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Research Article

STABILITY-INDICATING METHOD DEVELOPMENT AND VALIDATION OF ITRACONAZOLE AND TERBINAFINE HCL IN BULK AND PHARMACEUTICAL TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective of the present work was to develop and validate the stability-indicating method for the simultaneous estimation of itraconazole and terbinafine HCl in bulk and pharmaceutical tablet dosage form by reversed-phase high-performance liquid chromatography (HPLC). This combination of drugs is not reported for simultaneous HPLC analysis as of now.

Methods: The analysis of the developed method was carried on Shimadzu LC Prominence-i 2030 model with Lab Solution software and the separation was done on Shim-pack C18 GIST (250 mm×50 mm, 5 µm) column with a flow rate of 1.2 ml/min and run time of 12 min. The injection volume was 10 µl and mobile phase consisted of acetonitrile and 0.1% triethylamine in the ratio of 90:10 and 225 nm was used as a detection wavelength.

Results: The retention time was found to be 3.464 min and 8.705 min for itraconazole and terbinafine HCl, respectively. The calibration curve was found to be linear and r^2 values were 0.9989 and 0.9995 for itraconazole and terbinafine HCl, respectively.

Conclusion: The stability-indicating method was developed by subjecting itraconazole and terbinafine HCl marketed formulation to various stress conditions such as acidic, basic, oxidative, thermal, and water hydrolysis degradation conditions and the degraded product peaks were well resolved from sample peaks.

Keywords: Itraconazole, Terbinafine HCl, Reversed-phase high-performance liquid chromatography, Validation, Degradation.

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INTRODUCTION

Both itraconazole and terbinafine HCl are antifungal drugs. The International Union of Pure and Applied Chemistry name of itraconazole and terbinafine HCl is 4-[4-[4-[4-[[cis-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3dioxolan-4-yl] methoxy] phenyl] piperazin-1-yl] phenyl]-2-[(1RS)-1methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one] and (E)-N,6,6-trimethyl-N-(naphthalen-1-ylmethyl) hept-2-en-4-yn-1 amine hydrochloride respectively. The chemical formula of itraconazole and terbinafine HCl is $C_{35}H_{38}C_{l2}N_8O_4$ and $C_{21}H_{25}N{\cdot}HCl$, respectively, and molecular weight is 706 g/mol and 327.89084 g/mol, respectively [1,2]. Itraconazole and terbinafine HCl both are freely soluble in acetonitrile, methanol, and dimethyl sulfoxide but insoluble in water [1,2]. The chemical structure of both drugs is given in Figs. 1 and 2.

Combination of itraconazole and terbinafine HCl is used for the treatment of antifungal infections such as toenail onychomycosis and it stops the growth of fungi by preventing covering [3]. The literature survey reveals that there is no reversed-phase high-performance liquid chromatography (RP-HPLC) method reported for the estimation of itraconazole and terbinafine HCl in tablet dosage form [4-8]. Thus, the present work was carried out to develop novel, precise, accurate, rapid, and cost-effective stability-indicating method and to validate the method for simultaneous estimation of itraconazole and terbinafine HCl in tablet dosage form and its application for the separation of the peak of a degradation product.

METHODS

Instrument

RP-HPLC Shimadzu LC Prominence-i 2030 model and Lab Solution software were used for stability-indicating method development and validation of itraconazole and terbinafine HCl. The BIO-LAB (BL-135 D) centrifuge model was used for centrifugation of sample; Kroma Tech (KL-1.5) sonicator was used for sonication and hot air oven EXPO HI-TECH was used for thermal degradation.

Chemicals and reagents

Itraconazole and terbinafine HCl were provided by Alkem Laboratories, Navi Mumbai, Maharashtra, India, and commercial tablet dosage form Duofaze was purchased from a local market. The HPLC grade acetonitrile was purchased from Qualigens Thermo Fisher Scientific. Analytical grade triethylamine (TEA), hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from S.D Fine Chemicals.

Chromatographic conditions

The separation of itraconazole and terbinafine HCl was carried out using Shimadzu RP-HPLC system with Shim-pack GIST C18 (250 mm×4.6 mm, 5 µ) column. The mobile phase used was acetonitrile and 0.1% TEA in the ratio (90:10) at a flow rate of 1.2 ml/min, injection volume was 10 µl, column temperature was (30°C), and itraconazole and terbinafine HCl were detected at 225 nm using an ultraviolet (UV)-visible detector.

Selection of wavelength

Standard solutions of itraconazole (10 ppm) and terbinafine hydrochloride (10 ppm) were prepared and scanned by UV spectrophotometer separately, in the range of 200–400 nm and overlay UV spectra of itraconazole and terbinafine HCl obtained are shown in Fig. 3. The 225 nm wavelength was selected as detection wavelength for the separation of itraconazole and terbinafine HCl.

Preparation of 0.1% TEA

Add 1 ml of TEA in 1000 ml of deionized water.

Preparation of mobile phase

A mixture of 90 volumes of HPLC grade acetonitrile and 10 volumes of TEA was prepared and sonicated for 10–15 min to degas.

Preparation of standard solution

Standard solution of itraconazole and terbinafine HCl was prepared by dissolving 10 mg of itraconazole and 25 mg of terbinafine hydrochloride reference standards into 250 ml volumetric flask. About 150 ml of acetonitrile was added as a diluent and sonicated for 15–20 min and the volume was made up to the mark using acetonitrile, to obtain a concentration of 40 ppm of itraconazole and 100 ppm of terbinafine hydrochloride, respectively.

Preparation of sample solution

Ten tablets were weighed and finely powdered and quantity corresponding to 80 mg of (itraconazole + terbinafine HCl) was taken and transferred to a 250 ml volumetric flask and 150 ml of diluent was added. The flask was sonicated for 30–45 min with intermittent shaking. Volume was adjusted up to the mark with diluent. Sample solution was centrifuged at 5000 rpm for 10 min and then filtered through Whatman filter paper.

Method validation

The developed method for itraconazole and terbinafine HCl was validated for parameters such as system suitability, precision, linearity, accuracy, robustness, and solution stability as per ICH guidelines [9-12].

Forced degradation studies

Forced degradation is the process, in which pure drug and drug products are subjected to chemical and environmental stress conditions to know the degradation pathway of drug and degradation products which can be used to determine the stability of the drug [13]. For acid and alkali stress conditions, 5 ml of 0.1 N HCl and 0.1 N NaOH were added, respectively, and kept at 60°C for 1 h, for oxidative degradation, 5 ml of 30% H_2O_2 was added and kept at 60°C for 1 h, and 5 ml of water added and kept at 60°C for 1 h for water hydrolysis degradation. Thermal degradation was performed by keeping the sample in a Petri dish and then placed them in an oven at 60°C for 1 h.

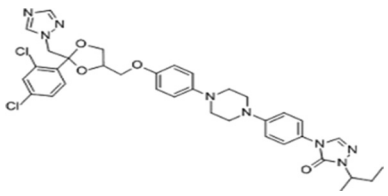

Fig. 1: Chemical structure of itraconazole

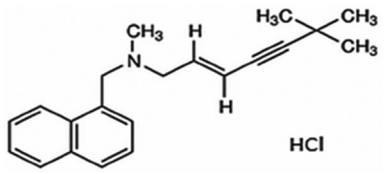

Fig. 2: Chemical structure of terbinafine HCl

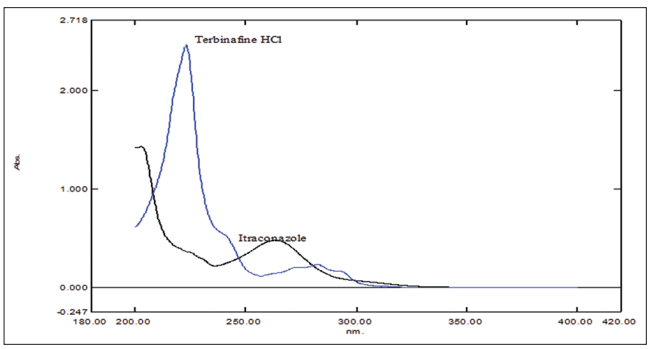

Fig. 3: Ultraviolet overlain spectra of itraconazole and terbinafine HCl

and kept at 60°C for 1 h for water hydrolysis degradation. Thermal degradation was performed by keeping the sample in a Petri dish and then placed them in an oven at 60°C for 1 h.

RESULTS AND DISCUSSION

Method development

A series of trials were carried out using different mobile phases such as acetonitrile:water (50:50), methanol:water (50:50), and acetonitrile:1% oil pollution act (90:10) and using different columns such as Inertsil ODS, Prontosil, and Shim-pack C18 to develop RP-HPLC method for simultaneous estimation of itraconazole and terbinafine HCl in marketed tablet dosage form. Finally, a typical chromatogram was obtained using acetonitrile and 0.1% TEA as mobile phase in a ratio of 90:10 on Shim-pack GIST C18 (250 mm×4.6 mm, 5 µ) column and injection volume of 10 µl. The flow rate was 1.2 ml/min and the run time was 12 min. The column temperature was 30°C and detection was carried out at 225 nm. The retention time was 3.4 min and 8.7 min for itraconazole and terbinafine HCl, respectively. Typical chromatograms of standard and sample solution of itraconazole and terbinafine HCl are shown in Figs. 4 and 5. The same developed method was applied for forced degradation studies of itraconazole and terbinafine HCl marketed tablet dosage form, and degraded product peak was well separated using this developed method. The optimized chromatographic conditions are tabulated in Table 1.

Table 1: Optimized chromatographic conditions for itraconazole and terbinafine HCl

Parameters	Optimized conditions
Column	Shimpack C18 (250 mm×4.6 mm, 5 µ)
Mobile phase	Acetonitrile and 0.1% triethylamine in the ratio of 90:10
Diluent	Acetonitrile
Column temperature	30°C
Wavelength	225 nm
Flow rate	1.2 ml/min
Injection volume	10 µl
Run time	12 min
Retention time	3.464 and 8.705 min

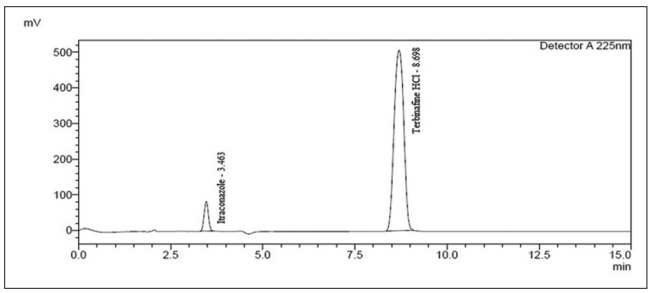

Fig. 4: Typical chromatogram of a standard mixture of itraconazole and terbinafine HCl

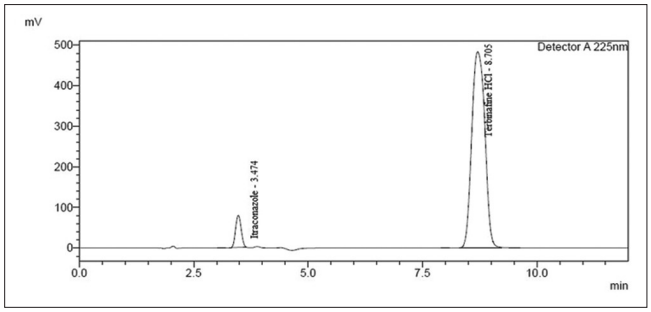

Fig. 5: Typical chromatogram of a sample of itraconazole and terbinafine HCl

System suitability

System suitability was done by injecting six replicates injection of the standard solution and retention time, tailing factor, and number of theoretical plate were evaluated. The standard solutions of itraconazole and terbinafine HCl were prepared as per the above method and injected into a chromatographic system. System suitability parameters such as number of theoretical plates, tailing factor, and resolution were evaluated. All the results of system suitability parameter are tabulated in Table 2 and all parameter results are within the limit.

Precision

The precision of an analytical procedure may be defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision and method precision were performed by injecting six injections of itraconazole and terbinafine HCl standard and sample of the same concentration [14]. The percentage relative standard deviation (% RSD) was calculated from the chromatogram area and it is <2%. From precision results, it was found that the method is precise. The data of system and method precision are tabulated in Table 3.

Accuracy

The accuracy of itraconazole and terbinafine HCl was performed by calculating recovery studies of the test sample at three different concentration levels (50%, 100%, and 150%) by the standard addition method. At each level, three replicates were injected into a chromatographic system. The mean percentage recovery for itraconazole and terbinafine HCl was found within a limit of 98–101%, and from percentage recovery results, it was found that the developed method is accurate. The percentage recovery results are tabulated in Tables 4 and 5.

Table 2: System suitability parameters

Parameters	Itraconazole	Terbinafine HCl
Retention time	3.469	8.705
Tailing factor	1.08	1.15
Number of theoretical plate	3806	5276

Table 3: System precision results

S. No.	Itraconazole (40 ppm)	Terbinafine HCl (100 ppm)
	Peak area	Peak area
1.	662,869	9,209,276
2.	663,860	9,203,549
3.	667,701	9,196,756
4.	663,904	9,203,818
5.	667,018	9,226,983
6.	667,498	9,235,940
Average	665,475	9,212,720
SD	2159	15,314
% RSD	0.32	0.17

SD: Standard deviation, RSD: Relative standard deviation

Table 4: Method precision results

S. No.	Itraconazole	Terbinafine HCl
	% assay	% assay
1.	101.2	99.3
2.	102.0	99.6
3.	101.7	100.0
4.	101.5	99.9
5.	101.4	99.5
6.	101.0	98.7
Average	101.5	99.5
SD	0.36	0.47
% RSD	0.35	0.47

SD: Standard deviation, RSD: Relative standard deviation

Linearity

The linearity of the developed method was determined at different concentration levels ranging from 20 ppm to 60 ppm for itraconazole and from 50 ppm to 150 ppm for terbinafine HCl. The linearity curve was constructed by plotting peak area versus concentration and the regression coefficient (r^2) was found to be 0.9989 for itraconazole and 0.9995 for terbinafine hydrochloride. From linearity results, it was found that the developed method is linear (Figs. 6 and 7). Results are shown in Tables 6 and 7.

Robustness

The developed method was evaluated for robustness by small deliberate changes in optimized method parameters which were done such as

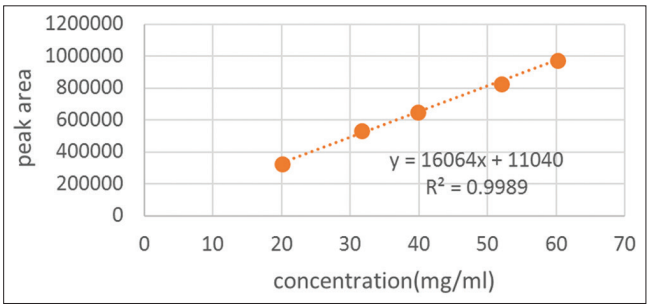

Fig. 6: Linearity graph of itraconazole

Table 5: % recovery results for itraconazole

Level	% recovery	Average	SD	% RSD
50%	99.0 100.4 99.6	99.7	0.70	0.70
100%	99.9 99.6 100.1	99.9	0.25	0.25
150%	99.5 99.8 99	99.4	0.40	0.40

SD: Standard deviation, RSD: Relative standard deviation

Table 6: % recovery results for terbinafine HCl

Level	% recovery	Average	SD	% RSD
50%	99.7 98.8 99.6	99.4	0.49	0.49
100%	99.9 100 99.9	99.9	0.06	0.06
150%	100.4 98.4 99.1	99.3	1.01	1.01

SD: Standard deviation, RSD: Relative standard deviation

Table 7: Linearity results for itraconazole and terbinafine HCl

Concentration (ppm)		Area	
Itraconazole	Terbinafine HCl	Itraconazole	Terbinafine HCl
20	50	331,698	4,371,822
32	80	530,320	7,242,916
40	100	652,900	9,245,345
52	120	833,091	11,074,374
60	150	984,345	13,763,467
Slope	Slope	16,064	94,174
Intercept	Intercept	11,040	−277,853
Correlation	Correlation	0.9989	0.9995

flow rate (±0.2 ml), wavelength (±2 nm), and temperature (±2°C) [15]. It was found that none of the above parameters caused an alteration in the peak area and retention time. The % RSD was found to be within the limits, and the method was found to be robust. The robustness results are shown in Table 8.

Solution stability

Sample solution of itraconazole and terbinafine HCl was injected at different time intervals and percentage assay was calculated. The solution stability of 24 h shows that the sample solution can be used over a period of 24 h without any degradation of the solution and solution stability results are shown in Table 9.

Assay of marketed formulation

For analysis of marketed formulation (Duofaze: 100 mg itraconazole and 250 mg terbinafine hydrochloride), 10 tablets were weighed and finely powdered was weighed the quantity of powder containing 80 mg of (itraconazole + terbinafine HCl) and transfer to a 250 ml volumetric flask and 150 ml of diluent was added. The flask was sonicated for 30–45 min with intermittent shaking. Volume was adjusted up to mark with diluent. The sample solution was centrifuged at 5000 rpm for 10 min and filtered through Whatman filter paper. The percentage assay for the marketed formulation was found to be 100.5% for itraconazole and 99.8% for terbinafine HCl as shown in Table 10.

Forced degradation studies

Forced degradation studies were carried out on itraconazole and terbinafine HCl marketed tablet formulation by treating the marketed formulation under stress conditions such as acidic, alkaline, hydrolysis, thermal, and oxidative conditions to estimate the ability of the developed method to separate itraconazole and terbinafine HCl from its degradation products as shown in Figs. 8-12. The forced degradation results are within the limit and it is tabulated in Table 11.

Acid degradation

In acid degradation condition (0.1 N HCl), both itraconazole and terbinafine HCl degraded and degradation was 17.2% and 0.1% for itraconazole and terbinafine HCl, respectively, and no peak of degradation of the product was observed in the chromatogram (Fig. 8).

Table 8: Robustness results for itraconazole and terbinafine HCl

Parameters	Itraconazole		Terbinafine HCl	
	RT	% assay	RT	% assay
Minus flow (1.0 ml/min)	4.145	101.1	10.410	99.9
Plus flow (1.4 ml/min)	2.985	101	7.432	99.7
Minus temperature (28°C)	3.485	101.4	8.747	100.1
Plus temperature (32°C)	3.483	100.7	8.744	98.7
Minus wavelength (223 nm)	3.489	101.7	8.742	100.2
Plus wavelength (227 nm)	3.487	100	8.729	98.2

RT: Retention time

Table 9: Solution stability results

Time interval	Itraconazole	Terbinafine HCl
	% assay	% assay
Initial	101.8	100.6
6 h	102.4	100.7
16 h	101.7	100.4
24 h	101.4	99.9

Table 10: % assay of marketed formulation

Tablet	Drug	% assay
Duofaze (itraconazole 100 mg+ terbinafine HCl 250 mg)	Itraconazole Terbinafine HCl	100.5 99.8

Base degradation

In alkali degradation (0.1 N NaOH), both itraconazole and terbinafine HCl degraded and degradation was 4.3% and 0.7% for itraconazole and terbinafine HCl, respectively, and no peak of degradation of the product was observed in the chromatogram (Fig. 9).

Water hydrolysis degradation

In hydrolysis degradation, both itraconazole and terbinafine HCl did not get degrade and percentage degradation was 0.05% and 0.4% for itraconazole and terbinafine HCl, respectively; no peak of degradation of the product was observed in the chromatogram (Fig. 10).

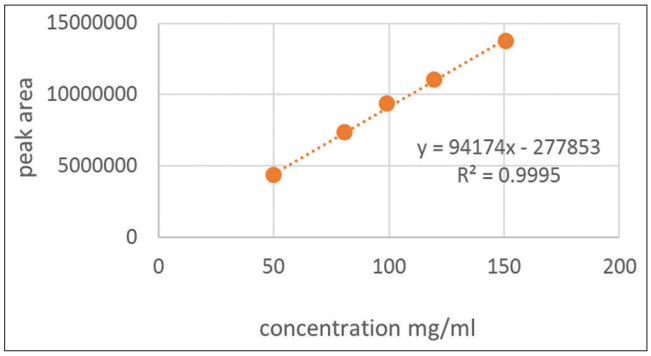

Fig. 7: Linearity graph of terbinafine HCl

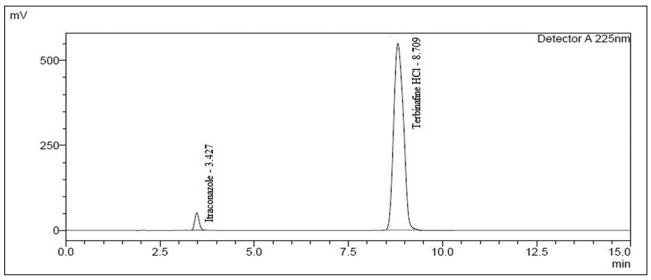

Fig. 8: Chromatogram of acid degradation

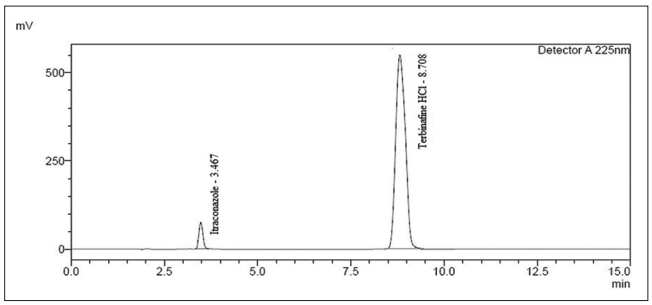

Fig. 9: Chromatogram of base degradation

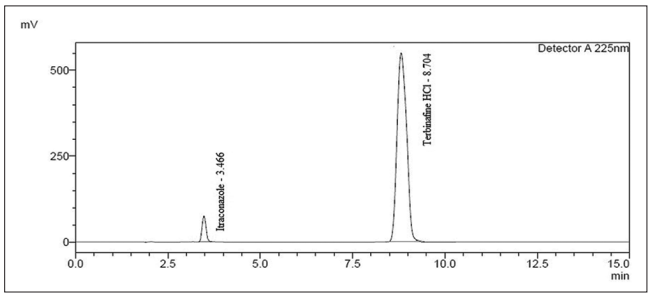

Fig. 10: Chromatogram of water hydrolysis degradation

Table 11: Forced degradation studies results of itraconazole and terbinafine HCl

Conditions	Itraconazole		Terbinafine HCl	
	% assay	Difference w.r.t. control	% assay	Difference w.r.t. control
Control sample	101.5	NA	99.5	NA
Acid-treated sample	84.2	17.2	99.3	0.1
Base-treated sample	97.1	4.3	98.7	0.7
Water-treated sample	101.4	0.05	99.0	0.4
Heat-treated sample	101.3	0.1	99.4	0.09
Peroxide-treated sample	85.4	16.0	87.4	12.0

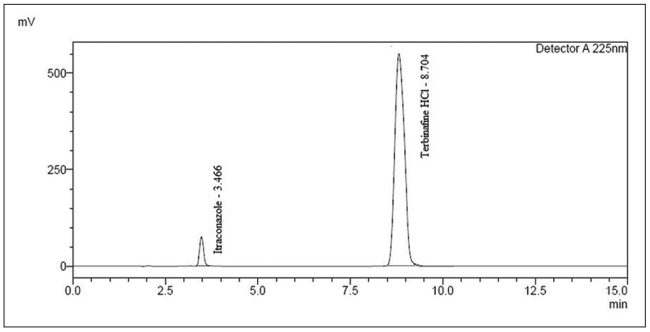

Fig. 11: Chromatogram of thermal degradation

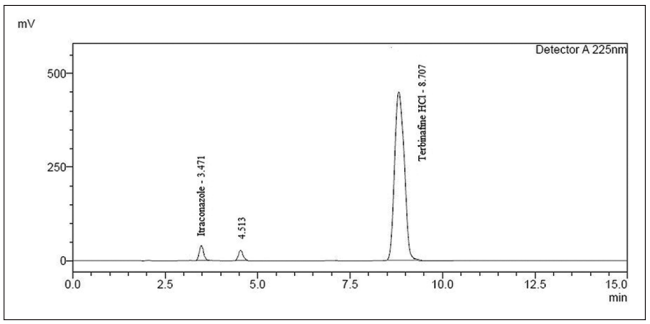

Fig. 12: Chromatogram of oxidative degradation

Thermal degradation

In thermal degradation, both itraconazole and terbinafine HCl did not degrade and percentage degradation was 0.1% and 0.09% for itraconazole and terbinafine HCl, respectively; no peak of degradation of the product was observed in the chromatogram (Fig. 11).

Oxidative degradation

In oxidative degradation (30% H_2O_2), both itraconazole and terbinafine HCl were get degraded. The percentage degradation was 16.0% and 12.0% for itraconazole and terbinafine HCl, respectively. The degradant product peak was observed at 4.513 min and from literature; it may be itraconazole oxidative degradation product (Fig. 12) [16].

CONCLUSION

The stability-indicating method has been developed and validated for the simultaneous estimation of itraconazole and terbinafine HCl in bulk and pharmaceutical tablet dosage form. The developed method was successfully applied for forced degradation studies of itraconazole and terbinafine HCl. Forced degradation results indicate that the developed method can be successfully used for the separation of degraded products from the sample. The developed method is novel, simple, cost effective, and accurate for the determination of itraconazole and terbinafine HCl, and it can be used for routine analysis of the itraconazole and terbinafine HCl in the formulation.

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AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

The author declares that there are no conflicts of interest regarding the publication of this article.

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