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Simultaneous Estimation of Itraconazole and Terbinafine HCl in Bulk and Pharmaceutical Tablet Dosage Form by Using UV Spectrophotometric Method

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ABSTRACT

Objective: The objective of present work was to develop and validate a novel and simple UV spectrophotometric method for simultaneous estimation of Itraconazole and Terbinafine HCl in bulk and pharmaceutical tablet dosage form. This combination of drugs is not reported for simultaneous UV spectrophotometric analysis as of now. **Method:** The developed method was carried on UV Shimadzu 1800 model and acetonitrile was used as a solvent. The wavelength of 235 nm was used as λ_{max} for Terbinafine HCl and 263 nm was used as λ_{max} for Itraconazole. **Results:** The %RSD of method precision and system precision of Itraconazole and Terbinafine HCl were found to be less than 2%. The percent recovery of Itraconazole and Terbinafine HCl were found to be in the range of 98 – 102 %. The calibration curve was found to be linear and r^2 values were 0.9994 and 0.9995 for Itraconazole and Terbinafine HCl respectively. The solution of Itraconazole and Terbinafine HCl was found to be stable up to 24 hours. **Conclusion:** A novel and simple UV spectrophotometric method were developed and validated for the simultaneous estimation of Itraconazole and Terbinafine HCl in bulk and pharmaceutical tablet dosage form. The results of the developed method were found to be within limit; hence method was found to be precise, accurate, linear and stable.

INTRODUCTION:

Both Itraconazole and Terbinafine HCl are antifungal drugs. The IUPAC 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 $C_{35}H_{38}Cl_2N_8O_4$ and $C_{21}H_{25}N{\cdot}HCl$ respectively and the molecular weight is 706 g/mol and 327.89084 g/mol respectively. Itraconazole and Terbinafine HCl both are freely soluble in acetonitrile, methanol, and DMSO but insoluble in water [1-2]. The chemical structure of both drugs is given in Figure 1 and 2.

Combination of Itraconazole and Terbinafine HCl are used for the treatment of antifungal infections such as toenail onychomycosis and it stops the growth of fungi by preventing covering [3-4]. The literature survey reveals that there is no RP- HPLC method reported for the estimation of Itraconazole and Terbinafine HCl in tablet dosage form [5-10]. Thus, the present work was carried out to develop novel, precise, accurate, rapid and cost-effective stability-indicating the 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.

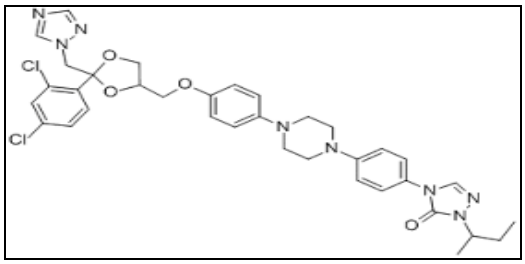

Figure no.1: Chemical structure of Itraconazole

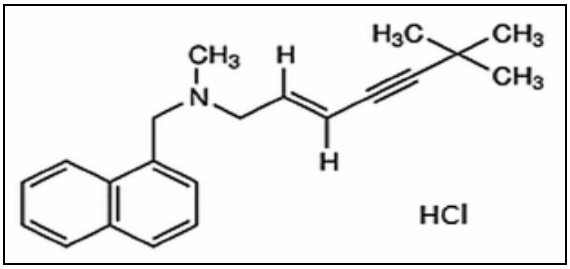

Figure no.2: Chemical structure of Terbinafine HCl

MATERIALS AND METHODS

Instrument

UV Shimadzu 1800 model used for method development and validation of Itraconazole and Terbinafine HCl. The Remi R-8C centrifuge model was used for centrifugation of sample, Kroma Tech (KL-1.5) sonicator was used for sonication.

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 Fischer Scientific.

Selection of wavelength

Standard solutions of Itraconazole and Terbinafine HCl were prepared and scanned by UV spectrophotometer separately, in range of 200-400 nm. The 235 nm wavelength was selected as λ_{max} for Terbinafine HCl and 263 nm as λ_{max} for Itraconazole. The overlay spectra of Itraconazole and Terbinafine HCl is shown in Figure 3.

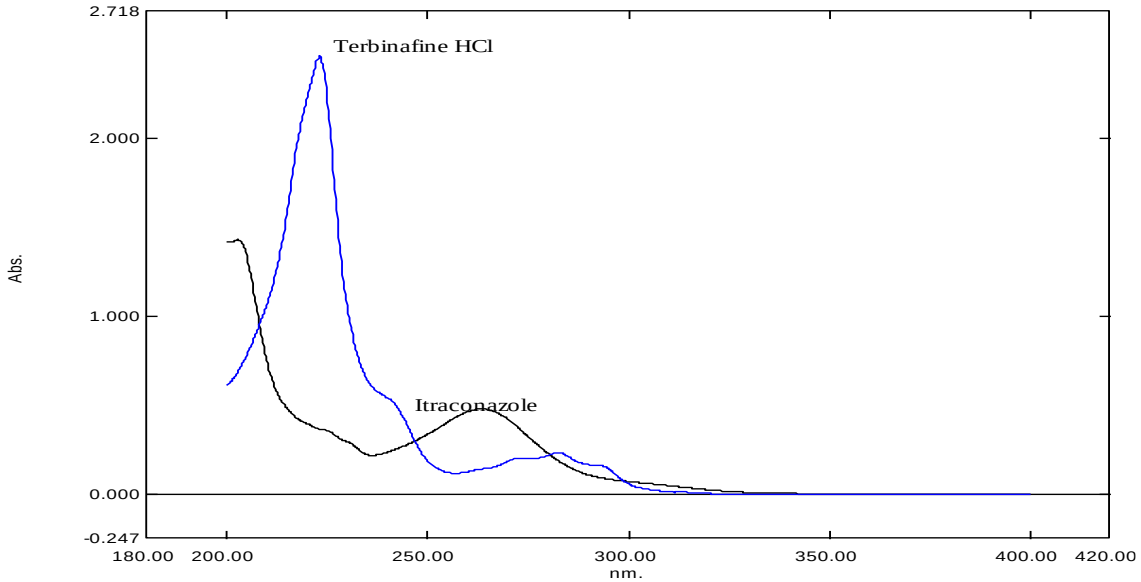

Figure no 3: UV overlain spectra of Itraconazole and Terbinafine HCl

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 by using acetonitrile. Further pipetted out 5 ml of stock solution and transferred into 50 ml of volumetric flask and the volume was made up to the mark by using acetonitrile to obtain a concentration of 4 ppm of Itraconazole and 10 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. The sample solution was centrifuged at 5000 rpm for 10 minutes and then 5 ml of centrifuged sample was pipetted out into a 50 ml volumetric flask and the volume was made up to the mark by using acetonitrile and then filtered through Whatman filter paper.

Method validation

The developed method for Itraconazole and Terbinafine HCl was validated for parameters like precision, linearity, accuracy, ruggedness and solution stability as per ICH guidelines [11-15].

RESULTS AND DISCUSSION

Method development

A series of trials were carried out using different solvents such as water, methanol, and acetonitrile. Both Itraconazole and Terbinafine were found to be soluble and stable in acetonitrile. Therefore, acetonitrile is used as solvent throughout the analysis of Itraconazole and Terbinafine HCl.

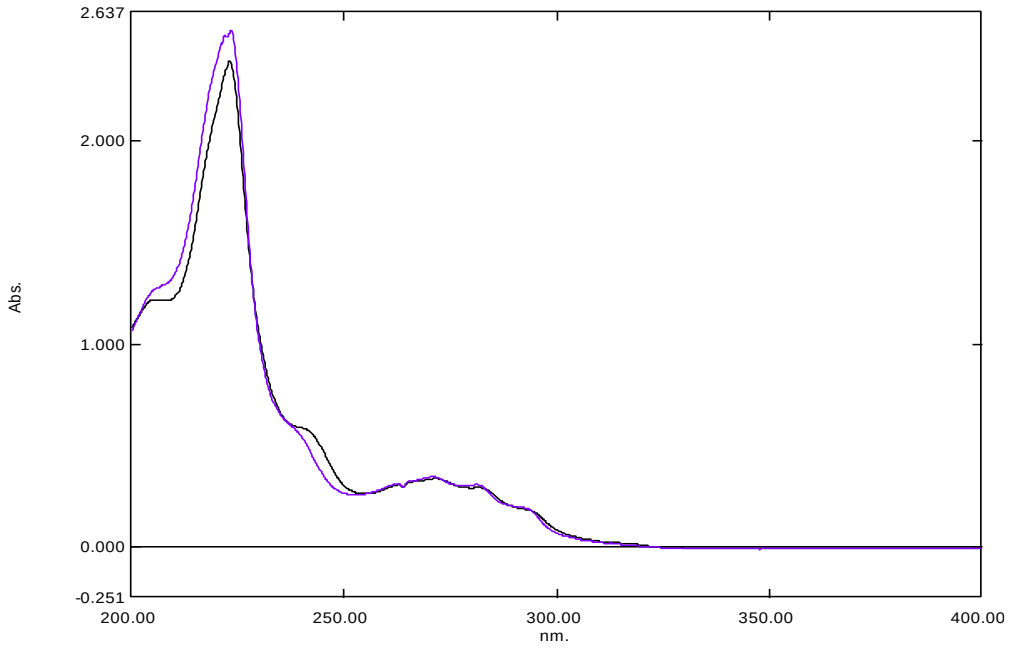

Figure no 4: Overlain spectrum of standard and sample solution of Itraconazole and Terbinafine HCl

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 six replicates of absorbance of Itraconazole and Terbinafine HCl standard and sample of the same concentration [16-17]. The %RSD was calculated from the absorbance and it was found to be less than 2%. From precision results, it was found that the method is precise. The data of system precision is tabulated in Table 1 and the data of method precision is tabulated in Table 2. The spectra of system and method precision of Itraconazole and Terbinafine HCl are shown in Fig. 5 and 6 respectively.

Table no 1: System precision results

Sr. No	Itraconazole (4 ppm)	Terbinafine HCl (10 ppm)
	Absorbance	Absorbance
1	0.327	0.675
2	0.328	0.677
3	0.327	0.674
4	0.324	0.672
5	0.326	0.674
6	0.323	0.673
Average	0.325833	0.674167
SD	0.001941	0.001722
% RSD	0.595639	0.255486

SD: Standard deviation, RSD: Relative standard deviation

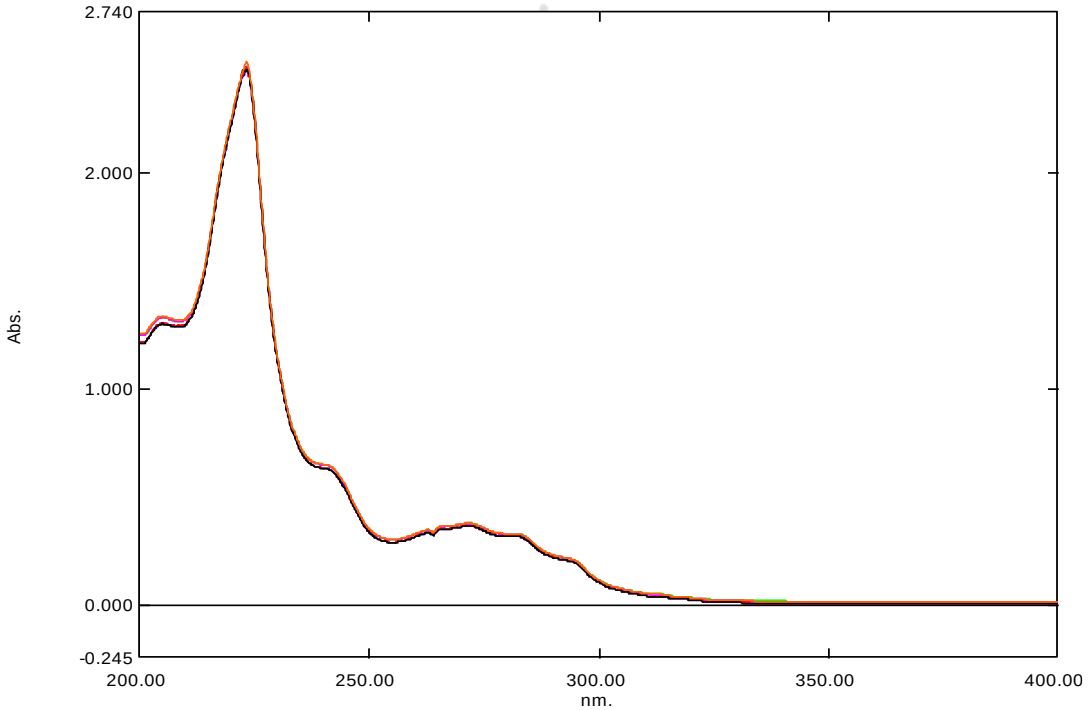

Figure no 5: Spectrum of system precision

Table no 2: Method precision results

Sr. No	Itraconazole	Terbinafine HCl
	% Assay	% Assay
1	101.19	100.72
2	100.56	101.94
3	99.61	100.57
4	100.87	100.42
5	99.92	102.09
6	101.51	101.33
Average	100.61	101.1783
SD	0.733839	0.71965
% RSD	0.72939	0.711269

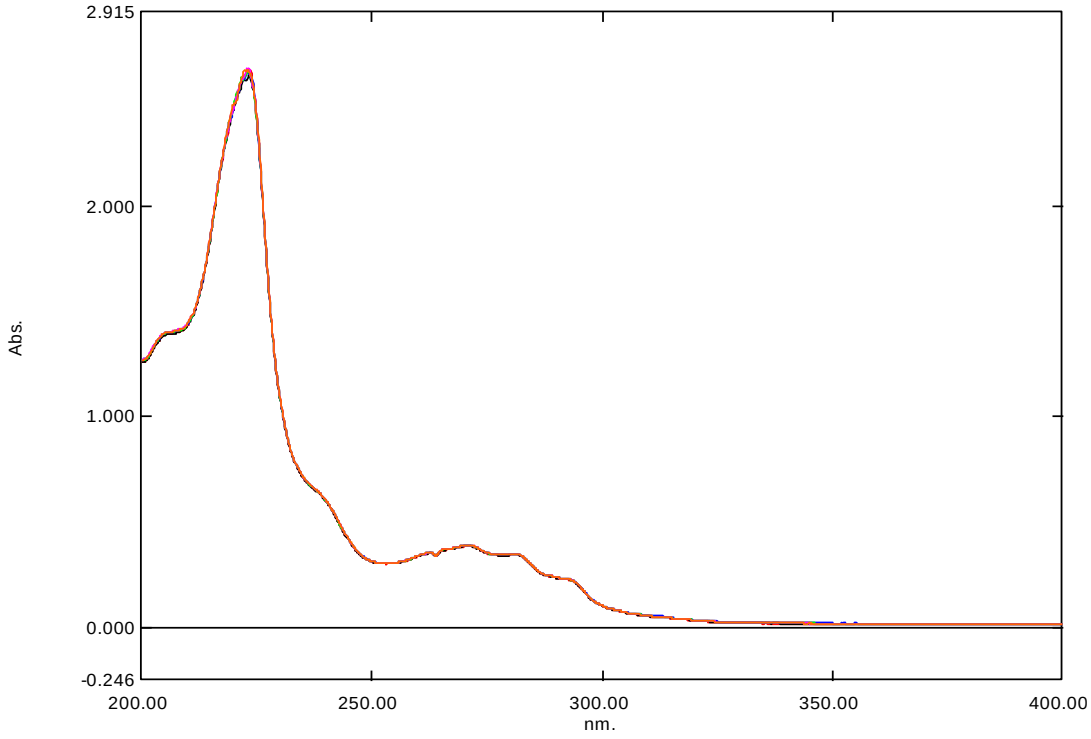

Figure no 6: Spectrum of method precision

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%, 150%) by the standard addition method. The mean % recovery for Itraconazole and Terbinafine HCl was

found within a limit of 98-101% and from % recovery results it was found that the developed method is accurate. % recovery results are tabulated in Tables 3 and 4.

Table no 3: % Recovery results for Itraconazole

Level	% Recovery	Average	SD	% RSD
50%	100.03	99.96	0.16	0.16
	99.78			
	100.08			
100%	100.05	100.36	0.68	0.68
	101.14			
	99.89			
150%	99.98	100.03	0.14	0.14
	99.92			
	100.19			

Table no 4: % Recovery results for Terbinafine HCl

Level	% Recovery	Average	SD	% RSD
50%	100.61	100.47	0.14	0.14
	100.45			
	100.34			
100%	100.55	100.65	0.39	0.39
	100.32			
	101.08			
150%	101.11	101.12	0.03	0.03
	101.15			
	101.09			

Linearity

The linearity of the Itraconazole and Terbinafine HCl was determined at different concentration levels ranging from 2 ppm to 6 ppm for Itraconazole and from 5 ppm to 15 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 0.9994 for itraconazole and 0.9995 for Terbinafine HCl. From linearity results, it was found that the developed method is

linear (Figure 6 and 7). Results are shown in Table 5. The spectra of linearity of Itraconazole and Terbinafine HCl were shown in Fig. 7 and 8 respectively.

Table no 5: Linearity results for Itraconazole and Terbinafine HCl

Concentration (mcg/ml)		Absorbance	
Itraconazole	**Terbinafine HCl**	**Itraconazole**	**Terbinafine HCl**
2	5	0.161	0.318
3.2	8	0.264	0.544
4	10	0.327	0.677
5.2	12	0.429	0.916
6	15	0.487	1.053
Slope	**Slope**	- 0.0818	- 0.051
Intercept	**Intercept**	0.0002	-277853
Correlation	**Correlation**	0.9994	0.9995

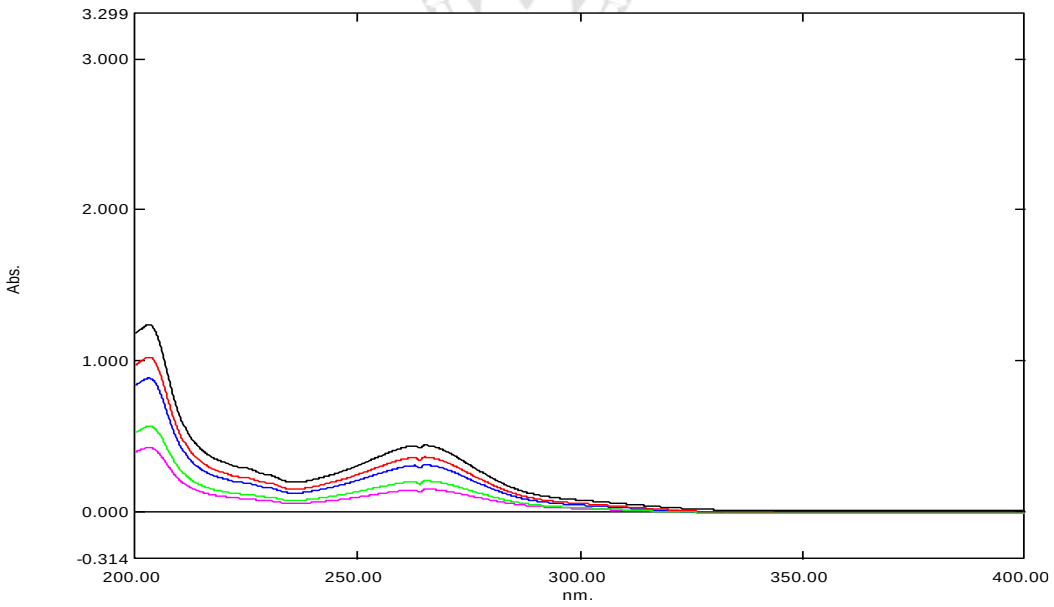

Figure no 7: Linearity spectrum of Itraconazole

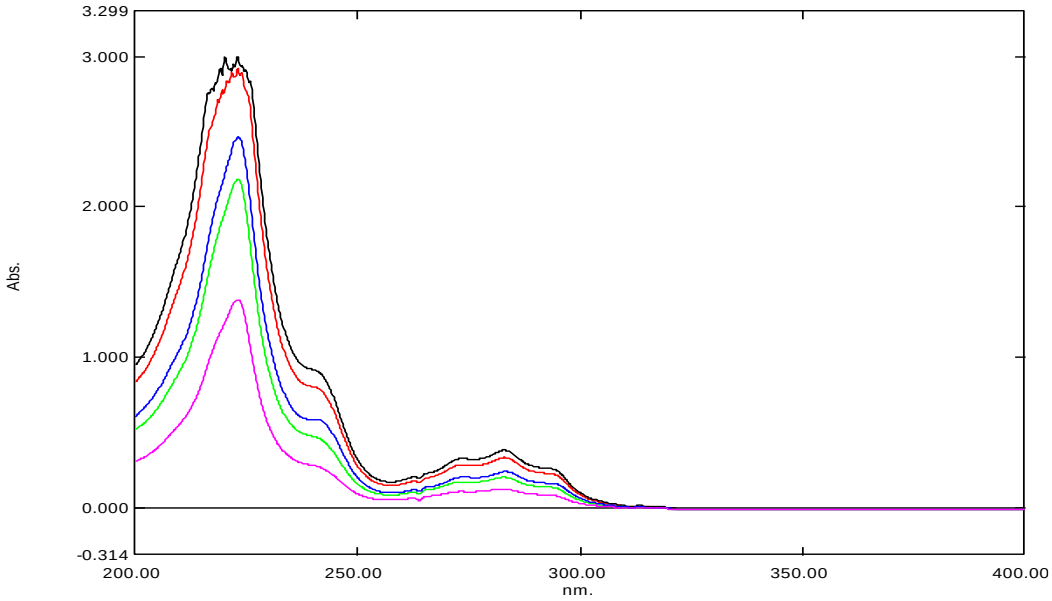

Figure no 8: Linearity spectrum of Terbinafine HCl

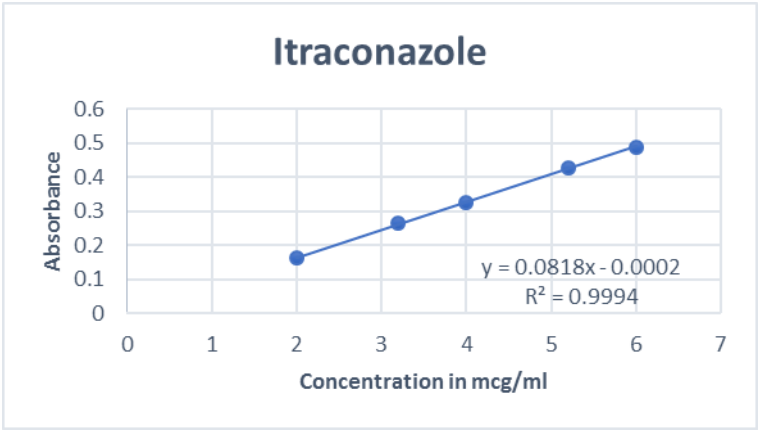

Figure no 9: Linearity graph of Itraconazole

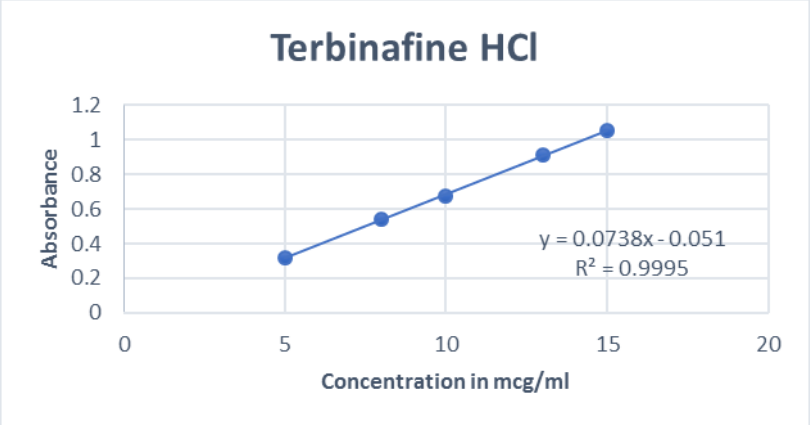

Figure no 10: Linearity graph of Terbinafine HCl

Solution stability

The absorbance of the sample solution of Itraconazole and Terbinafine HCl was measured at different time intervals and % assay was calculated. The solution stability of 24 hrs shows that the sample solution can be used throughout 24 hrs without any degradation of the solution and solution stability results are shown in Table 6.

Table no 6: Solution stability results

Time interval	Itraconazole	Terbinafine HCl
	% Assay	% Assay
Initial	100.23	101.24
6 Hrs	100.15	100.94
16 Hrs	100.04	100.54
24 Hrs	99.82	100.27

Assay of marketed formulation

For analysis of marketed formulation (Duofaze: 100mg Itraconazole and 250 mg Terbinafine hydrochloride), Ten tablets were weighed and finely powdered and quantity corresponding to 80 mg (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. The sample solution was centrifuged at 5000 rpm for 10 minutes and then pipetted out 5 ml of centrifuged sample into a 50 ml volumetric flask and make and the volume was made up to the mark by using acetonitrile and then filtered through Whatman filter paper.

Table no 7: % Assay of marketed formulation

Tablet	Drug	% Assay
Duofaze (Itraconazole 100 mg + Terbinafine HCl 250 mg)	Itraconazole	99.74%
	Terbinafine HCl	100.56%

CONCLUSION

A novel and simple UV spectrophotometric method were developed for simultaneous estimation of Itraconazole and Terbinafine HCl in bulk and pharmaceutical tablet dosage

form. The developed method was validated as per the ICH guidelines and results of the developed method were found to be within limit; hence method was found to be precise, accurate, linear and stable and it can be used for routine analysis of the Itraconazole and Terbinafine HCl in the formulation.

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