Original Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF TERCONAZOLE

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ABSTRACT

A simple, sensitive, and accurate stability indicating RP-HPLC method has been developed and validated for estimation of Terconazole in bulk and pharmaceutical dosage form. An isocratic, reverse phase HPLC method was developed and validated using $HiQSilC_{18}$ column(250 x 4.6 mm, 5 µm) and Acetonitrile:0.05 M KH_2PO_4 buffer(pH 3.4) (60:40 v/v) as mobile phase with detection carried out at 220nm. The retention Time (t_R) for Terconazole was found to be 5.0min ± 0.03. Stress testing of Terconazole was carried out according to the International conference of harmonization (ICH) guidelines Q1A (R_2). The drug was subjected to acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validatedasperICH guidelines Q2 (R_1). The data of linear regression analysis indicated a good linear relationship over the range of 2-12 µg/ml, with correlation coefficient of 0.9998. The accuracy of the method was established based on the recovery studies. The LOD and LOQ were 0.0016 µg/ml and 0.0047 µg/ml respectively. Terconazole showed considerable degradation under alkali, oxidative and neutral hydrolytic condition.

Keywords: Terconazole, High Performance Liquid Chromatography (HPLC), Validation, Stability-Indicating Method.

INTRODUCTION

Terconazolechemically is 1-[4-[[(*2S,4S*)-2-(2,4-Dichlorophenyl)-2- (1,2,4-triazol-1-ylmethyl)- 1,3-dioxolan-4-yl] methoxy] phenyl]- 4-propan-2-yl-piperazine, isan anti-fungal medication, primarily used to treat vaginal fungal infections (Fig. 1) [1]. It is official in British pharmacopoeia [2].

Literature search reveals only UV spectrophotometric method [3] and stability indicating HPLC method [4]reported for the estimation of Terconazole in Bulk and in pharmaceutical dosage form. To the best of our knowledge, no stability indicating RP-HPLC method has been reported for Terconazole. The present work describes a simple stability indicating RP-HPLC method for the determination of Terconazole, Stability testing was carried out according to the international conference on harmonization (ICH) guidelines, Q1A (R_2) [5,6] The method was validated according to the ICH guidelines [7].

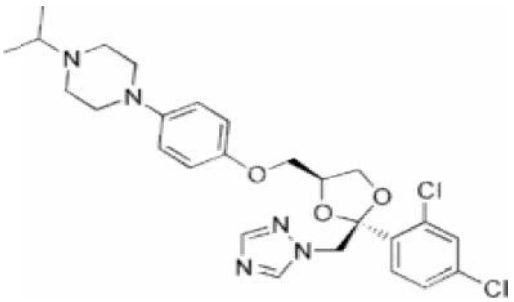

Figure 1: Structure of Terconazole

EXPERIMENTAL

MATERIALS AND METHODS

Chemicals and Reagents:

Terconazole was provided as a gift sample by Nifty Labs Pvt Ltd, Hyderabad and was used as such without any further purification.Acetonitrile (HPLC grade), KH_2PO_4(AR Grade) was purchased from S. D. fine chemical Laboratories, Mumbai, India.

Chromatographic Conditions:

HPLC system used was Agilent Model G4288A, Series 1100 Compac Lab,HPLC system comprising:G1310A- Isocratic pump,G1328A-Manual sample injection port 20 µl, HiQSilC_{18} Column, G1314A-Variable wavelength detector and Ezchrome- software.The mobile phase consisting of Acetonitrile: 0.05MKH_2PO_4 buffer (adjusted to pH 3.4 with o-phosphoric acid) in the ratio of 60:40 *v/v*, was filtered through 0.45µ membrane filter, sonicated and was pumped from the solvent reservoir. The flow rate of mobile phase was maintained at 1ml/min and the response was monitored at 220 nm with a run time of 10min.

Preparation of Standard Solution of Terconazole:

Stock solution of Terconazole was prepared by dissolving 10 mg of drug in 10 ml of Acetonitrileto get a concentration of 1000 µg/ml. From this further dilutions were made by dilutionof appropriate volume of stock solution with mobile phase to get the final concentration of 10 µg/ml.

Selection of Detection Wavelength:

From the standard stock solution further dilutions were done using mobile phase and scanned over the range of 200 - 400 nm and the spectrum was obtained. It was observed that the drug showed considerable absorbance at 220 nm. Also degradants peaks observed at 220 nm (Fig. 2).

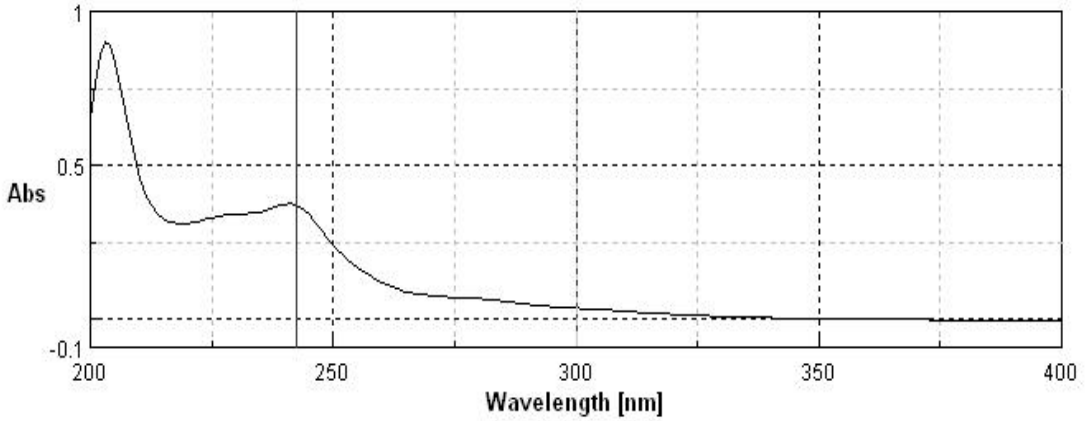

Figure 2: UV –Visible spectra of Terconazole. (10µg/ml)

Preparation of sample solution (Cream Formulation Analysis):

Formulation Analysis was carried out by preparing blend of cream as per label claim of the marketed formulation (Terazol 7 cream 0.4 %). 2.5 gm of cream (equivalent to 10 mg Terconazole) was weighed and extraction of drug was carried with Acetonitrile with vigorous shaking, the solution was filtered and volume made up to 10 ml with mobile phase. Form this solution was further diluted in mobile phase to get 4 µg/ml of drug solution, and was injected.

STRESS DEGRADATION STUDIES OF BULK DRUG

Stability studies are carried out to provide evidence on how the quality of drug varies under the influence of variety of environmental conditions like hydrolysis, oxidation, temperature, etc. and to establish specific storage conditions, shelf-life and retest period. For each studies 10 µg/ml solution was injected.

Alkaline treatment:

1 ml working standard solution of Terconazole (1000 µg/ml) was mixed with 1 ml of 1 N NaOH and volume was made upto 10 ml with Acetonitrile,Solution was kept for 24 hrs in dark place, after exposure 1ml of resultant solution was diluted to 10 ml in mobile phase and injected.

Acid treatment:

1 ml working standard solution of Terconazole (1000 µg/ml) was mixed with 1 ml of 1N HCl and volume was made upto 10 ml with Acetonitrile. Solution was kept for 24 hrs in dark place, after exposure 1ml of resultant solution was diluted to 10 ml in mobile phase and injected.

Neutral Hydrolysis:

1 ml working standard solution of Terconazole (1000 µg/ml) was mixed with 1 ml of 1ml of water and volume was made upto 10 ml with Acetonitrile,Solution was kept for 24 hrs in dark place, after exposure 1ml of resultant solution was diluted to 10 ml in mobile phase and injected.

Oxidation degradation:

1 ml working standard solution of Terconazole (1000 µg/ml) was mixed with 1 ml of 30% H_2O_2 and volume was made upto 10 ml with Acetonitrile,Solution was kept for 24 hrs in dark place, after exposure 1ml of resultant solution was diluted to 10 ml in mobile phase and injected.

Degradation under dry heat:

Dry heat study was performed by keeping Terconazole in oven (60^0 C) for a period of 8 hr. A sample was withdrawn after 8hr, weighed and dissolved in Acetonitrile to get solution of 1000 µg/ml and further diluted with mobile phase to get 10 µg/ml as final concentration and was injected.

Photo-degradation:

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hours, Sample was weighed, dissolved and diluted to get 10 µg/ml and injected.

TABLE 1: SUMMARY OF STRESS DEGRADATION STUDY OF TERCONAZOLE

Sr. No.	Stress Degradation Conditions	% Recovery	% of degradant
1	Base (1 N NaOH, Kept for 24 hr).	Complete Degradation	D1 (25.45 %) D2 (72.59 %)
2	Acid (1 N HCl,Kept for 24 hr).	76.83	No peak observed
3	Neutral (kept for 24 hr.)	69.74	D3 (16.2 %) D4 (16.58 %)
4	H_2O_2, 30% (kept for 6 hr.)	81.04	D5 (16.9 %)
5	Dry heat (60^0C for 8 hr.)	77.72	D6 (20.37%)
6	Photo stability (UV,200 watt hrs/square meter and Florescence,1.2 million Lux. Hrs)	91.53	No peak observed

RESULT AND DISCUSSION

Optimization of chromatographic conditions:

The primary target in developing this stability indicating RP-HPLC method is to achieve the resolution of Terconazole and its degradation products. This was achieved using Hi Q SilC_{18}column (250 x 4.6 mm, 5 µm) and Acetonitrile:0.05 M KH_2PO_4buffer(pH 3.4) (60:40 v/v) as mobile phase, The retention Time (t_R) for Terconazole was found to be 5.0 min ± 0.03. Forced degradation study showed the method is highly specific and no degradation products were eluted at retention time of drug (Fig. 3).Summary of stress degradation study is given in Table 1. The unaffected assay of Terconazole in the Cream confirms the stability indicating power of the method. The percent assay was found to be 98.95 ± 0.010

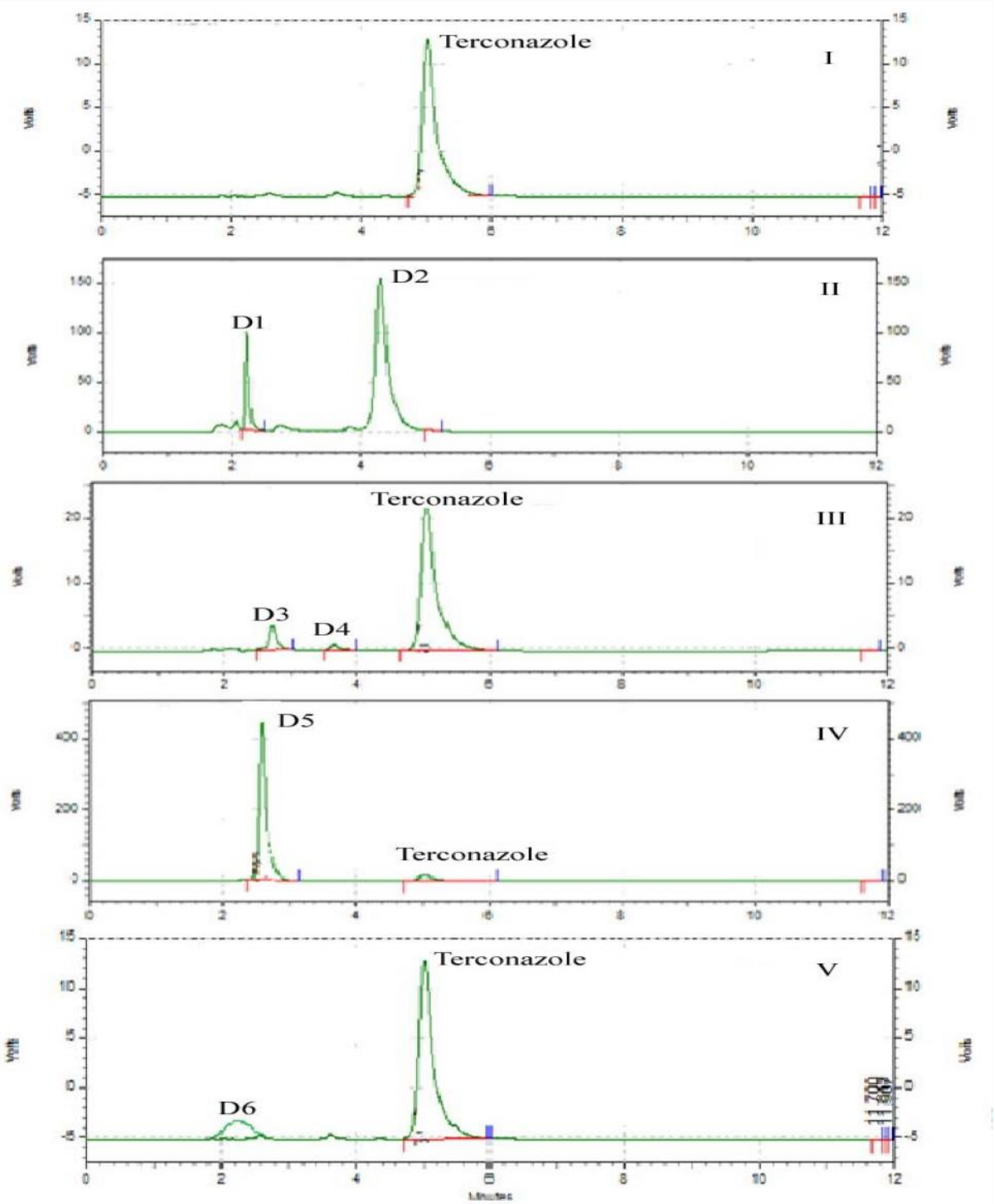

Figure 3: Chromatogram of I- Terconazole Standard, II- Alkali treated Terconazole, III- Neutral treated Terconazole, IV-oxidation treated Terconazoleand V-Under dry heat treated Terconazole.

METHOD VALIDATION

Specificity:

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 9991, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity:

The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 2-12 µg/ml for terconazole, five replicates per concentrations were analyzed, the equation of calibration curve was found to be
$y=70359x+199.03$, with $r^2=0.999$.

Precision:

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 6 replicates of Terconazole (4, 6,8 µg/ml) were analyzed in a day. For the inter day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and % RSD were calculated. % RSD for intra-day and inter-day precision was found to be in between 0.70to 1.05%and 0.13 to 0.78%(Table 2).

Accuracy:

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 4 µg/ml of Terconazole from cream solution. The % recovery was calculated from linearity equation. The results obtained are shown in Table 3.

Limit of detection (LOD) and limit of quantification (LOQ):

LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 0.0016 µg/ml and 0.0047 µg/ml.

Robustness studies:

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, pH, flow rate were altered and the effect on the area were noted.The method was found to be robust as %RSD was found to be less than 2%.

TABLE 2: INTRA-DAY AND INTER-DAY PRECISION OFTERCONAZOLE

	%Recovery (Intraday Precision)			%Recovery (Interday Precision)		
Conc. (µg/ml)	4	6	8	4	6	8
1st replicate	94.36	98.41	98.48	98.63	100	100
2nd replicate	95.82	98.67	98.48	98.63	100	100
3rd replicate	96.3	99.73	100	97.87	100	101
Mean	95.49	98.93	98.98	97.86	99.92	100.3
SD	1.01	0.69	0.87	0.76	0.13	0.57
%RSD	1.05	0.70	0.88	0.78	0.13	0.57

TABLE 3: RECOVERY STUDIES OFTERCONAZOLE

Level(%)	Sample(µg/ml)	Standard (µg/ml)	Recovered conc.	%Recovery± % RSD
50	4	2	5.96	99.38 ± 0.81
100	4	4	7.95	99.47 ± 0.52
150	4	6	9.92	98.98 ± 1.06

DISCUSSION

The developed method was found to be simple, sensitive, selective, accurate, and repeatable for analysis of Terconazole in bulk and in cream without any interference from the excipients. The results indicated the suitability of the method to study stability of Terconazole under various forced degradation conditions like hydrolysis, dry heat and photolytic degradation and in routine quantitative analysis.

CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of Terconazole in bulk drug and pharmaceutical dosage form. The developed method is specific, selective, robust, and precise.

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