Assay of Terbinafine Hydrochloride by Extractive-Spectrophotometry with Alizarin Red S-A Modified Approach

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Abstract

Terbinafine hydrochloride (TBH) is a new antifungal agent widely used for the treatment of many contagious diseases. Two spectrophotometric methods based on ion-pair complexation reaction are described for the determination of TBH. The first method is based on the formation of ion-pair complex between TBH and alizarin red S (ARS) dye in HCl medium followed by its extraction into methylene chloride and measurement at 450 nm (method A). The other method entails breaking of drug-dye complex in alcoholic KOH medium and measuring the absorbance of the blue dye at 570 nm (method B). The experimental conditions for selective and sensitive assay were optimized, and the drug-dye stoichiometry was found to be 1:1. Beer's law was obeyed over the concentration ranges 2.5-60, and 4-24 μg mL^{-1} for method A and method B, respectively, and the corresponding molar absorptivity values were 5.92×10^3 and 4.93×10^3 L mol^{-1} cm^{-1}, respectively. The limits detection (LOD) and quantification (LOQ) values were calculated to be 0.22 & 0.66 (method A) and 0.35 & 1.05 (method B). In addition, the methods were also validated with respect to accuracy, precision, robustness, ruggedness and selectivity. The two methods were applied to the analysis of tablets with no evidence of interference from excipients.

Keywords:

Terbinafine hydrochloride; Alizarin red S; spectrophotometry; Assay, Ion-pair complex .

1. Introduction

Antifungal drugs have been widely used for the control of many contagious diseases [1]. The allylamine derivatives are a new class of synthetic antifungal agents [2] which are well absorbed and excreted, mainly through urine. Terbinafine HCl (TBH), chemically known as (E)– N– [6,6– dimethyl– 2– heptan– 4– ynyl]– N– methyl– 1– naphthalene methanamine hydrochloride, belongs to the allylamine class of antifungal agents and was first synthesized in 1978 [3]. It is available for both systemic and topical use [4], and is now the drug of choice in dermatophyte nail and skin infections because of its fungicidal mode of action which leads to high cure rates, and low relapse rates over a short treatment duration. This antibiotic substance has a novel of mechanism of action involving the selective inhibition of fungal squalene epoxidation [5].

The drug is official in European Pharmacopoeia (EP) [6], British Pharmacopeia (BP) [7] and the United States Pharmacopoeia (USP) [8], the EP and BP describe a titrimetric procedure in which drug is dissolved in 96% ethanol, 5 mL of 0.01 mol L^{-1} HCl added followed by potentiometric titration with 0.1 mol L^{-1} NaOH. The USP recommends a HPLC

method with C_{18} column as stationary phase and triethylamine buffer, pH 7.5- acetonitrile-methanol (95:2:5 v/v/v) as mobile phase with gradient elution at a flow rate of 0.8 mL mm^{-1} with UV- detection at 280 nm.

Other than the official methods [6-8] the drug has been determined by electrochemical [9-11], spectrofluorimetric [12], non-aqueous titrimetric [13], chromatographic [14-23] and capillary electrophoretic [24,25] methods.

There are a few reports dealing with the visible spectrophotometric determination of TBH in bulk and dosage forms based on redox and charge-transfer complexation [26] and ion-pair complexation [27-29] reactions. Ion-pair extractive spectrophotometry has received considerable attention for the assay of several pharmaceutical compounds [30-40] because of its sensitivity and selectivity. Alizarin red S (ARS) has previously been used for the assay of TBH, but the procedure requires strict pH control using a buffer solution.

In the present communication, ion-pair extractive spectrophotometry is described for the assay of TBH using ARS as the ion-pair reagent, but the use of buffer in circumvented and 0.1M HCl is used in its replace to yield the required pH for complex formation (method A). Going further, a second method was developed by breaking the ion-pair complex with alcoholic KOH and measuring the absorbance of the free dye at 570 nm (method B). Both the methods, when applied to commercial tablets, yielded results which were accurate and precise besides being robust and rugged.

2. Experimental

2.1. Apparatus

A Systronics model 166 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) with matched 1-cm quartz cells was used for absorbance measurements.

2.2. Reagents and materials

All Chemicals used were of analytical reagent grade and double distilled water was wherever necessary. Spectroscopic grade methylene chloride was used. A 0.05% alizarin red S (ARS) solution was prepared by dissolving 50 mg of the dye (S.D. Fine Chem., Mumbai. India) in 100 mL of water in a volumetric flask. A 0.1 mol L^{-1} HCl was prepared by appropriate dilution of concentration acid (Merck, Mumbai, India, sp. gr. 1.18) with water.

Pure terbinafine hydrochloride (TBH) was received as gift from Dr. Reddy's Laboratories Limited, Hyderabad, India, and used as received. A 100 µg mL^{-1} standard drug solution was prepared by dissolving 10 mg of TBH in 0.1 mol L^{-1} HCl, and diluted to volume with the same solvent in a 100 mL volumetric flask, and mixed well.

Sebifin–250 (Glaxo Smith Kline Pharmaceuticals Limited, India) and Terbiforce-250 (Lifestar Pharmaceuticals Pvt. Ltd., India) tablets were purchased from commercial stores.

2.3. Procedures

2.3.1. Preparation of calibration graphs

2.3.1.1. Method A

In to a series of 125 mL separating funnels, different volumes (0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0) of 100 µg mL^{-1} TBH solution were accurately measured and the volume was adjusted to 9 mL with 0.1 mol L^{-1} HCl and mixed with 6 mL of ARS dye; the total volume was brought to 20 mL with water and mixed well. The drug-dye complex formed was extracted with 10 mL of methylene chloride (accurately measured) by shaking for 1 min.

After phase separation, the organic layer was separated, dried over anhydrous Na_2SO_4 and absorbance measured at 450 nm against the reagent blank simultaneously prepared.

2.3.1.2. Method B

The drug-dye complex (40 µg mL^{-1} with respect to drug) obtained above was used in method B. Known Aliquots (1-6 mL) of the above complex were accurately measured in to a set of 10 mL calibrated flasks. To each flask was added 0.5 mL of 1% alcoholic KOH and the contents were diluted to the mark with ethanol and mixed. Absorbance of each solution was measured at 570 nm against a reagent blank as reference.

A standard graph in each case was prepared, and the concentration of the unknown was calculated using the regression equation derived using the beer's law data.

2.3.2. Procedure for tablets

Twenty tablets were weighed and powdered. An amount of powder equivalent to 10 mg of TBH was shaken with 50 mL of 0.1 mol L^{-1} HCl in a 100 mL calibrated flask, diluted to mark with same acid and mixed. The insoluble matter was filtered off using a quantitative filter paper to obtain a solution of 100 µg mL^{-1} TBH. Three mL aliquot was taken for analysis in five replicates, as described under the procedure for bulk drug (method A). In method B, five mL aliquot of ion-pair complex (40 µg mL^{-1}) was taken in five replicates, and the general procedure was followed.

2.3.3. Procedure for placebo blank and synthetic mixture analyses
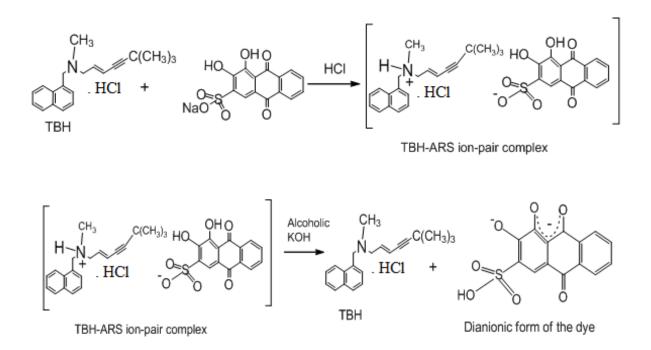
A placebo blank of the composition: talc (25 mg), starch (25 mg), acacia (20 mg), methyl cellulose (10 mg), sodium citrate (15 mg), magnesium stearate (20 mg) and sodium alginate (15 mg) was prepared by uniform mixing. Ten mg of placebo was treated with 0.1 mol L^{-1} HCl and its extract prepared as described under procedure for tablets. Five mL of the extract was objected to analysis as described earlier. To 10 mg of the placebo, 10 mg of pure drug was added, homogenized, and its extract prepared as described under procedure for tablets, 3 mL was taken analysis (n=5). Method B was applied to 4 mL aliquot of complex (40 µg mL^{-1} in TBH) from tablet extract to complete the assay
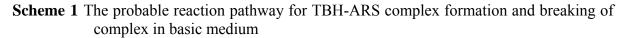
2.3.4. Procedure for composition study

Job's method of continuous variations [41] was used; 3.05×10^{-4} mol L^{-1} solutions of ARS and TBH were prepared. A series of solutions was prepared by mixing them in varying volume ratios in such a way that the total volume of the drug and TBS was kept at 10 mL in a total volume of 20 mL of aqueous phase. The absorbance of the extracted ion-pair in each instance was measured and plotted against the mole-fraction of the drug.

3. Results and Discussion

TBH was found to yield a clear yellow coloured product with ARS in acidic medium which extractable with methylene chloride for measurement at 450 nm. The coloured product is the ion-pair complex formed as show in scheme 1. The complex was found to be formed in HCl medium without the need for a buffering solution as reported by Elazazy et al. [27]. Therefore, investigations were carried out to establish the most favourable conditions for the formation of the coloured product.

Scheme 1 The probable reaction pathway for TBH-ARS complex formation and breaking of complex in basic medium

3.1. Method development

3.1.1. Method A

The influence of acid concentration on the reaction was studied. Constant and maximum absorbance was obtained with 3 mL of 0.1 mol L^{-1} HCl in a total of 20 mL aqueous phase (Fig. 1). Hence, 3 mL of 0.1 mol L^{-1} HCl was added to drug solution before the addition of dye solution and diluting to 20 mL with water.
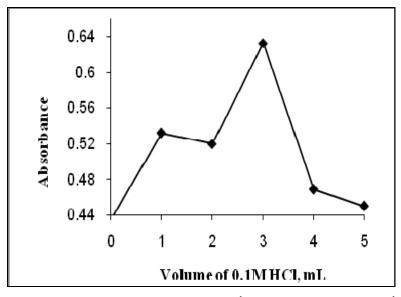

Fig. 1 Effect of volume of 0.1 mol L^{-1} HCl (TBH: 35 μg mL^{-1})

The effect of ARS concentration was examined. As shown in Fig. 2, 6 mL of 0.05% dye solution in 20 mL aqueous solution found adequate for complexation.
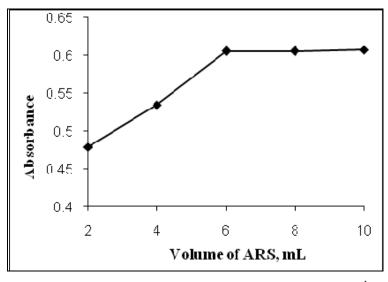
Fig. 2 Effect of volume of ARS dye (TBH: 35 µg mL^{-1})

The choice of organic solvent for quantitative extraction of the complex was also investigated. Methylene chloride was found to be an ideal solvent compared to many other solvents tried (Fig. 3). A single extraction with 10 mL methylene chloride was sufficient for quantitative recovery of the complex.
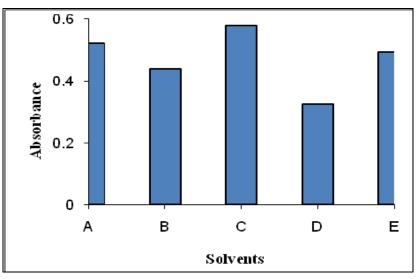

Fig. 3 Effect of different solvents; A. chloroform, B.1,2-dichloroethane, C. methylene chloride, D. carbon tetrachloride, and E. hexane (TBH: 35 µg mL^{-1})

The reaction was found to be spontaneous, and shaking time of 1-4 min yielded the same absorbance, hence one min shaking time was selected as optimum. The separation of two phases was complete in one min. the extracted drug-dye complex was stable for over 4 days. The aqueous to organic ratio of 2:1 resulted in maximum and constant absorbance values.

The reaction stoichiometry as found from Job's method of continuous variations was 1:1 (drug:dye) as shown in Fig. 4.
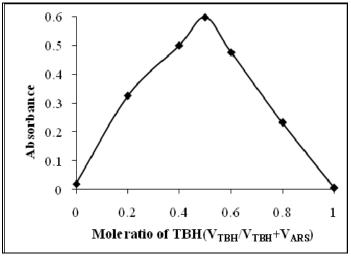
Fig. 4. Job's plot

3.1.2. Method B

The optimum volume of alcoholic KOH (1%) required to break the complex was determined to be 0.5 mL. The reaction was instantaneous, but unlike the drug-dye complex, the blue colour of free dye was stable for only 10 min under the described conditions.

3.2. Method validation

3.2.1. Linearity and sensitivity

The methods were validated with respect to linearity, sensitivity, accuracy, precision, robustness, ruggedness and selectivity as per the current *ICH* guidelines [42].

The absorbance-concentration plots (Fig. 5) were found to be linear over the concentration ranges stated in Table 1. The regression parameters calculated from the calibration graphs along with the standard deviations of slope (m) and intercept (b), the limits of detection (LOD) and quantification (LOQ), the molar absorptivity (ε) and Sandell sensitivity values are also summarized in Table 1.
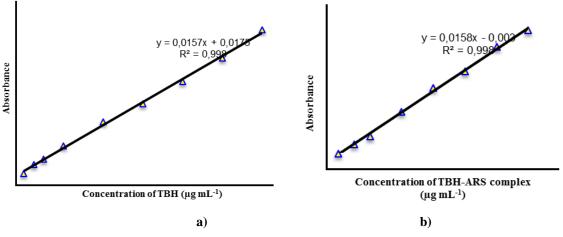

Fig. 5. Calibration plots: a) Method A b) Method B

Table 1. Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	450	570
Colour stability	4 day	10 min
Linear range, $\mu g\ mL^{-1}$	2.5-60	2-24
Molar absorptivity (ε), $L\ mol^{-1}\ cm^{-1}$	5.92×10^3	4.93×10^3
Sandell sensitivity[*], $\mu g\ cm^{-2}$	0.0555	0.0665
Limit of detection (LOD), $\mu g\ mL^{-1}$	0.22	0.35
Limit of quantification (LOQ), $\mu g\ mL^{-1}$	0.66	1.05
Regression equation, Y^{**}		
Intercept (a)	0.0237	0.0045
Slope (b)	0.0156	0.0159
Standard deviation of a (S_a)	3.92×10^{-3}	1.08×10^{-4}
Standard deviation of b (S_b)	1.76×10^{-3}	4.97×10^{-3}
Regression coefficient (r)	0.9991	0.9988

*Limit of determination as the weight in $\mu g\ mL^{-1}$ of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area $1\ cm^2$ and l = 1 cm. **Y=a+bX, Where Y is the absorbance, X is concentration in $\mu g\ mL^{-1}$, a is intercept and b is slope.

3.2.2. Accuracy and precision

The assay described under general procedure was repeated seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day) of the methods. The assay was performed on three levels of the analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were $\leq 1.46\%$ (intra-day) and $\leq 1.36\%$ (inter-day) indicating high precision of the methods. The accuracy of the methods was determined as percent mean deviation from known concentration, % bias = (concentration found - known concentration) $\times$ 100 / known concentration. Bias was calculated for each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of $\leq 2.34\%$ demonstrated high accuracy of the methods.

Table 2. Evaluation of intra-day and inter-day accuracy and precision

Method	TBH taken ($\mu g\ mL^{-1}$)	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=7)		
		TBH found[a] ($\mu g\ mL^{-1}$)	RSD[b] %	RE[c] %	TBH found ($\mu g\ mL^{-1}$)	RSD[b] %	RE[c] %
A	15.0	15.23	0.72	1.53	14.65	0.34	2.34
	30.0	29.51	1.36	1.63	29.59	1.21	1.37
	45.0	44.43	1.45	1.27	44.34	1.27	1.47
B	6.0	5.98	0.59	0.33	6.12	0.83	2.00
	12.0	12.22	1.46	1.83	11.89	1.36	0.92
	18.0	17.92	0.47	0.44	18.11	0.41	0.61

[a] Mean value of seven determinations; [b] Relative standard deviation (%); [c] Relative error (%).

3.2.3. Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in volume of HCl and ARS (method A) and volume of alcoholic KOH (method B) and the effect of these changes on the absorbance of colored systems was studied. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD ($\leq 1.86\%$). Method ruggedness was demonstrated by having the analysis

done by three analysts using the same instrument, and also by a single analyst performing analysis on three different instruments in the same laboratory. Intermediate precision values (%RSD) of this study were in the range 0.92-2.71% indicating acceptable ruggedness. These results are compiled in Table 3.

Table 3. Method robustness and ruggedness expressed as intermediate precision (% RSD)

Method	TBH taken ($\mu g\ mL^{-1}$)	Robustness			Ruggedness	
		Parameters altered*			Inter-analysts (%RSD), (n=3)	Inter-instruments (%RSD), (n=3)
		Volume of HCl	Volume of ARS	Volume of ethanolic KOH		
A	30	1.56	1.86	-	0.92	2.71
B	12	-	-	1.07	1.16	2.24

*In method A, the volume of HCl was 2.5, 3.0 and 3.5 mL, in method B the volumes of ethanolic KOH added were 0.4, 0.5 and 0.6 mL.

3.2.4. Selectivity

The placebo blank extract on subjecting to analysis using the developed methods yielded signals almost equal to those yielded by reagent blanks. The analysis of the synthetic mixture for the active ingredient yielded precision recoveries of 98.45% and 96.38%, respectively, for method A and method B with standard deviation < 2.5%. These studies revealed the absence of interference from the tablet excipients and additives in the assay of active ingredient.

3.3. Application to tablets

The proposed methods were applied for the determination of TBH in commercial tablets and the results are given in Table 4. The same batch tablets were analyzed by the European Pharmacopoeial method [6] for comparison, and the results of the proposed methods are in good agreement with the reference with respect to accuracy and precision as revealed by the Student's t- and F-values shown in Table 4.

Table 4. Results of analysis of formulations by the proposed methods and statistical comparison of the results with the official method

Tablet brand name	Nominal amount	Found* (% of nominal amount ± SD)		
		Official method	Proposed methods	
			Method A	Method B
Terbiforse	250	99.45±1.15	99.27±1.09 t = 0.25 F= 1.11	100.3±1.43 t = 1.03 F= 1.55
Sebifin	250	98.92 ± 1.02	98.45±0.92 t = 0.75 F= 1.15	99.54±0.81 t = 1.07 F= 1.59

*Mean value of five determinations.
(Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77).
(Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39).

3.4. Accuracy by recovery study

To further examine the accuracy of the proposed methods, recovery experiment following the standard addition procedure was conducted. Tablet powder (pre-analyzed) was spiked with pure TBH at three levels and the total was found by the proposed methods, and the percent recovery of pure drug was calculated. Determination at each concentration level

was repeated thrice. The percent recovery values, compiled in Table 5, reveal that the methods are not affected by the matrix, and the method is accurate to be used in routine analysis.

Table 5. Results of recovery study *by* standard-addition method.

Tablet studied	Method A				Method B			
	TBH in formulation, μg mL^{-1}	Pure TBH added, μg mL^{-1}	Total found, μg mL^{-1}	Pure TBH recovered (Percent±SD*)	TBH in formulation, μg mL^{-1}	Pure TBH added, μg mL^{-1}	Total found, μg mL^{-1}	Pure TBH recovered (Percent±SD*)
Terbiforse	19.85	10.0	29.44	98.63±1.81	8.02	4.0	11.82	98.31±2.09
	19.85	20.0	40.61	101.9±2.05	8.02	8.0	16.26	101.5±1.89
	19.85	30.0	49.65	99.60±2.12	8.02	12.0	20.44	102.1±2.21
Sebifin	19.69	10.0	29.40	99.02±1.41	7.96	4.0	11.91	99.57±1.42
	19.69	20.0	39.64	99.87±1.63	7.96	8.0	15.55	97.41±2.36
	19.69	30.0	50.29	101.2±2.18	7.96	12.0	19.62	98.29±2.31

*Mean value of three determinations.

4. Conclusions

Unlike the chromatographic and electrophoretic methods reported for TBH, the suggested methods are simple, inexpensive and easy to perform. The methods are based on chemical reactions rather than the sophistication of the instrument used. The reagent (ARS) utilized is cheap and easily available and the reaction does not require critical pH adjustment unlike other reported spectrophotometric methods. The methods are more sensitive compared to many available methods and have wide linear dynamic ranges, and are free from common tablet excipients and additives.

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