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A Derivative Spectrophotometric Method for the Vierordt's Detection of Terbinafine and Itraconazole in Topical Dosage Form

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ABSTRACT

The goal of the current work was to simultaneously estimate the dosage forms of pharmaceutical topical dosage form and bulk itraconazole and terbinafine HCl by developing and validating a novel and straight forward UV spectrophotometric approach. As of right now, no reports of this medication combination for simultaneous UV spectrophotometric analysis exist. Acetonitrile was utilized as a solvent in the UV Shimadzu 1800 model, which was employed to test the developed approach. For Terbinafine HCl, the wavelength at which the λmax was measured was 235 nm, while for itraconazole, it was 263 nm. It was discovered that the percent RSD of both the system and technique precision for itraconazole and terbinafine HCl was less than 2%. In summary, a new and straightforward UV spectrophotometric technique was created and verified for the simultaneous measurement of terbinafine HCl and itraconazole in pharmaceutical topical dosage form and bulk dose forms. The created approach was determined to be precise, accurate, linear, and stable because the results were found to be within the given range.

KEYWORDS

Itraconazole, Terbinafine HCl, UV spectrophotometric, Validation, Simultaneous estimation method, vierordt's method

1. INTRODUCTION

Terbinafine HCl and itraconazole are both antifungal medications. Itraconazole and Terbinafine HCl have the IUPAC names 4-[4-[4-[4-[[cis2,4-dichlorophenyl-2-(-)2 -(1H-1,2,4-triazol-1-ylmethyl)[-1,3dioxolan-4-yl] piperazin-1-yl] phenyl] methoxy] phenyl](1RS)- 1-methylpropyl] -2-[-2,4-triazol-3-one, 2,4-dihydro-3H and (E)-N,6,6-trimethyl-N-(hept-2-en-4-yn-1 amine hydrochloride, naphthalen 1-ylmethyl), respectively. Itraconazole and Terbinafine HCl have the chemical formulas C35H38Cl2N8O4 and C21H25N·HCl, respectively, and their corresponding molecular weights are 706 g/mol and 327.89084 g/mol. Both itraconazole and Terbinafine HCl are insoluble in water but easily soluble in acetonitrile, methanol, and DMSO (1,2).

When taken in conjunction with terbinafine HCl, it inhibits the growth of fungi by inhibiting their covering, making it an effective treatment for antifungal illnesses like toenail onychomycosis (3–5). According to a review of the literature, there isn't a published RP-HPLC method for estimating the dosage of itraconazole and terbinafine HCl in tablets (6–11). Therefore, the current work was done to develop a new, precise, accurate, quick, and affordable stability-indicating method as well as to validate the method for estimating Itraconazole and Terbinafine HCl simultaneously in tablet dosage form and using it to separate a degradation product's peak.

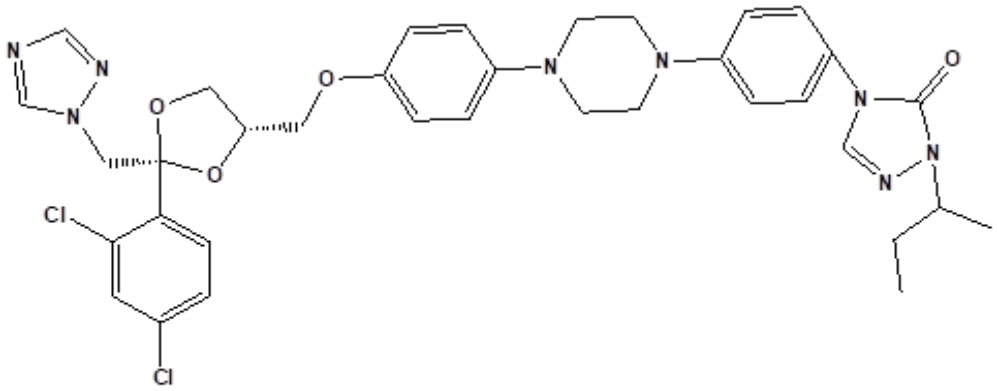

Figure No. 1 ITRACONAZOLE

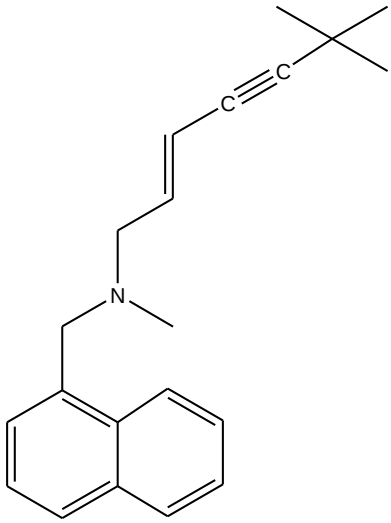

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Figure No. 2 TERBINAFINE HYDROCHLORIDE

2. MATERIALS AND METHODS

(a)Instrument

Itraconazole and Terbinafine HCl method development and validation were conducted using the UV Shimadzu 1800 model. Sample centrifugation was performed using the Remi R-8C centrifuge model, and sonication was performed using the TEMPO sonicator.

(b) Chemicals and reagents

Nosch Lab Private limited, Kukalpatty, Hyderabad, India supplied the itraconazole and BLD Pharm supplied terbinafine HCl, while DUOFAZE, a commercial topical dosage form, was bought from a nearby market. Thermo Fischer Scientific's Qualigens supplied the HPLC grade acetonitrile.

(c) Selection of wavelength

Itraconazole and Terbinafine HCl standard solutions were made, and each was examined separately using a UV spectrophotometer set to scan in the 200–400 nm range. For Terbinafine HCl, the wavelength at which the λmax was chosen was 235 nm, while for itraconazole, it was 263 nm.

3. Preparation of Standard solution

10 mg of itraconazole and 25 mg of reference standards for terbinafine hydrochloride were dissolved into a 250 ml volumetric flask to create the standard solution of itraconazole and terbinafine HCl. Acetonitrile (around 150 ml) was added as a diluent and sonicated for 15 to 20 minutes, or until the volume reached the desired level. After that, 5 ml of the stock solution was pipetted out and put into a 50 ml volumetric flask. The volume was then adjusted with acetonitrile to get the desired concentrations of 10 ppm Terbinafine hydrochloride and 4 ppm Itraconazole, respectively.

Preparation of Sample solution

After ten tablets were weighed and coarsely pulverized, 80 mg of itraconazole plus Terbinafine HCl were consumed, transferred, and mixed with 150 ml of diluent in a 250 ml volumetric flask. For 30 to 45 minutes, the flask was sonicated with sporadic shaking. Diluent was used to modify the volume to the desired level. The sample solution was centrifuged for 10 minutes at 5000 rpm. Five millilitres of the centrifuged sample were then pipetted into a 50-millilitre volumetric flask, where the volume was adjusted with acetonitrile before being filtered through Whatman filter paper.

UV Spectrophotometric Methods: Simultaneous Equation Method: Simultaneous equation method, also known as vierordt's method, is used to estimate drug combinations containing two or more drugs in combined dosage form. The simultaneous equation method is an analysis created for the absorption of the drugs itraconazole (A) and terbinafine B at their maximum wavelength. This provides assurance and specificity for the identification of the drug entities in the pharmaceutical dosage form.

Two absorbing drugs if present in the sample; each of drug absorbs at the other's λ max, by the simultaneous equation method, it can be possible to identify both drugs. Two wavelengths were selected to develop the simultaneous equations: 229nm and 223nm. The absorptivity and absorbance at these wavelengths were put in equation 1 and 2 to get the concentration of both drugs.

Cx = $\boldsymbol{A2ay1 - A1ay2}$ / $\boldsymbol{ax2ay1 - ax1ay2}$ (1)

Cy = $\boldsymbol{A1}$ax2 − $\boldsymbol{A2}$ax1 / $\boldsymbol{ax2ay1 - ax1ay2}$ (2)

A1, A2=Absorbance of the diluted sample at λ1 and λ2. The absorptivity's of X atλ1 = ax1 and λ2 = ax2. The absorptivity's of Y at λ1 =ay1 and λ2 =ay2. The absorbance' of sample at λ1 =A1 andλ2 =A2. Cx and Cy be the concentration of X and Y, respectively.

Absorbance Ratio Method/Q Value Method: This method also knows as Q value method. This method is used for multicomponent analysis by using UV Spectrophotometer. In a sample solution or formulation, separating different components is unnecessary. The absorbance ratio method is a modified version of the simultaneous equation method. It involves the measurement of absorbance at two different wavelengths, one being the λmax of one drug and the other being an iso-bestic wavelength. 225 and 229nm are two wavelengths selected from the overlaid spectrum of itraconazole and terbinafine and used for the calculation of absorbance ratio method.

Cx =(QM-QY / QX-QY) × A1 / ax1

Cy = (QM-QX / QY-QX) × A1 / ay1

A1 and A2 are absorbance of mixture at 225 and 229nm respectively, ax1 and ay1 are absorptivity's of Itraconazole and Terbinafine at 225nm ax2 and ay2 are absorptivity's of Itraconazole and Terbinafine at 229nm respectively,

QM=A2/A1, QX=ax2/ax1 and QY=ay2/ay1

3.3.3 Method validation

In accordance with ICH recommendations,(12–16) the developed technique for is itraconazole and terbinafine HCl was verified for attributes including precision, linearity, accuracy, ruggedness, and solution stability.

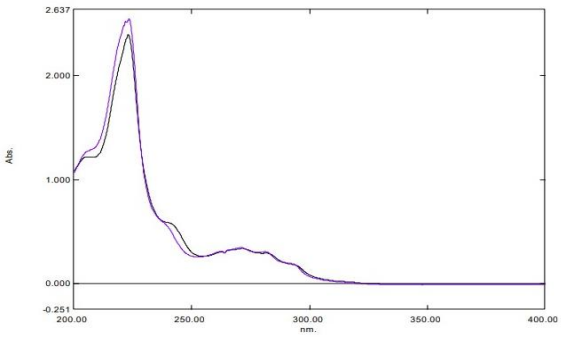

Figure No.3 Overlain spectrum of standard and sample solution of itraconazole and terbinafine

4.4.4 RESULTS AND DISCUSSION

4.4.4(a) Method development

A number of experiments were conducted with various solvents, including acetonitrile, methanol, and water. It was discovered that terbinafine and itraconazole were both stable and soluble in acetonitrile. Thus, throughout the investigation of itraconazole and terbinafine HCl, acetonitrile is utilized as a solvent.

4.4.4(b) Precision

The degree of agreement between several measurements taken from the same homogenous sample under certain conditions can be used to determine the precision of an analytical method. Six replicates of the absorbance of the Terbinafine HCl standard and sample with the same concentration, together with itraconazole, were used to measure the precision of the system and method (17,18). After calculating the percentage RSD using the absorbance, it was discovered to be less than 2%. It was discovered via precision results that the procedure is accurate. Table 1 contains the system precision data, and Table 2 contains the technique precision data. Figs. 5 and 6 display the system and technique precision spectra of itraconazole and terbinafine HCl.

Table no 1: System precision results

Sr. No	Itraconazole (4 ppm) [abs.]	Terbinafine HCl (10 ppm) [abs.]
1	0.326	0.674
2	0.328	0.678
3	0.326	0.674
4	0.322	0.671
5	0.327	0.675
6	0.322	0.674
Average	0.325166667	0.674333333
SD	0.002562551	0.002250926

%RSD	0.788073033	0.333800158

Table no 2: Method precision results

Sr. No	Itraconazole (% Assay)	Terbinafine HCl (% Assay)
1	101.18	100.74
2	100.58	101.96
3	99.62	100.55
4	100.89	100.44
5	99.94	102.06
6	101.53	101.37
Average	100.6233333	101.1866667
SD	0.73191985	0.715085077
%RSD	0.727385812	0.706698916

4.4.4(c) Accuracy

By computing recovery studies of the test sample at three distinct concentration levels (50%, 100%, and 150%) using the conventional addition method, the accuracy of itraconazole and terbinafine HCl was assessed. The devised method was found to be accurate based on the percentage recovery data, and the mean percentage recovery for itraconazole and terbinafine HCl was determined to be within a range of 98-101%. The recovery percentage findings are listed in Tables 3 and 4.

Table no 3: % Recovery results for Itraconazole

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

	100.19			

Table no 4: % Recovery results for Terbinafine HCl

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

4.4.4(d) Linearity

At various concentration levels, ranging from 2 ppm to 6 ppm for Itraconazole and from 5 ppm to 15 ppm for Terbinafine HCl, the linearity of both compounds was assessed. Plotting peak area against concentration allowed for the construction of a linearity curve, and the regression coefficient (r2) for itraconazole and terbinafine HCl, respectively, was determined to be 0.9994 and 0.9995. The devised approach was determined to be linear based on linearity results (Figure 6 and 7). Table 5 displays the results. Figures 7 and 8 display the linearity spectra of itraconazole and terbinafine HCl, respectively.

Table no 5: Linearity results for Itraconazole and Terbinafine HCl

Concentration Itraconazole	Concentration Terbinafine HCl	Absorbance Itraconazole	Absorbance Terbinafine HCl
2	2	0.163	0.316
4	4	0.266	0.546
6	6	0.329	0.679
8	8	0.427	0.918
10	10	0.489	1.055
Slope	**Slope**	0.0821	0.0765
Intercept	**Intercept**	0.0026	0.0625
Correlation	**Correlation**	0.9999	0.9844

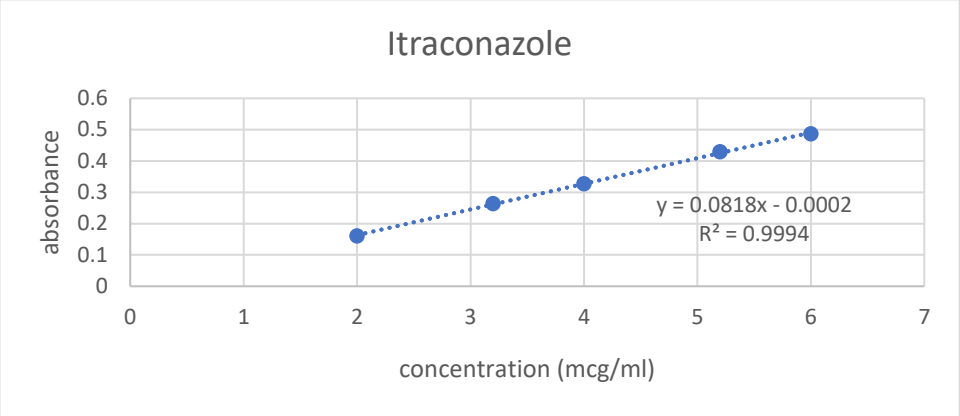

Figure no 4: Linearity graph of Itraconazole

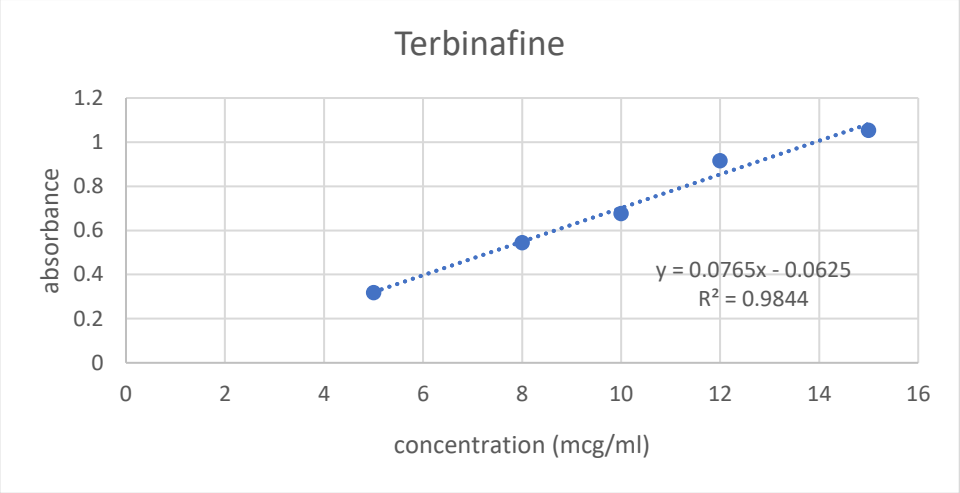

Figure no 5: Linearity graph of Terbinafine HCl

4.4.4(e) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Limit of detection and limit of quantitation were measured by standard deviation method with a calibration standard and by using this formula LOD= 3.3 (SD/S) and LOQ= 10 (SD/S) where (SD) is the standard deviation of the response and (S) is slope of the calibration curve. The LOD and LOQ of itraconazole is 0.265 µg/ml and 0.803 µg/ml and for terbinafine is 0.211 µg/ml and 0.640 µg/ml.

Table No. 6: (LOD) And (LOQ) Of Itraconazole and Terbinafine

S. No.	Drugs	LOD (µg/ml)	LOQ (µg/ml)
1.	Itraconazole	0.265	0.211
2.	Terbinafine HCl	0.803	0.640

4.4.4(f) Robustness:

The robustness of the developed method was determined by small changes in UV parameters, such as wavelength ± 5. The result was indicated as % RSD.

Table No. 7: Robustness Of Itraconazole and Terbinafine

S. No.	Drugs	Wavelength	Absorbance	Mean absorbance	Standard deviation	%RSD
1.	Itraconazole	229nm	0.84356			
			0.84562	0.84	0.00160412	0.19
			0.84246			
2.	Itraconazole	226nm	0.86255			
			0.86352	0.86	0.00055717	0.06
			0.86256			
3.	Itraconazole	227nm	0.82352			
			0.82428	0.83	0.00206749	0.25
			0.82742			
4.	Terbinafine	223nm	0.75235			
			0.75423	0.75	0.00261685	0.35
			0.75752			
5.	Terbinafine	225nm	0.74263			
			0.74653	0.74	0.0020618	0.28
			0.74342			
6.	Terbinafine	227nm	0.79832			
			0.79632	0.80	0.00241109	0.30
			0.79352			

4.4.4(g) Ruggedness:

The ruggedness of the proposed method was determined by two analysts at 10µg/ml concentration of Itraconazole and 25µg/ml concentration of Terbinafine. The result was designated as % RSD.

Table No. 8: Ruggedness Of Itraconazole and Terbinafine

S. No.	Drug	Concentration	Mean absorbance	SD	%RSD	Mean absorbance	SD	%RSD
1.	Itraconazole	10 (µg/ml)	0.4538	0.00225	0.496669	0.4661	0.002762	0.59262
2.	Terbinafine	25 (µg/ml)	0.92533	0.00295	0.318819	0.9544	0.000794	0.083165

4.4.4(h) Solution stability

At various intervals, the absorbance of the Terbinafine HCl and Itraconazole sample solution was measured, and the assay percentage was computed. The sample solution may be utilized for the full 24 hours without experiencing any degradation, according to the solution stability test. The results of this test are displayed in Table 6.

4.4.4(i) Assay of marketed formulation

80 mg of topical cream (Itraconazole + Terbinafine HCl) was taken. This quantity was then transferred to a 250 ml volumetric flask, and 150 ml of diluent was added for the analysis of the marketed formulation (Duo faze: 100 mg Itraconazole and 250 mg Terbinafine hydrochloride). For 30 to 45 minutes, the flask was sonicated with sporadic shaking. Diluent was used to modify the volume to the desired level. The sample solution was centrifuged for 10 minutes at 5000 rpm. Five millilitres of the centrifuged sample were then pipetted into a 50-millilitre volumetric flask, where the volume was adjusted with acetonitrile before being filtered through Whatman filter paper.

DISCUSSION

It was discovered that the percentages of recovery for both itraconazole and terbinafine HCl fell between 98 and 102%. It was discovered that the calibration curve was linear, with R2 values for itraconazole and terbinafine HCl of 0.9994 and 0.9995, respectively. In the simultaneous equation method also known as vierordt's method, maximum absorption was found at 229 nm for itraconazole and at 223 nm for terbinafine. The linearity was found in the range of 5 -25µg/ml for both drugs and methods, with the correlation coefficient (R^2) of itraconazole and terbinafine is 0.999 for simultaneous equation method. It was discovered that the mixture of itraconazole and terbinafine HCl remained stable for a full day.

5.5.5 CONCLUSION

For the simultaneous measurement of itraconazole and terbinafine HCl in bulk and pharmaceutical tablet dosage form, a novel and straightforward UV spectrophotometric approach was devised. It was determined that the developed method was exact, accurate, linear, and stable, and that it could be used for routine analysis of the formulation's itraconazole and terbinafine HCl after it was validated in accordance with ICH recommendations and the results were found to be within limit.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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