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Newer RP-HPLC Method Development and Validation for the Simultaneous Estimation of Terbinafine and Itraconazole in Combined Dosage Form

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ABSTRACT

A simple, accurate, precise and stability-indicating RP-HPLC method was developed for the simultaneous estimation of terbinafine and itraconazole in the tablet dosage form. The chromatogram was run through Kromasil $C_{18,}$ column (250 x 4.6 mm, 5µm) consisting of the mobile phase, 0.01M potassium dihydrogen orthophosphate buffer (pH 4): acetonitrile took in the ratio of 50:50 v/v, pumped at a flow rate of 1.0 mL/min. The temperature was maintained at 30°C and the optimized wavelength selected for the present investigation was 250 nm. Retention time of terbinafine and itraconazole were found to be 2.289 and 2.998 minutes, respectively. LOD, LOQ values obtained from regression equations of terbinafine and itraconazole were 0.95, 2.87 and 0.24, 0.74 mcg/mL respectively. Hydrolysis, oxidation, photolysis and thermal degradation are evaluated by subjecting the drug substance to stress conditions. Good separation of the drugs and their degradation products were observed using this method. This validated method can be applied for the simultaneous estimation of itraconazole and terbinafine in a commercially available formulation sample.

INTRODUCTION

Terbinafine hydrochloride (Figure 1) is a synthetic allylamine antifungal[1, 2]. It is highly lipophilic and tends to accumulate in the skin, nails, and fatty tissues. Like other allylamines, terbinafine inhibits ergosterol synthesis by inhibiting the fungal *squalene monooxygenase (squalene 2, 3-epoxidase)*, an enzyme that is part of the fungal cell wall synthesis pathway. Itraconazole (Figure 2), is one of the triazole antifungal agents[3] that inhibits cytochrome P-450-dependent enzymes resulting in impairment of ergosterol synthesis. It has been used against histoplasmosis, blastomycosis, cryptococcal meningitis & aspergillosis**.**

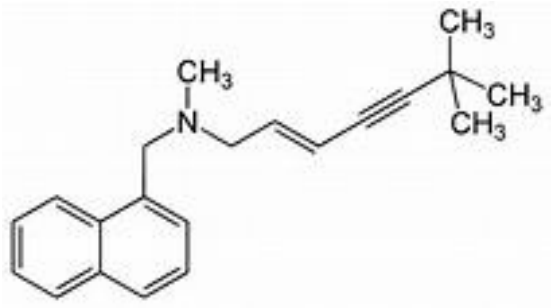


Figure No. 1: Chemical structure of terbinafine

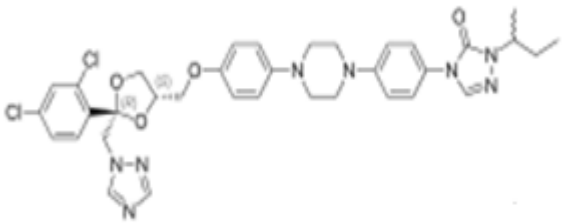


Figure No. 2: Chemical structure of Itraconazole

There are few reported HPLC and other analytical methods[4-8] for the determination of terbinafine and itraconazole in single and combination with other formulations. Recently, Rode and rao[9] have published, stability-indicating LC Method for the determination of itraconazole and terbinafine. The published method is having a retention time of 3.4 and 8.7 minutes. But the present article uses a different mobile phase and the retention time obtained for both the drugs was less than 3 minutes. The drugs were thoroughly validated as per the specifications recommended in ICH Guidelines.

MATERIALS AND METHODS

Materials and Chemicals

Authentic samples of itraconazole and terbinafine were procured from Dr. Reddy's Laboratories Limited (Hyderabad, India). Itroterb tablets (containing itraconazole (100mg) and terbinafine (250 mg) were manufactured by Fourrts India Laboratories Ltd, India) procured from a local pharmacy. HPLC-grade chemicals were purchased from Spectrochem Pvt. Ltd. (Mumbai, India). potassium dihydrogen orthophosphate (Rankem, Mumbai, India) and orthophosphoric acid (Qualigens Fine Chemicals, Mumbai, India) were analytical reagent grade.

Apparatus

Chromatography was performed on a Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with an isocratic HPLC pump (Shimadzu LC-20AT) and a Photodiode array detector (Shimadzu SPD-20AV) with a Rheodyne syringe-loading sample fixed loop (20 µL) injector (7725). The LC separations were performed at ambient temperature on a Kromasil $C_{18,}$ column (250 x 4.6 mm, 5µm), (Torrance, CA). Data were acquired and processed by the use of Spinchrom (CFR version 2.4.1.93) software. Degassing of the mobile phase was done by sonication in an Ultrasonic bath (Ultrasonics Selec, Vetra, Italy). The standard substances were weighed on a Precisa (205 ASCS Swiss Quality, Switzerland) analytical balance. Photostability studies were carried out in a photostability (NEC-103R Neutronic, Mumbai, India) chamber, which was set at 25ºC ± 1ºC. A thermal stability study was carried out in a hot air oven (Sedko Laboratory, Equipment, Ahmedabad, India).

Chromatographic separations

HPLC studies were individually carried out for all the reaction solutions and in a mixture of the solutions in which decomposition was observed. The separation was carried out under isocratic elution with potassium dihydrogen orthophosphate buffer (adjusted pH 4 with 0.5% orthophosphoric acid)–acetonitrile (50:50 v/v) as the mobile phase. The mobile phase was filtered through a 0.45-µm nylon filter and degassed before use. The flow rate was 1 mL/min, and the detection wavelength was 260 nm.

Preparation of standard stock solutions: Accurately weighed 62.5 mg of terbinafine, 25 mg of itraconazole transferred to 25ml volumetric flask and 3/4th of diluents was added to these flasks and sonicated for 10 minutes. Flask was made up with diluents (Water: Acetonitrile, 50:50) and labeled as a standard stock solution (2500µg/ml of terbinafine and 1000 µg/ml of itraconazole).

Preparation of standard working solutions: 1ml from each stock solution was pipetted out and taken into a 10 mL volumetric flask and made up with diluent to get the final concentration of 250µg/ml of terbinafine and 100 µg/ml of itraconazole.

Preparation of sample stock solutions: 20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to one tablet was transferred into a 100 mL volumetric flask, 50 mL of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters to get the concentration of (2500µg/ml of terbinafine and 1000 µg/ml of itraconazole).

Preparation of Sample working solutions: 0.5ml of filtered sample stock solution was transferred to a 10 mL volumetric flask and made up with diluent to get the concentration of 250µg/ml of terbinafine and 100 µg/ml of itraconazole.

Method validation

The developed method was validated according to ICH guidelines[10-12] concerning system suitability parameters, specificity, precision, linearity, accuracy, Limit of detection (LOD), Limit of quantitation (LOQ). The system suitability parameters were determined by preparing standard solutions of terbinafine (250 ppm) and itraconazole (100 ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%. The specificity of the method is found to be correct if the interfering peaks in blank and placebo at retention times of these drugs were not found. The linearity of the system was studied by preparing the standard stock solution ranging between 62.5 to 375 µg/ml and 25- 150 µg/ml for terbinafine and itraconazole. Accuracy was carried out by % recovery studies of Terbinafine and Itraconazole at three different concentration levels (50%, 100%, and 150%). Precision was carried out by inter and intraday analysis. Robustness of the method was carried out by small deliberate changes in a method like flow rate, mobile phase ratio, and temperature but no recognized change in the method would be found. Robustness

conditions like Flow minus (0.9 ml/min), Flow plus (1.1ml/min), mobile phase minus (65Buffer: 35 Acetonitrile), mobile phase plus (55Buffer:45Acetonitrile), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate and %RSD was calculated. The LOD and LOQ value can be obtained by the calibration curve and the assay was carried out in triplicate (*n*= 3) at three different concentration levels for terbinafine and itraconazole respectively.

Forced Degradation studies

Oxidation degradation:

To 1 ml of stock solution of terbinafine and itraconazole, 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min. For the HPLC study, the resultant solution was diluted to obtain 250 µg/ml & 100µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Acid degradation:

To 1 ml of stocks solution terbinafine and itraconazole, 1mL of 2N Hydrochloric acid was added and refluxed for 30 mins. The resultant solution was diluted to obtain 250 µg/mL & 100µg/mL solution and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Alkali degradation:

To 1 ml of stock solution terbinafine and itraconazole, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins. The resultant solution was diluted to obtain 250 µg/ml & 100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Dry Heat degradation (Thermal):

The standard drug solution was placed in oven at105°C for 1hour to study dry heat degradation. For the HPLC study, the resultant solution was diluted to 250 µg/ml & 100µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photolytic degradation:

The photochemical stability of the drug was also studied by exposing the 2500µg/ml & 1000µg/ml to UV Light by keeping the beaker in UV Chamber for 1day or 4000 Watt-hours/m2 in photostability chamber. For the HPLC study, the resultant solution was diluted to obtain 300 µg/ml & 50µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral Degradation:

Stress testing under neutral conditions was studied by refluxing the drug in water for 1 hour at a temperature of 60º. For the HPLC study, the resultant solution was diluted to 250 µg/ml & 100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

HPLC method development and optimization

Different parameters like buffer, organic modifier ratio, and pH were optimized[13] to achieve good separation between terbinafine and itraconazole, and the degradation products formed under various conditions. Initial studies on individual reaction solutions were carried out using buffer–methanol (50:50 v/v) as the mobile phase. Several studies were carried out by decreasing the percentage of methanol from 50% to 35% until a satisfactory resolution was obtained. Another attempt was made by substituting HPLC water with methanol. The advantages observed were smoothening of baseline, but peaks were not well resolved. After several attempts, it was found that good resolution was obtained with 50% buffer (potassium dihydrogen orthophosphate adjusted with pH 4), and 50% acetonitrile gave sufficient separation as well as symmetrical peak shape. So, finally, the above mobile phase was selected for both validations as well as for assay. It was then applied to a mixture of those stressed samples in which there were recognizable different degradation products were formed. The method worked well with the mixture of degradation solutions and was even applicable to formulations.

Validation of the method

The method was validated concerning the following parameters.

Linearity. Linear calibration plots of each drug for the previously mentioned method were obtained over the calibration ranges 25–150 µg/mL and 62.5–375 µg/mL itraconazole and terbinafine respectively; the correlation coefficient obtained was greater than 0.999 for both drugs (Table 1). The results show that a good correlation existed between the peak area and concentration of the analyte.

Table No. 1: Linearity table of terbinafine and itraconazole.

Terbinafine		Itraconazole	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
62.5	549110	25	209523
125	1072833	50	410173
187.5	1589712	75	611744
250	2147753	100	827802
312.5	2673684	125	1032449
375	3184415	150	1229481

LOD and LOQ. The LOD values for terbinafine and itraconazole were shown in (Table 2).

Table No. 2: LOD and LOQ value of terbinafine and itraconazole

Molecule	LOD(mcg/ml)	LOQ(mcg/ml)
Terbinafine	0.95	2.87
Itraconazole	0.24	0.74

Precision. Data obtained from the analysis of the samples on the same day (n = 3) and consecutive days (n = 3) are given in Table 3. As evident, the % RSD values of the data obtained were well below 2%.

Table No. 3: System precision table of terbinafine and itraconazole

Sr. No.	Area of terbinafine	Area of itraconazole
1.	2202273	847467
2.	2214284	854513
3.	2179595	841914
4.	2182257	840124
5.	2182304	835717
6.	2222395	852575
Mean	2197185	845385
S.D	18479.7	7383.1
% RSD	0.8	0.9

Accuracy. Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. As shown from the data in Table 4, good recoveries were made at each added concentration, confirming that the method was accurate.

Table No. 4: Accuracy study of terbinafine and itraconazole

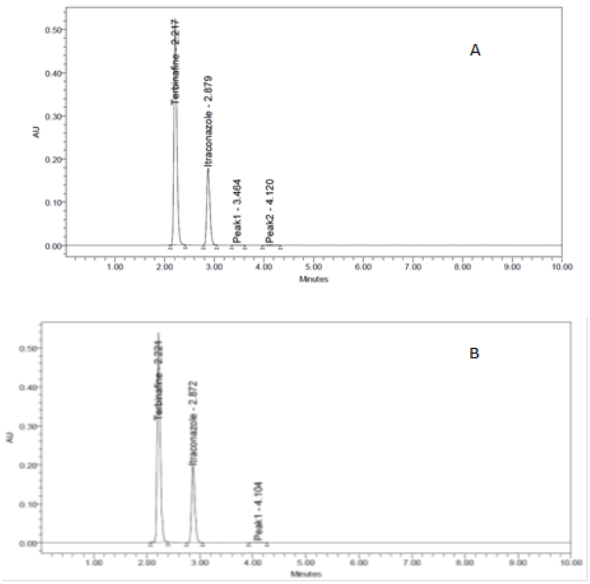
% level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% recovery	Mean % Recovery	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% recovery	Mean % Recovery
50%	125	124.75	99.80	99.66%	50	50.47	100.94	99.58%
	125	124.66	99.72		50	49.44	98.87	
	125	125.01	100.01		50	49.52	99.05	
100%	250	248.87	99.55		100	99.35	99.35	
	250	248.56	99.42		100	98.53	98.53	
	250	247.88	99.15		100	99.04	99.04	
150%	375	375.08	100.02		150	149.60	99.73	
	375	373.26	99.54		150	150.32	100.21	
	375	374.01	99.74		150	150.79	100.53	

Degradation studies. A good resolution was obtained between the drugs and the degradation products formed under the various stress conditions, indicating the specificity of the method. The resolution factor (Rs) from acidic, alkaline, neutral, oxidative, and thermal degradation

products was always ≥ 1.8, which ensured the complete separation of itraconazole and terbinafine from their degradation products. The drugs underwent degradation in acidic, basic, oxidative, thermal, neutral and photolytic degradation (Table 5 & Figure 3). Studies performed to determine the purity of terbinafine and itraconazole peaks using a PDA detector showed purity angle (PA) values of 0.074 and 0.063 and purity threshold (TH) values of 0.256 and 0.272 for terbinafine and itraconazole respectively. The PA value was found to be less than the TH value, indicating that the terbinafine and itraconazole were free from any co-eluting peak.

Table No. 5: Degradation data of terbinafine and itraconazole

Type of degradation	Terbinafine			Itraconazole		
	Peak area	% of drug recovered	% of drug degraded	Peak area	% of drug recovered	% of drug degraded
Acid	2059259	93.35	6.65	792242	93.34	6.66
Base	2116310	95.93	4.07	811257	95.58	4.42
Peroxide	2112761	95.77	4.23	814413	95.95	4.05
Thermal	2170551	98.39	1.61	832115	98.04	1.96
UV	2174370	98.57	1.43	832239	98.05	1.95
Neutral	2188908	99.22	0.78	841029	99.09	0.91



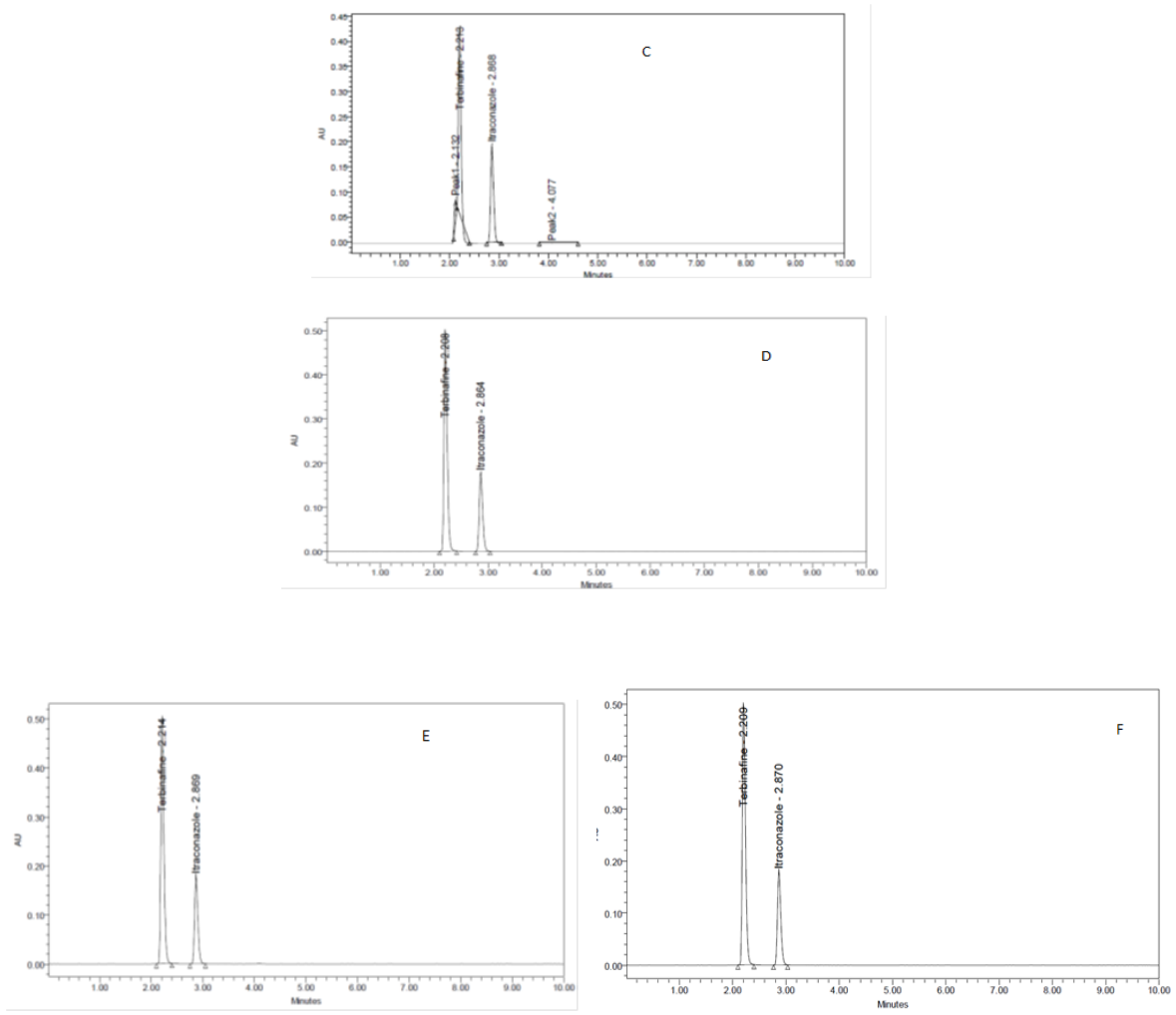


Figure No. 3: Forced degradation chromatograms of terbinafine and itraconazole (A: Acid degradation, B: Base degradation, C: Peroxide degradation, D: Thermal degradation, E: UV degradation, F: Neutral degradation)

Robustness. The results presented in Table 6 indicate that the selected factors remained unaffected by a slight variation of these parameters. It was also found that acetonitrile from the different manufacturers does not have a significant influence on the determination. Insignificant differences in peak areas and less variability in retention times were observed.

Table No. 6: Robustness data for itraconazole and terbinafine.

Sr. No.	Condition	%RSD of terbinafine	%RSD of itraconazole
1	Flow rate (-) 0.9ml/min	0.2	0.2
2	Flow rate (+) 1.1ml/min	0.9	0.8
3	Mobile phase (-) 65B:35A	0.2	0.2
4	Mobile phase (+) 55B:45A	0.4	0.2
5	Temperature (-) 25°C	0.4	0.4
6	Temperature (+) 35°C	1.3	1.4

B: Buffer and A: Acetonitrile

System suitability. The results (Table 7) obtained from system suitability tests agree with the United States Pharmacopoeia requirements. The variation in retention times among six replicate injections of itraconazole and terbinafine standard solutions was very low, rendering RSD of less than 2 %, respectively.

Table no 7: System suitability parameters for itraconazole and terbinafine

Sr. No.	Terbinafine			Itraconazole			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.218	7757	1.28	2.879	9404	1.20	6.0
2	2.222	7763	1.28	2.883	9973	1.19	5.8
3	2.224	7214	1.24	2.884	10323	1.19	5.8
4	2.224	7067	1.28	2.886	11121	1.18	5.9
5	2.234	7758	1.22	2.924	9819	1.21	6.3
6	2.289	7245	1.30	2.998	9545	1.21	6.0

Applicability of the developed method to marketed formulation

The developed method (Figure 4a) was successfully applied to analyze itraconazole and terbinafine in the marketed formulation (Figure 4b). A clear separation of the drugs and degradation products was achieved in the tablet with no interference from excipients (Table 8).

Table No 8: Analysis of tablets containing terbinafine and itraconazole in combination (n=3)

Tablet	Drug (mg/tab)	% Drug Obtained ± SD	Std error of estimation
Itroterb tablets	Terbinafine (250 mg)	**99.38±0.15**	**0.109**
	Itraconazole (100mg)	**98.31±0.16**	**0.114**

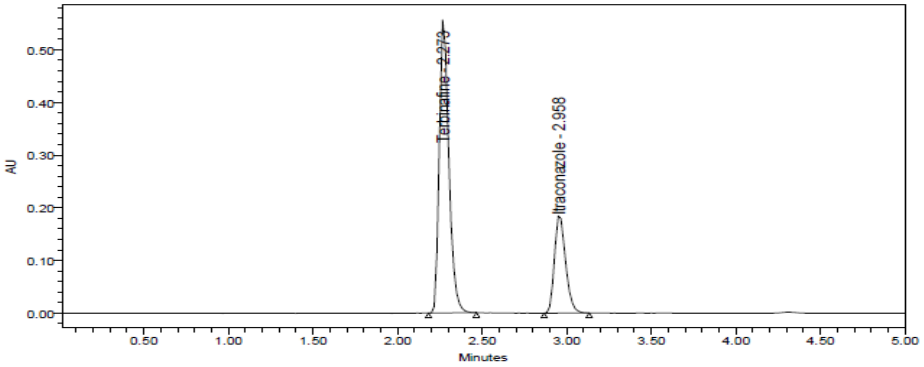


Figure No 4 a: Standard Chromatogram of terbinafine and itraconazole (showing retention times at 2.273 and 2.958 minutes, respectively)

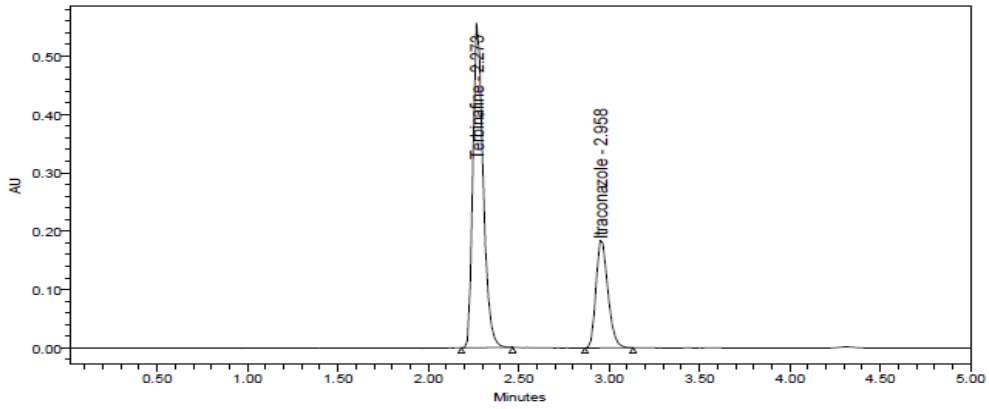


Figure No. 4 b: Sample Chromatogram of terbinafine and itraconazole (showing retention times at 2.273 and 2.958 minutes, respectively)

CONCLUSION

In this study, terbinafine and itraconazole were subjected to stress studies under various ICH-recommended conditions. The additional findings in this study show that the drugs underwent degradation in acidic, basic, oxidative, thermal, photolytic and neutral degradations. The method was validated for parameters like linearity, accuracy, specificity, robustness, and

system suitability. The application of this method for the analysis of terbinafine and itraconazole in tablet dosage form shows that there is no interference of excipients or degradation products in the analytical determination. Thus, the proposed method could be regarded as the stability-indicating method for the simultaneous estimation of terbinafine and itraconazole either in bulk drug or in pharmaceutical formulations.

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Conflicts of interest: Nil

REFERENCES

1. Kumudhavalli V. Isocratic RP-HPLC, UV method development and validation of itraconazole in capsule dosage form. Int J Pharm Sci Res 2011; 2:3269-71.
2. Gupta A, Bharti T, Salahuddin NN. Analytical method development and validation of terbinafine HCl in formulated product using reverse phase ultra-performance liquid chromatography (RP-UPLC). Eur J Biomed Pharm Sci 2016:3:542-7.
3. Available from: https://www.1mg.com/generics/itraconazole-terbinafine-405211.
4. Rao TM, Ratna VJ, Rao SY, Kumar HT. Development and validation of RP-HPLC method for the determination of itraconazole in bulk and capsule dosage form. Int J Pharm Sci Rev Res 2015;31:39, 221-5.
5. Gupta MK, Rajput S. Development and validation of RP-HPLC method for quantitation of itraconazole in tablets dosage form. Int J Pharm Res Rev 2015;4:23-9.
6. Hamsa K, Mohamed AA**.** High-performance liquid chromatography method for the determination of terbinafine hydrochloride in the semi-solids dosage form. Int J Pharm Sci Rev Res 2013;21:58-61.
7. Patel MM, Patel HD, Development and validation of RP-HPLC method for simultaneous estimation of terbinafine hydrochloride and mometasone furoate in the combined dosage form. Int J Pharm Pharm Sci 2016;6:106-9.
8. Rao DD, Sait SS, Reddy SP, Mukkanti K. A stability-indicating LC method for the assay estimation of itraconazole in pharmaceutical dosage form. Anal Chem Indian J 2009;8:271-6.
9. Devyani M Rode, Dr. Nutan Rao. Stability indicating method development and validation of itraconazole and terbinafine HCl in bulk and pharmaceutical tablet dosage form. *Asian J Pharm Clin Res, Vol 12, Issue 9, 2019, 51-55.*
10. ICH Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonisation 2005.
11. ICH Stability Testing: Photostability Testing of New Drug Substances and Products Q1B, International Conference on Harmonisation 2005.
12. ICH Stability Testing of New Drug Substances and Products Q1A (R2). International Conference on Harmonisation 2005.
13. The United States Pharmacopoeia, USP-24, NF-19, United States Pharmacopoeial Convention, INC, Rockville, MD, Asian Edition, 2000, pp. 2149- 51.