Extractive spectrophotometric methods for determination of terbinafine hydrochloride in pharmaceutical formulations using some acidic triphenylmethane dyes

M Chennaiah, T Veeraiah, T Vinod Kumar & G Venkateshwarlu*

Department of Chemistry, Nizam College, Hyderabad, 500 001, India

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Three simple and sensitive extractive spectrophotometric methods have been described for the assay of terbinafine hydrochloride either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) in acidic medium. The extracted complexes show absorbance maxima at 410 nm for all three methods. Beer’s law is obeyed in the concentration ranges 2.0-25, 2.0-25 and 2.0-25 µg/mL with BTB, BPB and BCG respectively. The effects of concentration of dye, pH, and interference of excipients have been studied and optimized. The limits of detection and quantification have been determined for three methods. All the three methods have been validated as per the guidelines of ICH. The methods have been applied for the determination of drug in commercial tablets and the results of analysis are validated statistically through recovery studies.

Keywords: Bromocresol green, Bromophenol blue, Bromothymol blue, Spectrophotometry, Terbinafine hydrochloride

However methods on spectrophotometric determination of this drug involving ion pair complexes with common and versatile acidic dyes, viz. Bromothymol blue (BTB), Bromophenol blue (BPB) and Bromocresol green (BCG) are not reported yet. This prompted the authors to develop extractive spectrophotometric methods for the determination of TFH using the above-mentioned dyes.

Experimental Procedure

TFH was procured from Hetero Drugs Pvt. Ltd., Hyderabad as a gift sample. The dyestuffs, viz. BTB, BPB and BCG (AR grade), and chloroform (HPLC grade) supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. 0.025% solution of the dyestuffs and sodium acetate-hydrochloric acid buffers were prepared in doubly distilled water. The spectra of ion-pair complexes were recorded on SHIMADZU 140 double beam spectrophotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-Visible single beam spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 pH meter was used for pH measurement.

Procedure for construction of calibration curves

Different aliquots of drug solution were transferred into 125 mL separating funnel. To this, 5 mL of buffer (pH 2.5, 2.8 and 3.5) and 5 mL of dye were added, and the total volume was made up to 20 mL with water. 10 mL of chloroform was added and the contents were shaken for 5 min. The two layers were allowed to separate for 5 min. The organic layer was separated and the absorbance of yellow colored solution which is stable at least for 3h was measured at 410 nm against blank similarly prepared. The same procedure of analysis was followed either for assay of pure drug or for dosage form. The calibration graphs are found to be linear over the concentration ranges (Table 1). The optical characteristics and statistical data for the regression equation of the proposed methods are also given in Table 1.

Procedure for assay of dosage forms

Ten tablets of Terbicip 250 mg were powdered and dissolved in doubly distilled water and stirred thoroughly, filtered through a Whatman No. 42 filter...
paper. This solution was transferred into 100 mL standard volumetric flask and diluted with doubly distilled water as required. Different solutions of drug in the range of calibration curve were chosen and the assay was estimated using the calibration curve. The results of the recovery experiments are given in Table 2.

**Stoichiometry of the complexes**

In order to establish molar ratio between TFH and dyes used, the Job’s method of continuous variation has been applied using equimolar solutions of drug and dye (8 x 10^{-5}M). The results show the formation of 1:1 complex by the drug with each of the dye. The formation constants of the complexes were evaluated from Job’s plots by the methods described in literature^7,8.

**Optimisation of assay parameters**

The influence of pH on the ion-pair formation of terbinafine hydrochloride with various dyestuffs has been studied using sodium acetate-hydrochloric acid buffer. It is observed that the absorbance of complexes with BTB, BPB and BCG is constant within the pH ranges 2.2-3.3, 2.0-3.0 and 2.8-3.8 respectively. Thus, all the absorbance measurements were made at pH 2.8, 2.5 and 3.5 with BTB, BPB and BCG respectively.

The effect of dye concentration was also studied by adding different volumes of dye solution to a constant

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**Table 1—Optical characteristics and statistical analyses for the regression equation of the proposed methods**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BTB</th>
<th>BPB</th>
<th>BCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{max}, \text{nm})</td>
<td>410</td>
<td>410</td>
<td>410</td>
</tr>
<tr>
<td>Beer’s law limit, (\mu g \text{ mL}^{-1})</td>
<td>2.0-25</td>
<td>2.0-25</td>
<td>2.0-25</td>
</tr>
<tr>
<td>Molar absorptivity, (L \text{ mol}^{-1} \text{ cm}^{-1})</td>
<td>1.9x10^5</td>
<td>2.02x10^5</td>
<td>2.07x10^5</td>
</tr>
<tr>
<td>Formation constant (K), (M^{-1})</td>
<td>1.34 x 10^6</td>
<td>1.126 x 10^6</td>
<td>1.2 x 10^6</td>
</tr>
<tr>
<td>Sandell sensitivity, (\mu g \text{ cm}^{-2})</td>
<td>0.0179</td>
<td>0.0177</td>
<td>0.0159</td>
</tr>
<tr>
<td>Slope (specific absorptivity), (b)</td>
<td>0.0557</td>
<td>0.0564</td>
<td>0.0627</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>-0.0051</td>
<td>0.0249</td>
<td>-0.0195</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9991</td>
<td>0.999</td>
<td>0.9994</td>
</tr>
<tr>
<td>Standard deviation of intercepts (% n=6)</td>
<td>0.00399</td>
<td>0.0048</td>
<td>0.0103</td>
</tr>
<tr>
<td>Limit of detection, (\mu g \text{ mL}^{-1})</td>
<td>0.2362</td>
<td>0.2808</td>
<td>0.5409</td>
</tr>
<tr>
<td>Limit of quantification, (\mu g \text{ mL}^{-1})</td>
<td>0.7086</td>
<td>0.8425</td>
<td>1.6226</td>
</tr>
<tr>
<td>Regression equation</td>
<td>(Y= 0.0557C-0.0051)</td>
<td>(Y= 0.0564C+0.0249)</td>
<td>(Y=0.0627C-0.0195)</td>
</tr>
</tbody>
</table>

^aWith respect to \(Y=bc+a\), where C is the concentration (\(\mu g \text{ mL}^{-1}\)) and Y is absorbance.

^bSix replicate samples.
amount of TFH (10 µg mL$^{-1}$). The maximum absorbance, in each case, was found with 2.0 mL of dyestuff, beyond which absorbance was constant. Thus, 5 mL of each dye was used for ion-pair formation throughout the experiment.

A systematic study on the effect of foreign species present along with TFH on the determination of the drug at 12.5 µg mL$^{-1}$ level was also carried out. This study was carried out by following the proposed procedures for a 10 mL sample system, by adding a known amount of foreign species to 12.5 µg mL$^{-1}$ solution of TFH. The excipients and their tolerance limits are microcrystalline cellulose 90 µg mL$^{-1}$, starch 175 µg mL$^{-1}$, lactose 135 µg mL$^{-1}$, magnesium stearate colloidal 85 µg mL$^{-1}$, silicon dioxide 95 µg mL$^{-1}$ and titanium dioxide 25 µg mL$^{-1}$.

Results and Discussion

Terbinafine hydrochloride contains tertiary amino group which is protonated in acid medium, while sulphonic acid group is present in BTB, BPB and BCG, which is the only group undergoing dissociation in the pH range 1-5. The colour of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group. It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally, the protonated terbinafine hydrochloride forms ion-pairs with the dyestuffs which are quantitatively extracted into chloroform. The possible reaction mechanisms are proposed and given in Scheme 1.

Validation of proposed method

All the three proposed methods have been validated in terms of guideline proposed by International Conference on Harmonization held at Geneva in 1996, considering selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation. The student t-test and variance F-test have been performed in comparison with a reference method (Table 2). To test the reproducibility of the proposed methods, six replicate determinations of 12.5µg mL$^{-1}$ of terbinafine hydrochloride were made. The coefficient of variation was found to be less than 1.2% for all the procedures.

The proposed methods have been successfully applied for the determination of TFH in pharmaceutical preparations. The performance order of the proposed methods is BTB>BCG>BPB. The results obtained (Table 2 ) have been compared with those obtained by a reference method by means of t-test at 95% confidence level. In all cases, the average results obtained by proposed method and reference method are statistically identical, as the difference between the average values is not significant at 95% confidence level.

Conclusion

TFH forms ion-pair complexes with acidic dyes in 1:1 proportion and is extractable into chloroform as well as offers a basis for assay of the drug. The developed methods are simple, sensitive, reproducible and can be used for routine analysis of TFH in pure and formulation forms.

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References