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Stability Indicating UV-Spectrophotometric Assay Of Terbinafine Hydrochloride In Dosage Forms.

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Abstract: Two sensitive, precise and cost-effective UV-spectrophotometric methods are described for the determination of terbinafine hydrochloride (TFH) in bulk drug and tablets. The methods are based on the measurement of absorbance of TFH in 0.1M HCl at 222 nm in method A and in 0.1M acetic acid at 282 nm in method B. As per the International Conference on harmonization ICH guidelines, the methods were validated for linearity, accuracy, precision, limits of detection (LOD), limit of quantification (LOQ), robustness and ruggedness. Beer's law is obeyed over the concentration ranges of 0.2-4.0 and 2.0-50.0 µg mL^{-1} TFH in method A and method B, respectively, and the corresponding molar absorptivity values are 8.7 x 10^4 and 7.9 x 10^3 L mol^{-1} cm^{-1}. The proposed methods were applied successfully to the determination of TFH in tablets with good accuracy, precision and without any detectable interference from tablets excipients. The validity and reliability of the proposed methods were further assed by the recovery studies *via* a standard addition method. In addition, forced degradation of TFH was conducted in accordance with the ICH guidelines. Acidic, basic and water hydrolysis, thermal stress, peroxide and photolytic degradation were used to assess the stability indicating power of the methods. Slight degradation was observed during base degradation in method A and substantial degradation was observed during oxidative degradation in both methods. No degradation was observed under other stress conditions.
Key words: Terbinafine Hydrochloride, UV-spectrophotometry, Stability indicating, Pharmaceuticals.

Introduction.

Terbinafine hydrochloride (TFH), chemically known as (E)-*N*-(6,6-dimethyl- 2 - hepten - 4 - ynyl) -*N*-methyl - 1 – naphthalene methanamine hydrochloride [29], is a potent antifungal agent of the allylamine class with broad spectrum activity against yeasts, dimorphic fungi, molds, and dermatophytes [23,22,2,4].

Various techniques have been used for the determination of TFH in body fluids and pharmaceuticals. The drug is official in European Pharmacopoeia [14], British Pharmacopoeia [6] and the United States Pharmacopoeia [30]. European Pharmacopoeia and British Pharmacopoeia describe acid-base titration in hydro-alcoholic medium, the end point being located potentiometrically [14,6]. United States Pharmacopoeia describes high performance liquid chromatographic method for the estimation of TFH [30] . High performance liquid chromatography (HPLC) has been applied for the determination of TFH and metabolites in human plasma [10], TFH and metabolites in human plasma, milk and urine [26], metabolites in human plasma [32] and the drug in tissues [31]. An improved high throughput liquid chromatographic/tandem mass spectrophotometric method for TFH in plasma [11] and ultra performance liquid chromatographic method for the drug and its metabolites in

human plasma and urine [5] are the other chromatographic methods reported for body fluids. In addition microbiological assay for the drug are also found in the literature [18, 21].

Several HPLC procedures [28,15,17,25,1,7,24] employing different columns and mobile phases have been reported for its assay in dosage forms when present alone [28,15,17,25,1,7] or in combination with bezafibrate [24]. High performance thin layer chromatography (HPTLC) has recently been applied for the assay of drug in tablets [27] and for the simultaneous determination of TFH and triamcinolone acetamide in compound tablets [13]. Non-aqueous titrimetry [8] visible spectrophotometry [12,16,9] and voltammetry [3] are the other analytical techniques available for the assay of TFH in its dosage forms.

UV-spectrophotometry is one of the simplest techniques routinely used in pharmaceutical quality control laboratories because of its sensitivity, speed, fair selectivity, low cost and ease of performance. However, the literature on TFH is poor with regard to UV-spectrophotometric methods. In a method reported by Cardoso and Schapoval [8], the absorbance of methanolic extract of tablets and creams was measured at 224 nm. Simultaneous determination of TFH and triamcilone acetamide by derivative spectrophotometry has been reported by EL-Saharty et.al [13].

Stability indicating assay are increasingly being applied to many pharmaceutically important compounds [19]. But, none of the methods available for TFH [28,15,17,25,1,7,24,27,13,8] including the UV-spectrophotometric methods [13,8] is stability indicating. The objective of the present study was to develop simple, sensitive and rapid uv-spectrophotometric methods which are stability indicating. The methods are based on the measurement of the absorbance of the drug in 0.1M HCl at 222 nm or in 0.1M acetic acid at 282 nm.

Materials and Methods.

Apparatus

Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer was used for absorbance measurements.

Materials

Pharmaceutical grade terbinafine hydrochloride (TFH) was received from Dr. Reddy's laboratories limited, Hyderabad, India, as gift sample and used as received. Zimig-250 (from Glaxo Smith Kline Pharmaceuticals Limited, India) and Terbiforce-250 (from Lifestar Pharma PVT. Ltd.) tablets were purchased from commercial sources in the local market. All reagents and chemicals used were of analytical reagent grade and distilled water was used throughout the study. Solutions of hydrochloric acid, 0.1M and 2M (Merck, Mumbai, India, Sp. gr. 1.18), acetic acid, 0.1M (Merck, Mumbai, India; Sp. gr.1.048-1.050), sodium hydroxide, 2M (Merck, Mumbai, India) and 5% hydrogen peroxide (Merck, Mumbai, India 30% w/v) were prepared in double distilled water and used for degradation studies.

Standard drug solution

Standard drug solutions of 10 µg mL^{-1} TFH prepared in 0.1M HCl for assay in method A and 100 µg mL^{-1} of TFH prepared in 0.1M acetic acid for assay in method B .

Procedures

Method A (Using 0.1M HCl)

Aliquots (0.00, 0.2, 0.5, 1.0, …4.0 mL) of TFH standard solution (10 µg mL^{-1}) were accurately transferred into a series of 10 mL calibrated flasks and the volume was made up to the mark with 0.1M HCl. The absorbance of each solution was measured at 222 nm against 0.1M HCl blank.

Method B (Using 0.1M acetic acid)

Aliquots (0.00, 0.2, 0.5, 1.0, ….5.0 mL) of TFH standard solution (100 µg mL^{-1}) were accurately transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with 0.1M acetic acid. The absorbance of each solution was measured at 282 nm against 0.1M acetic acid blank.

In both the cases, calibration curves were plotted and the concentration of the unknown was read from the calibration graph are computed from regression equation derived using Beer's law data.

Procedure for tablets

Twenty tablets were weighed accurately and ground into a fine power. An accurately weighed amount of the tablet powder equivalent to 10 mg of TFH was transferred into two 100 mL calibrated flasks. Sixty mL of 0.1 M HCl was added to one and 0.1 M acetic acid to other flask and the content was shaken thoroughly for 15-20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with the respective solvent, mixed well and filtered using a whatman No. 42 filter paper. The filtrate (100 µg mL^{-1}) was subjected to analysis by following procedures described above after appropriate dilution in method A.

Procedure for placebo blank analysis

A placebo blank of the composition: talc (50 mg), starch (50 mg), acacia (50mg), methyl cellulose (20 mg), sodium citrate (20 mg), magnesium stearate (20 mg) and sodium alginate (10 mg) was made and its solution was prepared by taking 20 mg as described under 'Procedure for tablets', and then analysed using the procedure described earlier.

Procedure for the determination of TFH in synthetic mixture

To 20 mg of the placebo blank of the composition described above, 10 mg of TFH was added and homogenized, transferred to a 100 mL calibrated flask and the solution was prepared as described under "Procedure for tablets", and then subjected to analysis by the procedure described above. This analysis was performed to study the interference by excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate.

Preparation of acid, base and water hydrolysis induced-degradation of sample

For acid, alkaline and water hydrolysis degradation studies to 2.0 mL of 10 µg mL^{-1} stock solution of TFH, 2.5 mL of 2M HCl or 2M NaOH or water were added separately in method A, and to 2.0 mL of 100 µg mL^{-1} stock solution of TFH, 2.5 mL of 2M HCl or 2M NaOH or water were added separately in three 10 mL calibrated flasks in method B. The flasks were kept on a water bath set at 80^oC for 3.0 hrs, then cooled to room temperature. Then the solutions were neutralized with equal volume of 2M NaOH or 2M HCl. The content of each flask was made up to the mark with 0.1 M HCl in method A and with 0.1M acetic acid in method B. The absorption spectrum was run from 200-400 nm.

Preparation of hydrogen peroxide induced-degradation of sample

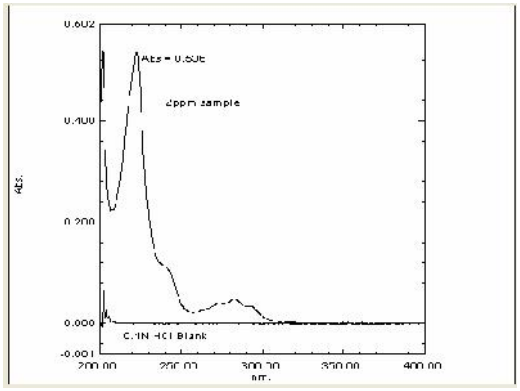
To 2.0 mL of TFH standard solution (10 µg mL^{-1} for method A and 100 µg mL^{-1} for method B) 2.5 mL of 5% hydrogen peroxide was added in 10 mL calibrated flasks. The flasks were kept on a water bath set at 80^0C for 3.0 hrs. The flasks were cooled room temperature, made up to the mark with 0.1M HCl, 0.1M NaOH in method A and method B respectively. The absorption spectrum of each was run from 200-400 nm.

Preparation of dry heat degradation and photo-degradation samples.

The powdered drug was stored in the oven at 105^0C for 48 hrs to study dry heat degradation, and exposed to 200 watt hr. m^{-2}, UV-radiation and 1.2 million lux hr. of visible radiation for study of photo degradation. After that solutions containing 2 µg mL^{-1} TFH in 0.1 M HCl and 20 µg mL^{-1} TFH in 0.1 M acetic acid were prepared for method A and method B, respectively. The absorption spectra were run from 200-400 nm.

Results And Discussions.

In the present work, two UV-spectrophotometric methods for the determination of TFH in bulk drug and in tablets have been described. In method A 0.1 M HCl was used as the solvent and the absorbance was measured at 222 nm (Figure 1) where in method B, 0.1M acetic acid was used as a solvent with the measurement being made at 282 nm (Figure 2).



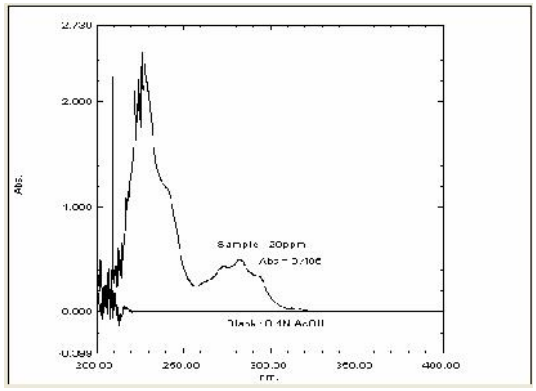
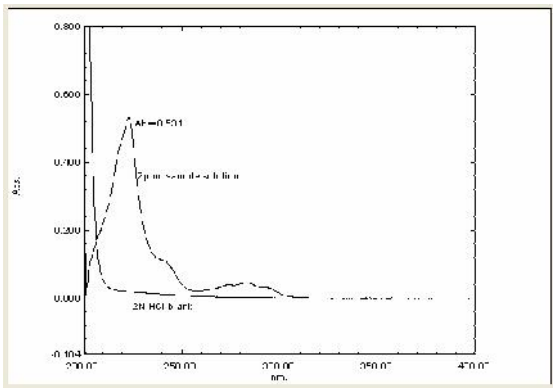


Figure 1 **Figure 2**

Figure 1: (Absorption spectra of 2 µg mL^{-1} TFH in 0.1M HCl, Method A))
Figure 2: (Absorption spectra of 20 µg mL^{-1} TFH in 0.1M Acetic acid, Method B)

Stability Property

The absorption spectra of the TFH solutions treated with acid, base, hydrogen peroxide, dry heat and UV radiation were run in the range of (200-400 nm). The degradation study was based on the comparison of the UV spectra of "stressed TFH samples" with that of the "standard TFH solution". The absorption spectrum of TFH solution treated with 2M hydrochloric acid (Figure 3 and 4) showed the same spectrum of the standard solution (Figure 1 and 2) which shows that TFH does not undergo degradation under acidic conditions in both methods.



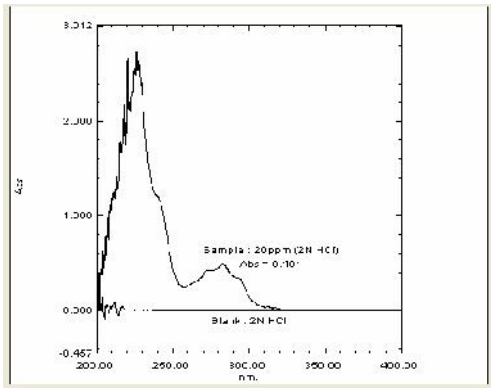
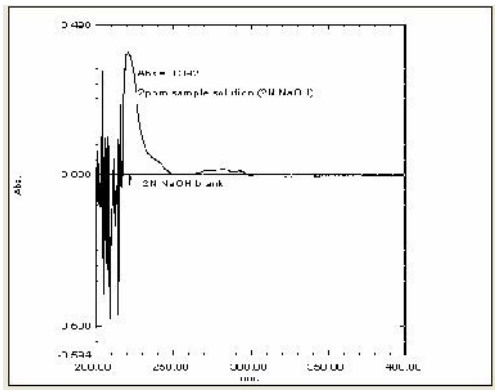


Figure 3 **Figure 4**

Figure 3: UV-spectra of TFH after subjected to acidic degradation (Method A).
Figure 4: UV-spectra of TFH after subjected to acidic degradation (Method B).

Also the spectra of TFH solution treated with 2M sodium hydroxide indicate slight degradation in method A (Figure 5) and no significant degradation in method B (Figure 6) when compared to standard (Figure 1 and 2).



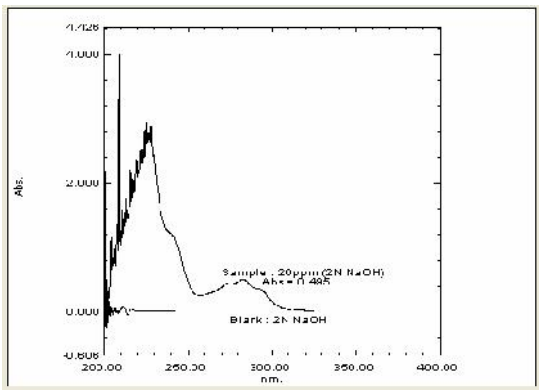
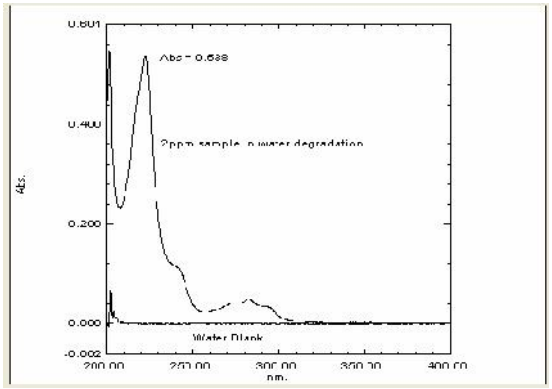


Figure 5 **Figure 6**

Figure 5: UV-spectra of TFH after subjected to base degradation (Method A).
Figure 6: UV-spectra of TFH after subjected to base degradation (Method B).

From Figure 7, 8, 9, 10, 11, and 12, it is clear that TFH is quite stable under water, dry heat and UV-Visible exposure stress conditions.



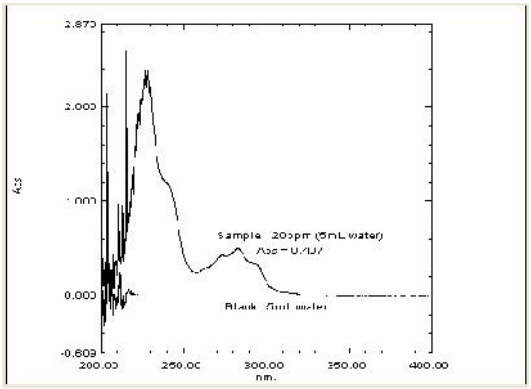


Figure 7 **Figure 8**

Figure 7: UV-spectra of TFH after subjected to water degradation (Method A).
Figure 8: UV-spectra of TFH after subjected to water degradation (Method B).

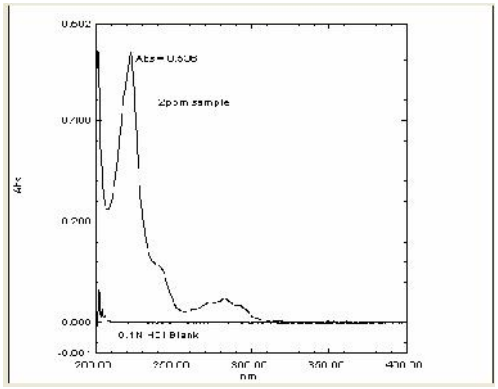


Figure 9

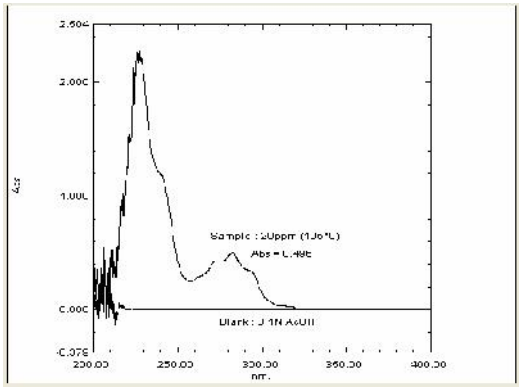


Figure 10

Figure 9: UV-spectra of TFH after subjected to thermal degradation (Method A).
Figure 10: UV-spectra of TFH after subjected to thermal degradation (Method B).

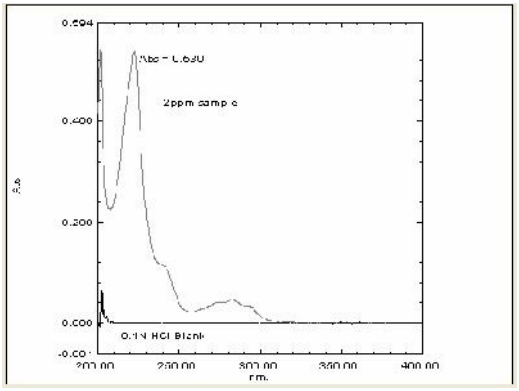


Figure 11

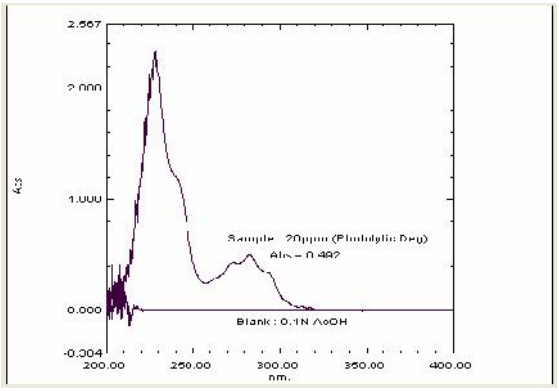


Figure 12

Figure 11: UV-spectra of TFH after subjected to photolytic degradation (Method A).
Figure 12: UV-spectra of TFH after subjected to photolytic degradation (Method B).

Contrary to the above discussions, the absorption spectrum of TFH solution subjected to oxidative stress conditions showed that TFH undergoes significant degradation (Figure 13, 14) in both the methods.

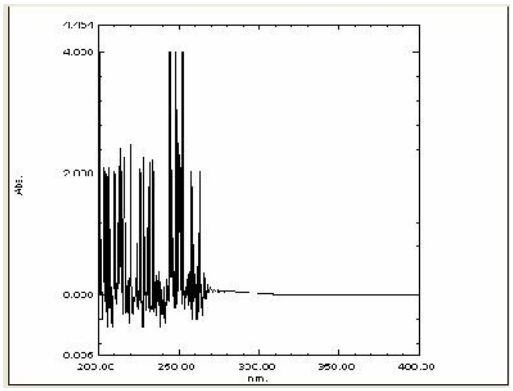


Figure 13

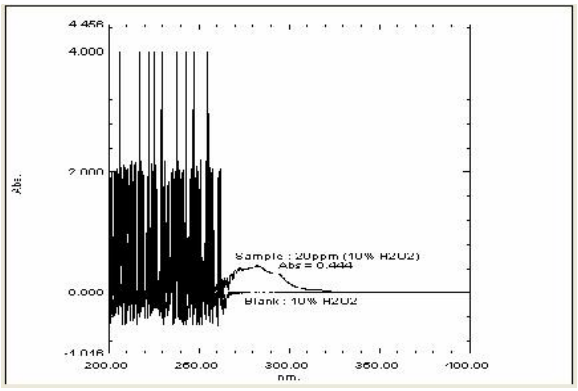


Figure 14

Figure 13: UV-spectra of TFH after subjected to oxidative degradation (Method A).
Figure 14: UV-spectra of TFH after subjected to oxidative degradation (Method B).

Method validation

The proposed methods were validated as per ICH guidelines [20] for linearity, sensitivity, precision, accuracy robustness, ruggedness and selectivity.

Linearity, sensitivity, limits of detection and quantification

A linear correlation was found between absorbance at λ_{max} and concentration of TFH in the ranges given in Table I. The graphs are described by the regression equation : Y= a +bX (where Y-absorbance of 1-cm layer of solution; a-intercept; b-slope and X-concentration in µg mL^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and regression coefficient (r^2) for each system and the values are presented in Table I. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values of the two methods are also given in Table I. The limits of detection (LOD) and quantification (LOQ) calculated according to ICH guidelines [20] using the formule : LOD = 3.3 S/b and LOQ = 10 S/b, (where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot) are also presented in Table I. The high values of ε and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.

Table I. Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	222	282
Linear range, µg mL^{-1}	0.2 – 4.0	2.0 – 50.0
Molar absorptivity (ε), L mol^{-1} cm^{-1}	8.72 X 10^4	7.97 X 10^3
Sandell sensitivity*, µg cm^{-2}	0.0038	0.0411
Limit of detection (LOD), µg mL^{-1}	0.003	0.09
Limit of quantification (LOQ), µg mL^{-1}	0.01	0.27
Regression equation, Y**		
Intercept (a)	0.0076	-0.0035
Slope (b)	0.2584	0.0247
Standard deviation of a (S_a)	0.003411	0.028083
Standard deviation of b (S_b)	0.001518	0.000925
Regression coefficient (r^2)	0.9999	0.9997

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

Accuracy and precision

Accuracy was evaluated as percentage relative error between the measured concentrations and the concentrations taken for TFH (Bias %). Precision of the method was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of TFH were analyzed in seven replicates during the same day (intra-day precision) and for six consecutive days (inter-day precision). RSD (%) values of the intra-day and inter-day studies showed that the precision was satisfactory. The results obtained are compiled in Table II and show that the accuracy is good.

Robustness and ruggedness

Method robustness and ruggedness were demonstrated by determination of TFH at three different conditions. Method robustness was tested by measuring the absorbance at different wave lengths where the method ruggedness was performed by four different analysts, and also with three different cuvettes by a single analyst. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in the Table III.

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

Method	TFH taken µg mL^{-1}	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=6)		
		TFH found, µg mL^{-1}	% RE	%RSD	TFH found, µg mL^{-1}	% RE	%RSD
A	1.5	1.50	0.30	0.14	1.50	0.20	0.14
	2.5	2.49	0.43	0.25	2.49	0.53	0.30
	3.5	3.48	0.71	0.32	3.48	0.45	0.32
B	15	14.86	0.94	0.17	14.81	1.26	0.27
	25	24.91	0.37	0.16	24.91	0.37	0.16
	35	34.86	0.40	0.27	34.77	0.64	0.30

%RE. Percent relative error, %RSD. Relative standard deviation.

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

Method	TFH taken µg mL^{-1}	Robustness# (%RSD)	Ruggedness	
			Inter-analysts (%RSD), (n=4)	Inter-cuvettes (%RSD),(n=4)
A	1.5	0.64	0.53	0.91
	2.5	0.76	0.58	0.56
	3.5	0.85	0.71	0.60
B	15	0.76	0.76	0.98
	25	0.88	0.81	0.78
	35	0.71	0.56	0.69

#Wavelengths used were 221, 222 and 223 in method A and 281,282 and 283 in method B.

Selectivity and interference

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. The placebo blank solution was subjected to analysis according to the recommended procedures and found that there was no interference from the inactive ingredients, indicating a high selectivity for determining TFH in its tablets. A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution yielded percent recoveries of 99.61±0.75 for method A and 99.56±0.85 for method B, indicating the non-interference by inactive ingredients.

Application to tablets

In order to evaluate the analytical applicability of the proposed method to the quantification of TFH in commercial tablets, results obtained by the proposed methods were compared to those of the reference method [6] by applying Students's t-test for accuracy and F-test for precision. The reference method which describes acid-base titration in hydro-alcoholic medium and the end point being located potentiometrically. The results showed that the Students's t- and F-values at 95% confidence level did not exceed the tabulated values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision (Table IV).

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analysed tablet powder was spiked with pure TFH at three concentration levels (50, 100 and 150% of that in tablet powder) and the total was found by the proposed methods. The added TFH recovery percentage values ranged from 99.2-100.5% with standard deviation of 0.38-0.87 indicating that the recovery was good, and that the co-formulated substance did not interfere in the determination (Table V).

Table IV. Results of analysis of tablets by the proposed methods and statastical comparison of the results with the reference method

Tablet brand name	Nominal amount (mg/tablet)	Found* (Percent of label claim ±SD)		
		Reference method	Method A	Method B
			99.12±0.72	97.48±0.60
Zimig®*	250	98.6±1.13	t =0.87	t=1.97
			F=2.46	F=3.55
			99.62±0.67	100.30±0.62
TERBIFORCE#	250	98.8±1.20	t =1.34	t =2.47
			F=3.21	F=3.75

* Mean value of 5 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.
Marketed by: *GlaxoSmithKline, #Lifestar Pharma pvt. Ltd.

Table V. Results of recovery study *via* standard-addition method.

Tablets studied	Method A				Method B			
	TFH in tablet, µg mL^{-1}	Pure TFH added, µg mL^{-1}	Total found, µg mL^{-1}	Pure TFH recovered (Percent±SD*)	TFH in tablet, µg mL^{-1}	Pure TFH added, µg mL^{-1}	Total found, µg mL^{-1}	Pure TFH recovered (Prercent±SD*)
Zimig®*	1.5	0.75	2.25	99.73±0.87	15	7.5	22.44	99.21±0.48
	1.5	1.5	2.99	99.51±0.78	15	15	29.91	99.38±0.64
	1.5	2.25	3.74	99.47±0.81	15	22.5	37.52	100.09±0.38
TERBIFORCE#	1.5	0.75	2.25	99.44±0.50	15	7.5	22.50	100.04±0.48
	1.5	1.5	3.00	100.19±0.78	15	15	30.03	100.23±0.74
	1.5	2.25	3.73	99.23±0.41	15	22.5	37.62	100.53±0.76

*Mean value of three determinations.

Conclusion

Two simple UV-spectrophotometric methods for the determination of TFH in bulk drug and in pharmaceutical dosage form were developed and validated as per ICH guidelines. The proposed methods rely on the use of simple technique but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Stress testing is an important aspect of the drug development process. The present methods require simple and low cost instrumental setup and involve minimal manipulation producing relatively more and accurate results. From this study it can be conclude that TFH quite stable to several stress conditions such as acid, hydrolysis, dry heat treatment and photo oxidation conditions whereas it undergoes degradation under base and oxidation conditions. Moreover, the proposed methods have the advantages of simplicity and high sensitivity and are free from such experimental variables as heating or extraction step. The methods have been demonstrated to be free from interference by common tablet excipients and additives, so they can be used as alternative for rapid and routine determination of bulk sample and formulation.

Acknowledgement

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