 N HCl.

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

Degradation procedure for oxidative degradation
Similar procedure as neutral hydrolysis was followed with 6% H2O2 and 3% H2O2.

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

Degradation procedure for photodegradation
TH (approximate amount) was exposed to UV light (in U.V chamber) at wavelengths 254nm and 365nm for 2 hrs. 10 μg/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 R²=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

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>AUC (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3092.5</td>
</tr>
<tr>
<td>4</td>
<td>5479.4</td>
</tr>
<tr>
<td>6</td>
<td>7966.2</td>
</tr>
<tr>
<td>8</td>
<td>10325.8</td>
</tr>
<tr>
<td>10</td>
<td>12940.9</td>
</tr>
<tr>
<td>12</td>
<td>16041.7</td>
</tr>
</tbody>
</table>

Fig. 3: Calibration curve of Terbinafine Hydrochloride

Table 2: Accuracy result of Terbinafine Hydrochloride

<table>
<thead>
<tr>
<th>Amount of sample (μg/ml)</th>
<th>Amount of drug added(μg/ml)</th>
<th>Amount of drug recovered (μg/ml)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>8 (80%)</td>
<td>17.8</td>
<td>98.88</td>
<td>0.3495</td>
</tr>
<tr>
<td>10</td>
<td>10 (100%)</td>
<td>20.12</td>
<td>100.6</td>
<td>0.8699</td>
</tr>
<tr>
<td>10</td>
<td>12 (120%)</td>
<td>21.7</td>
<td>98.63</td>
<td>0.5285</td>
</tr>
</tbody>
</table>
Table 3: Precision result of Terbinafine Hydrochloride

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Intraday precision</th>
<th>Interday precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC* % of TH* %RSD</td>
<td>AUC* % of TH* %RSD</td>
</tr>
<tr>
<td>10</td>
<td>13108.96 99.38 1.163</td>
<td>13274.57 100.6 0.949</td>
</tr>
</tbody>
</table>

*Average of three determination

Table 4: Repeatability result of Terbinafine Hydrochloride

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>AUC*</th>
<th>SD</th>
<th>RSD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>13080.93</td>
<td>123.8528</td>
<td>0.009469</td>
<td>0.94682</td>
</tr>
<tr>
<td>12</td>
<td>16016.13</td>
<td>22.17123</td>
<td>0.001384</td>
<td>0.138431</td>
</tr>
</tbody>
</table>

*Average of six determination

Table 4: System suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Statistical data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plate</td>
<td>3772-4580</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.9-1.4</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Degradation condition</th>
<th>% degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolytic degradation (neutral)</td>
<td>No degradation</td>
</tr>
<tr>
<td>Acidic hydrolysis</td>
<td>17% degradation with 1 N HCl</td>
</tr>
<tr>
<td>Basic hydrolysis</td>
<td>No degradation</td>
</tr>
<tr>
<td>Oxidative degradation</td>
<td>No degradation</td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>No degradation</td>
</tr>
<tr>
<td>Photodegradation</td>
<td>5 to 7 % degradation when exposed to both wavelengths.</td>
</tr>
</tbody>
</table>

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

Fig. 5: Chromatogram of TH subjected to acidic hydrolysis in 1N HCl
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. Statistical analysis proves that the developed method was able to discriminate between Terbinafine hydrochloride and its possible degradation products. Statistical analysis proves that the developed method was able to discriminate between Terbinafine hydrochloride and its possible degradation products.

ACKNOWLEDGEMENT

The authors are thankful to Cipla Ltd, Maharashtra, India for providing a gift sample of Terbinafine Hydrochloride and Modern College of pharmacy (for ladies), Moshi Pune, Maharashtra, India for providing necessary facilities for carrying out the work.

REFERENCES

2. European Pharmacopoeia 2011; Vol-II: 3024-3025.
18. Suma BV, Kannan K, Madhavan V, Nayar CR. HPTLC Method for determination of Terbinafine in the Bulk drug and Tablet


