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Stability Indicating RP-HPLC Method for Estimation of Itraconazole and Terbinafine in Bulk and Tablet Dosage Forms

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Abstract
Itraconazole is an antifungal medication used to treat number of fungal infections. On set of action within an hour and Last up to twenty-one hours. Terbinafine is an antifungal medication used to treat pityriasis versicolor, fungal nail infections and ringworm. On Set of action within an hour and Last up to 36 hours. To develop and validate simple, fast, economical and eco-friendly RP-HPLC method for the estimation of ITRA and TERB in bulk and tablet dosage form according to ICH guidelines**.** This method achieved by Shimadzu LC-20A instrument with isocratic elution with the mobile phase of methanol and water in the ratio of (9.5:0.5v/v) on Zodiac C 18 (250mm x 4.6mm, 5µm) with a flow rate of 1mL/min. at a wave length of 257nm with UV detector. Tablets were allowed to undergo different stress conditions like acid, base, oxidation, thermal degradation studies. Retention time of ITRA and TERB was found to be 4.288 and 2.551 respectively. The linearity of proposed method investigated in the range of 10-50µg/mL for both ITRA and TERB. The Limit of Detection of ITRA and TERB 1.25µg/mL and 8.00µg/mL respectively. The Limit of Quantification of ITRA and TERB are 3.79µg/mL and 24.00µg/mL respectively. From the above results, it can be concluded that the developed RP-HPLC method represents a good technique for determination of Itraconazole and Terbinafine contents in bulk and tablet formulation with good sensitivity, precision, and reproducibility.

Keywords
Itraconazole, Terbinafine, RP-HPLC, Forced degradation studies.

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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, 3 dioxolan-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 $C_{35}H_{38}Cl_2N_8O_4$ and $C_{21}H_{25}N{\cdot}HCl$, respectively, and molecular weight is 706 g/mol and327.89084 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. The literature survey reveals that there is only one reversed-phase high-performance liquid chromatography (RP-HPLC) method reported for the estimation of Itraconazole and Terbinafine HCL in tablet dosage form. 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.

MATERIALS AND METHODS

Instrument

RP-HPLC Shimadzu LC 20A model HPLC and Research lab fine chem industries solution were used for stability-indicating method development and validation of Itraconazole and Terbinafine HCl., Ultrasonics (1.5L50) sonicator was used for sonication.

Chemicals and reagents

Itraconazole and Terbinafine HCL were provided by K. P Laboratories, Hyderabad, India, and commercial tablet dosage form was purchased from a local market. The HPLC grade Methanol and Water, was purchased from Research lab fine chemical Industries.

Chromatographic conditions

The separation of Itraconazole and Terbinafine HCl was carried out using Shimadzu RP-HPLC system with Zodiac C 18 (250mm x 4.6mm, 5µm) column. The mobile phase used was Methanol and water at a ratio of (95:5) a flow rate of 1.0 mL/min, injection volume was 10 µl, column temperature was (30°C), and Itraconazole and Terbinafine HCL were detected at 257 nm using an ultraviolet (UV)-visible detector. Selection of wavelength Standard solutions of Itraconazole (10nppm) 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 257 nm wavelength was selected as detection wavelength for the separation of Itraconazole and Terbinafine HCL.

Preparation of mobile phase

A mixture of 95 volumes of HPLC grade Methanol and 5 volumes of Water was prepared and sonicated for 10–15 min to degas.

Preparation of standard solution

Accurately weighed 10mg Itraconazole and Terbinafine transferred into a 100mL of clean and dry volumetric flask, add about 30 mL of mobile phase and sonicate to dissolve and degas completely and make volume up to the mark with the mobile phase. Further dilutions like 3, 5, 10, 20,25, 30,40, 50, 70, 90, 100 µg/mL (10-100µg/mL) make up with mobile phase.

Preparation of sample solution

20 Tablets of mytra-T (250mg of TERB, 100mg of ITRA) were taken and powdered. A quantity of powder equivalent to 10mg of Itraconazole (equivalent to 20mg of Terbinafine) taken in to 10mg volumetric flask and make the volume by using methanol. The solution was filtered through 0.45µm filter. From the filtrate 0.1mL was taken in 10mL volumetric flask and made up the mark with methanol to get a sample solution concentration of 10µg/mL.

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

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% H2O2 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 Petridish and then placed them in an oven at 60°C for 1 hr.

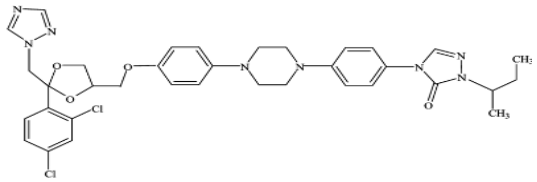

Fig No: 1 Itraconazole

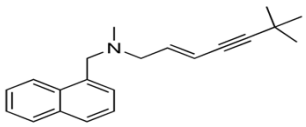

Fig No: 2 Terbinafine

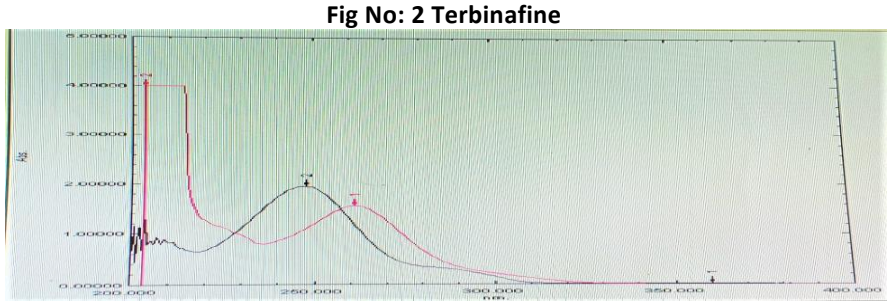

Fig No:3 UV Overlay of Itraconazole And Terbinafine

RESULTS AND DISCUSSION

Method development

A series of trials were carried out using different ratios of mobile phases such as Methanol: Water (96:4), methanol: water (94:6), and Change in flow rate method for simultaneous estimation of Itraconazole and Terbinafine HCl in marketed tablet dosage form. Finally, a typical chromatogram was obtained using Methanol and water as mobile phase in a ratio of 95:5 on Zodiac C18 (250 mm×4.6 mm, 5 µ) column and injection volume of 10 µl. The flow rate was 1.0 mL/min and the run time was 10 min. The column temperature was 30°C and detection was carried out at 257 nm. The retention time was 4.288 and 2.551 min for Itraconazole and Terbinafine HCl, respectively. Typical chromatograms of standard and sample solution of Itraconazole and Terbinafine HCL are shown in Fig.4. 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.

Instrument	: HPLC SHIMADZU
Column	: C_{18} (250x4.6mm i.d., 5µm)
Wavelength	: 257nm
Mobile phase	: Methanol: Water (95:5%v/v)
Flow rate	: 1mL/min
Detector	: UV detector
Injector	: Rheodyne injector
Injection volume	: 20µL
Type of elution	: Isocratic
Run time	:10min

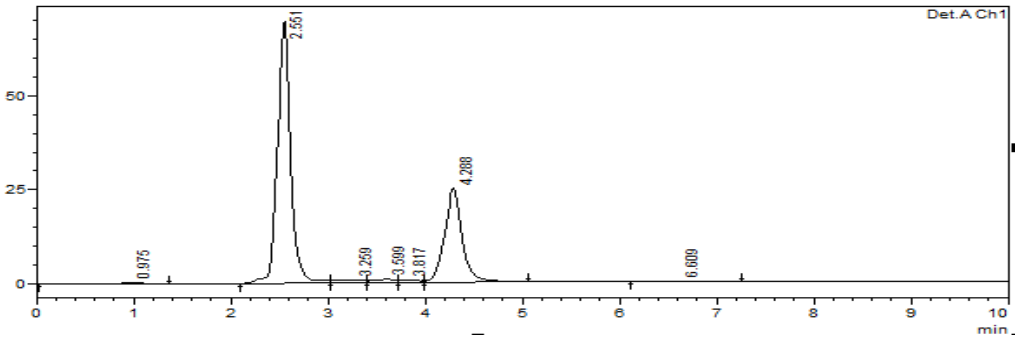

Fig No:4 A Typical Chromatogram of Itraconazole And Terbinafine

1. 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 (r2) 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.

2. 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 . 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.

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.

4. Robustness

The developed method was evaluated for robustness by small deliberate changes in optimized method parameters which were done such as flow rate (±0.2 ml), wavelength (±2 nm), and temperature (±2°C) . 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.

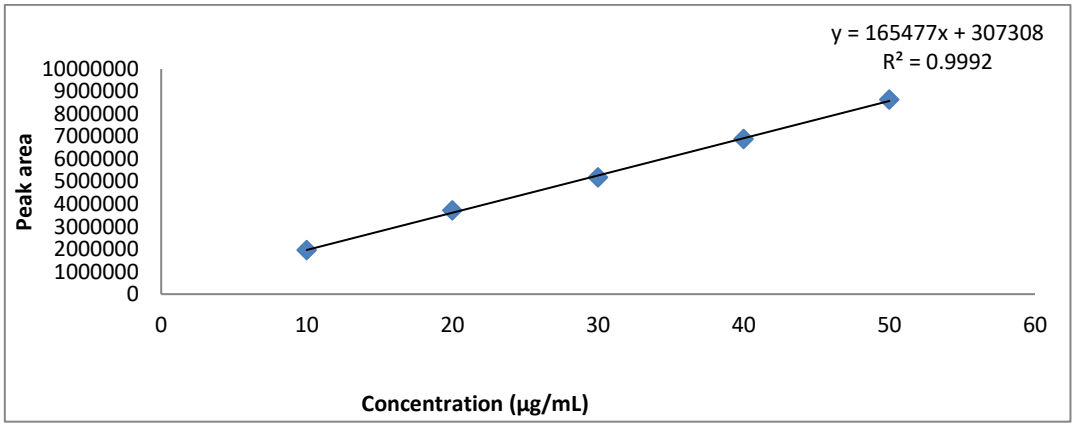

Fig No. 5: Linearity Graph of Itraconazole Table

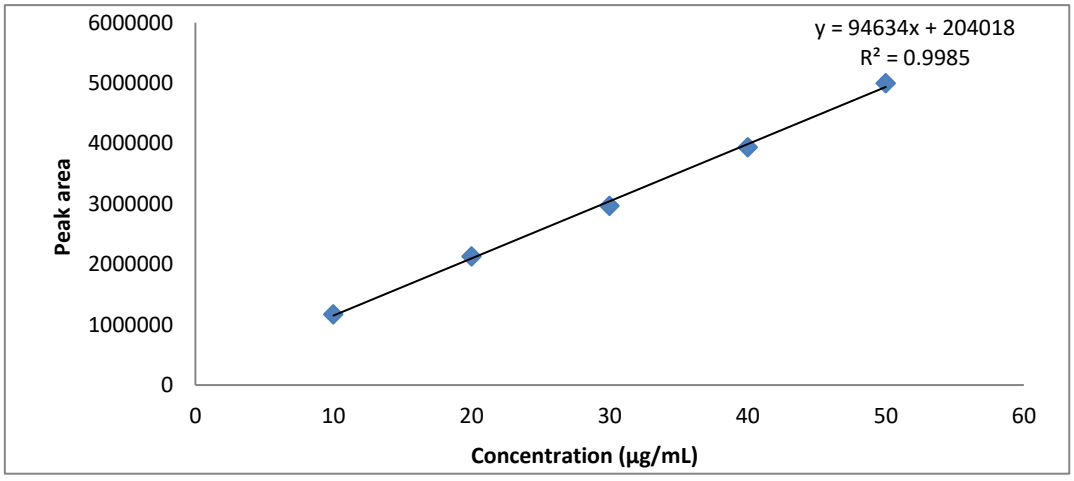

Fig No. 6: Linearity graph of Terbinafine Table

TABLE 1: Linearity results for Itraconazole and Terbinafine HCL

S.NO	CONCENTRATION (µg/mL)	PEAK AREAS OF TERBINAFINE	PEAK AREAS OF ITRACONAZOLE
1	10	1948427	1172032
2	20	3712036	2032206
3	30	5176156	2971042
4	40	6886397	3939981
5	50	8635108	4999870
Correlation Co-efficient (R^2)		0.999	0.998

Table 2: Results for the Intra-day precision of Terbinafine and Itraconazole

S.NO	Peak area of Terbinafine	Peak area of Itraconazole
1	3712036	2132263
2	3613420	1970843
3	3784862	2016349
4	3615412	2153834
Average	3726433	2143324
S.D	40135	35185
R.S.D	1.007	1.64

TABLE 3: Limit of Detection and limit of Quantification

Drug	LOD	LOQ
Terbinafine	8.00	24.00
Itraconazole	1.25	3.79

TABLE 4: Robustness results of Terbinafine and Itraconazole

S.NO	MOBILE PHASE	FLOW RATE (mL/min)	Max(nm)	%RSD OF TERBINAFINE	%RSD OF ITRACONAZOLE
1	97:3	1	257	1.09	1.03
2	96:4	1	257	1.61	1.34
3	95:5	0.8	257	0.99	1.50
4	95:5	1.2	257	1.42	1.77

TABLE 5: Accuracy Data of Terbinafine and Itraconazole

S.NO	Concentration	%Recovery of Terbinafine	%Recovery of Itraconazole	Mean Recovery of Terbinafine	Mean Recovery of Itraconazole
1	50%	90.0%	106%	94.33%	101.3%
2	100%	95.7%	98.4%		
3	150%	97.3%	99.6%		

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.

1. BASE DEGRADATION

The base degradation study observed with 0.1M of sodium Hydrochloride solution. The study has been observed for 1,3,5 days time intervals on HPLC.

2. ACID DEGRADATION

The base degradation study observed with 0.1M of Hydrochloric acid solution. The study has been observed for 1,3,5 days time intervals on HPLC.

3. OXIDATIVE DEGRADATION

The base degradation study observed with 0.1M of Hydrogen peroxide solution. The study has been observed for 1,3,5 days time intervals on HPLC.

4. THERMAL DEGRADATION

The thermal degradation was observed with methanol. The study has been performed for 1,3,5 days.

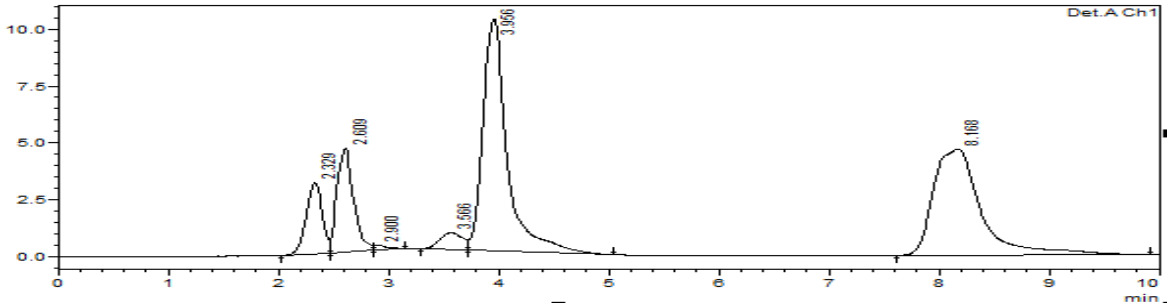

FIG No:7 chromatogram of Terbinafine and Itraconazole for base degradation 1 day.

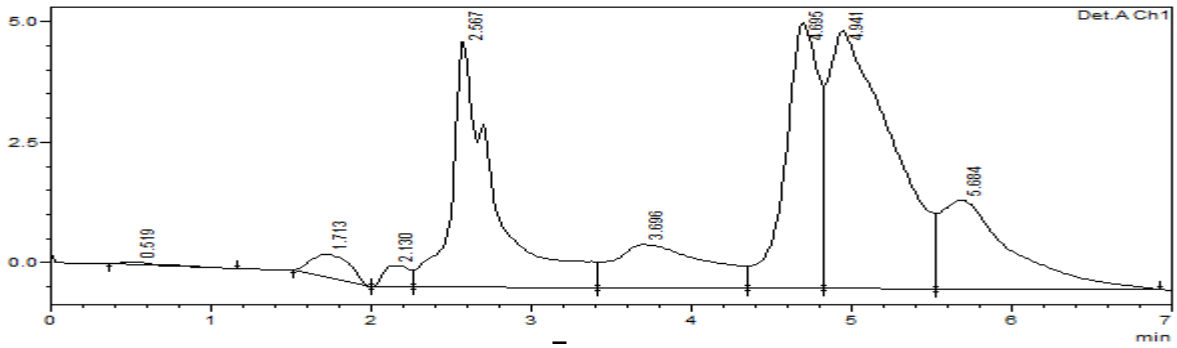

FIG No: 8 **chromatogram of** Terbinafine **and** Itraconazole **for base degradation 3 day**.

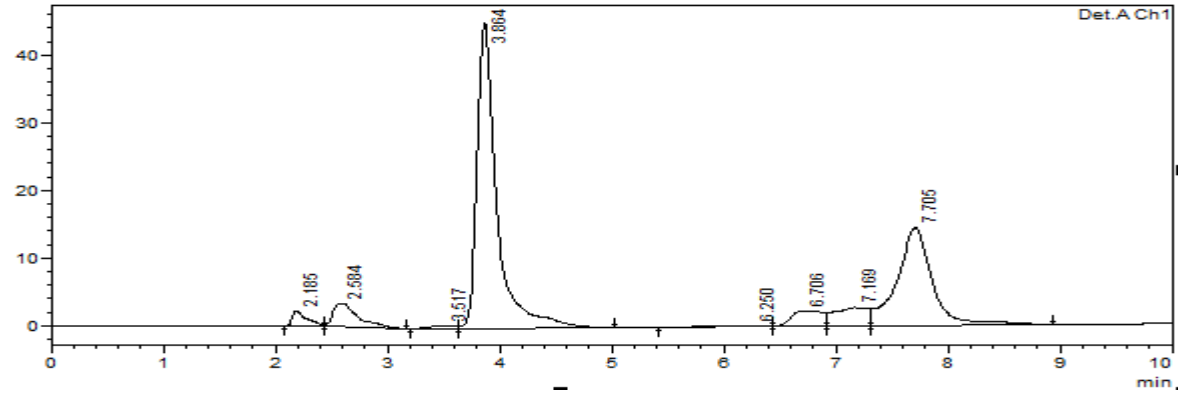

FIG No:9 chromatogram of Terbinafine and Itraconazole for acid **degradation 1 day**

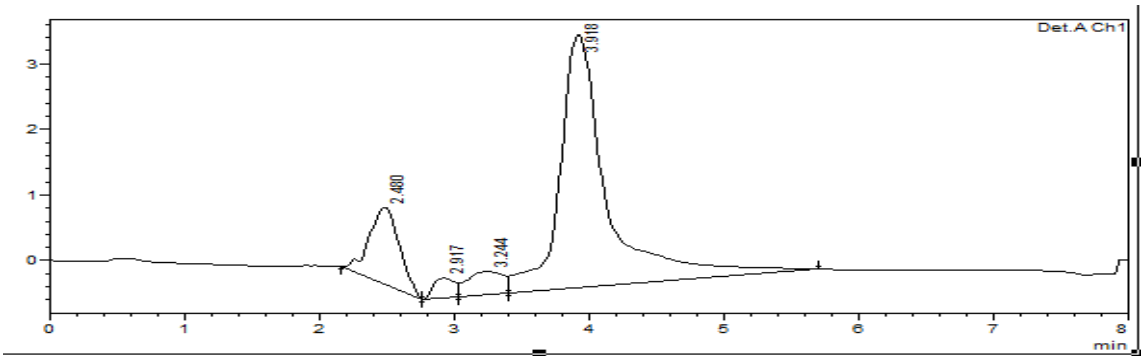

FIG No:10 chromatogram of Terbinafine and Itraconazole for acid degradation 3 day.

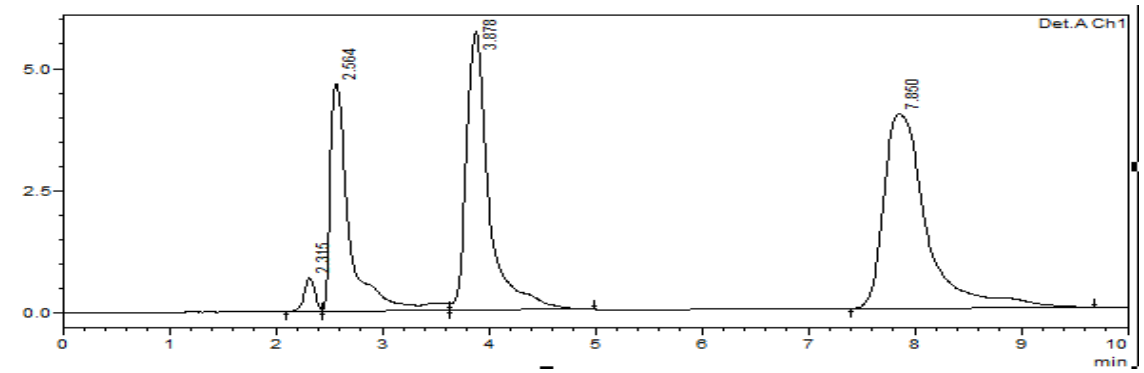

FIG No: 11 chromatograms of Terbinafine and Itraconazole for hydrogen peroxide degradation 1day.

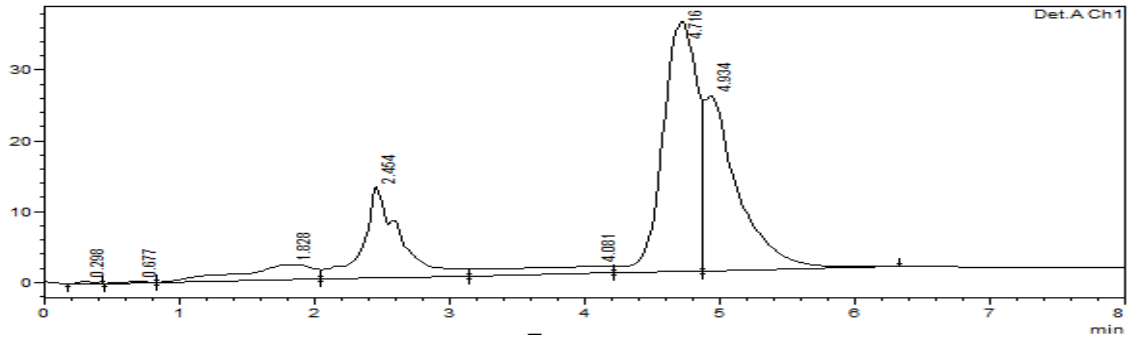

FIG No: 12 chromatograms of Terbinafine and Itraconazole for hydrogen peroxide degradation 3 day

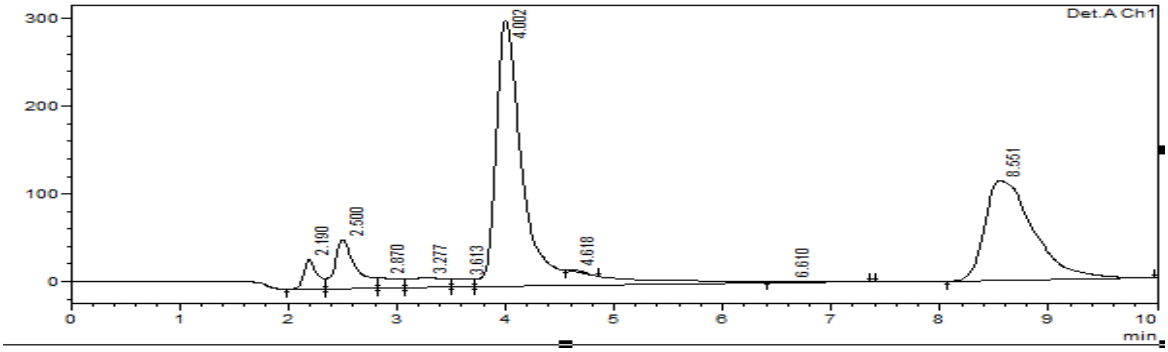

FIG No:13 Chromatogram of Terbinafine and Itraconazole for thermal degradation on 5 day

CONCLUSION

A new sensitive method was developed and optimized, the following parameters were validated according to the ICH guidelines .Mobile phase – Methanol: Water (95:5%v/v)Limit of detection (LOD)- 1.25, 8.0 µg/mL for Itraconazole and Terbinafine respectively Limit of quantification (LOQ)-3.75, 24.0 µg/mL for Itraconazole and Terbinafine respectivelyLinearity-10-50µg/mL. Precision, accuracy and robustness were performed. To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Terbinafine and Itraconazole was obtained with a mobile phase comprising of Methanol: Water (95:5v/v) at a flow rate of 1min/min to get better reproducibility and repeatability. Quantification was achieved of Terbinafine and Itraconazole at 257nm.the retention time for Terbinafine and Itraconazole was found to be 2.551 and 4.228 min respectively. Linear correlation was obtained between peak area versus concentration of Terbinafine and Itraconazole in the concentration ranges of 10-100µg/mL. The mean recovery obtained were of Terbinafine and Itraconazole, was 94.33% and 101.3%, which indicates the accuracy of the proposed method .The %RSD valve of Terbinafine and Itraconazole was found to be less than 2, which indicates the accuracy of the proposed method.

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