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# METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ITRACONAZOLE AND TERBINAFINE HCL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING UV VISIBLE SPECTROSCOPY

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**Keywords:**

Itraconazole, Terbinafine, UV spectroscopy, Method development

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**ABSTRACT:** In this present study, a simple, effective, and accurate method has been developed for estimating itraconazole and terbinafine in bulk and pharmaceutical dosage form using UV Visible spectrophotometer. Two methods were developed: the simultaneous equation method, also known as vierordt's method, and the other absorbance ratio method, also known as Q value method. In the simultaneous equation method, maximum absorption was found at 229 nm for itraconazole and at 223 nm for terbinafine. In the absorbance ratio method, two different wavelengths were selected, 229 is being the λmax of one drug and 225nm is another iso-bestic wavelength. 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. For the absorbance ratio method, itraconazole's correlation coefficient (R2) is 0.9953 and 0.9877, and terbinafine is 0.9903 and 0.9948. All validation parameters were studied after method development, including linearity, accuracy, precision, limit of detection, limit of quantitation, robustness, and ruggedness as per ICH guidelines. The method was found robust and %RSD was less than 2% for accuracy, precision, and ruggedness. The developed method was suitable for analyzing itraconazole and terbinafine in bulk and dosage form.

**INTRODUCTION:** Itraconazole (ITZ) is an antifungal drug. It is a white to almost white powder, with molecular formula $C_{35}H_{38}C_{l2}N_8O$, molecular weight 706 g/mol and chemical structure is shown in **Fig. 1** [1]. This anti-fungal medication is used to treat various fungal infections, including aspergillosis, blastomycosis, and histo-plasmosis. It may be given by mouth or intravenously. Some brands of itraconazole are not for use in treating fungal infections of the fingernails or toenails. Itraconazole has antimycotic properties. Formulated for both topical and systemic use [2].

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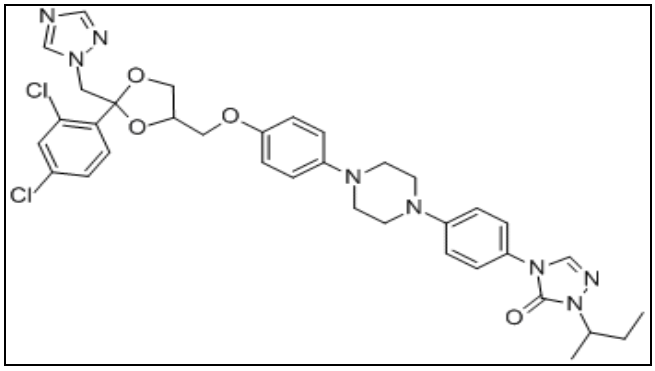

**FIG. 1: STRCTURE OF ITRACONAZOLE**

Terbinafine, sold under the brand name Lamisil, is an antifungal medication used to treat pityriasis versicolor fungal nail infection and ringworm, including joch itch and athlete's foot. It is either taken up mouth or applied to the skin as a cream ointment [3, 4]. The cream and ointment are not effective for nail infection [5]. Terbinafine hydrochloride (TFH) **Fig. 1** is an allylamine derivative. Chemically, it is [(2E)-6, 6-

dimethylhept - 2 - en - 4 – yn – 1 - yl] (methyl) (naphthalen-1-ylmethyl) amine hydrochloride [6].

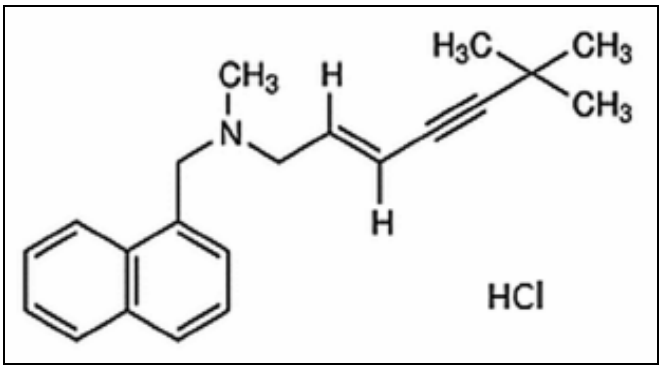


**FIG. 2: STRCTURE OF TERBINAFINE**

## MATERIALS AND METHOD:

**Instruments:** Double beam UV Visible spectro-photometer having 1 cm path length matched quartz cuvette within 1 cm light path with model LS-2704 was used to determine the λmax of standard compound in lenience lab Bhopal. Digital weighing balance was used for weighing sample.

**Reagents and Chemicals:** Standard sample of Itraconazole was gifted from biogenetic healthcare, Patiala (Punjab) and Standard sample of Terbinafine HCl was gifted from davis morgan lab, Chandigarh (Punjab). Tablet formulation (Gpitro-TR) was procured containing both drug from local market. Methanol and acetonitrile and water of HPLC grade were purchased from Rankem by Avantor Performance Materials India Ltd, Mumbai, India

**Preparation of Working Standard Solution:** 100mg of working standard itraconazole and 250mg of working standard terbinafine were accurately weighed and dissolved in 100 ml of methanol as diluents into separated 100ml of volumetric flask to get the concentration of 1000µg/ml solutions. From this solution take 1ml and diluted with 100ml methanol it gave concentration of 10 µg/ml or 10ppm as working stock solution.

**Scanning of λ max:** The standard drug samples namely Itraconazole and Terbinafine were used to scan in the range from 300 nm to 190 nm wavelength to find out the λ max values. The λ max value of the diluted standard stock of drug was reported to be 229 nm for Itraconazole with an absorbance value of 2.688, while λ max value for Terbinafine was observed to be 223 nm with an absorbance value of 2.568. The overlain spectrum shows maximum absorbance at 225nm. In simultaneous equation method, maximum absorption was found at 229nm for itraconazole and at 223nm for terbinafine. For absorbance ratio method 229 and 225nm, two wavelengths are selected for analysis. The graph is shown in **Fig. 3** and **4.**

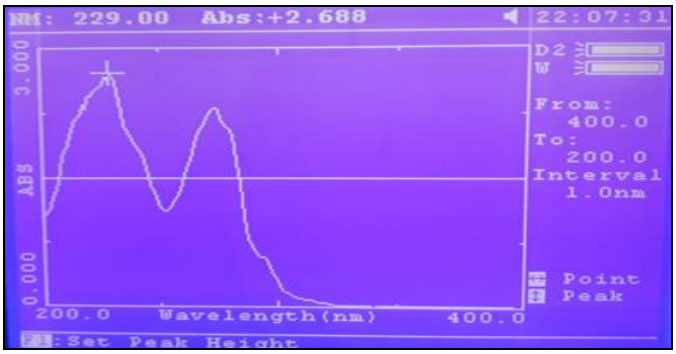


**FIG. 3: PEAKS GENERATED AFTER SCANNING THE STANDARD ANALYTE ITRACONAZOLE IN UV RANGE FROM 300 NM TO 190 NM ON UV-VISIBLE SPECTROPHOTOMETER**

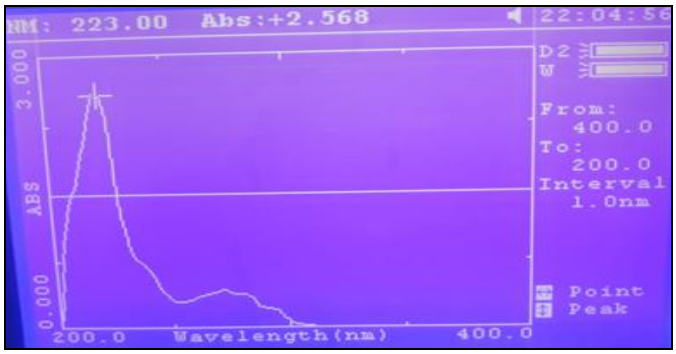


**FIG. 4: PEAKS GENERATED AFTER SCANNING THE STANDARD ANALYTE TERBINAFINE IN UV RANGE FROM 300 NM TO 190 NM ON UV-VISIBLE SPECTROPHOTOMETER**

**Preparation of Sample Solution:** Take twenty tablets of Gpitro-TR and weigh each tablet containing 100mg of itraconazole and 250mg of terbinafine and then determine the average weight of it. The tablets were crushed with mortar and pestle. The amount of powder equivalent to one tablet was transferred in a 100ml volumetric flask, and the volume was made up of methanol and mixed well for 2min to give a concentration of 1000 µg/ml. From this solution, take 1ml, transferred to 100 ml volumetric flask; volume was made up by methanol to give a concentration of 10

µg/ml. This solution was used as a working stock solution.

**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.

$$Cx = \boldsymbol{A_2 a y_1 - A_1 a y_2} \,/\, \boldsymbol{a x_2 a y_1 - a x_1 a y_2} \qquad (1)$$

$$Cy = \boldsymbol{A}1\boldsymbol{a}x_2 - \boldsymbol{A}_2 a x_1 \,/\, \boldsymbol{a x_2 a y_1 - a x_1 a y_2} \qquad (2)$$

$A_1$, $A_2$=Absorbance of the diluted sample at $\lambda_1$ and $\lambda_2$. The absorptivity's of X at$\lambda 1$ = ax1 and $\lambda_2$ = ax2. The absorptivity's of Y at $\lambda_1$ =ay1 and $\lambda_2$ =ay2. The absorbance' of sample at $\lambda_1$ =A1 and$\lambda_2$ =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.

$$Cx = (QM\text{-}QY \,/\, QX\text{-}QY) \times A1 \,/\, ax1$$

$$Cy = (QM\text{-}QX \,/\, QY\text{-}QX) \times A1 \,/\, ay1$$

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

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

**Method Validation:** The developed method by UV spectrophotometer was validated by various parameters such as linearity, accuracy, precision, the limit of detection, limit of quantitation, robustness, ruggedness as per as ICH guidelines.

**RESULT AND DISCUSSION:**

**Linearity:** From the stock solution, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml different concentration solutions were prepared. For the simultaneous equation method, the solution of 5-25 µg/ml concentration was scanned at 229 nm for itraconazole and at 223 nm for terbinafine. Similarly, the absorbance ratio method concentration of solutions of 5-25 µg/ml were scanned at 229 is the λ max of one drug and 225nm is another iso-bestic wavelength. The calibration curve was plotted by taking absorbance at y-axis and concentration at the x-axis. The correlation coefficient ($R^2$) of itraconazole and terbinafine is 0.999 for simultaneous equation method and the correlation coefficient ($R^2$) of itraconazole is 0.9953 and 0.9877 and for terbinafine is 0.9903 and 0.9948 for absorbance ratio method. Good linearity was shown at the concentration of 5-25. µg/ml for both methods are shown in **Table 1-6.**

**TABLE 1: UV LINEARITY RANGE OF ITRACONAZOLE AND TERBINAFINE FOR SIMULTANEOUS EQUATION METHOD**

| Itraconazole at 229nm | | Terbinafine at 223nm | |
|---|---|---|---|
| **Conc. inµg/ml** | **Absorbance** | **Conc. in µg/ml** | **Absorbance** |
| 5 | 0.3635 | 5 | 0.7623 |
| 10 | 0.5215 | 10 | 0.8632 |
| 15 | 0.6716 | 15 | 0.9524 |
| 20 | 0.8206 | 20 | 1.0408 |
| 25 | 0.9736 | 25 | 1.1342 |

**TABLE 2: UV LINEARITY RANGE OF ITRACONAZOLE FOR ABSORBANCE RATIO METHOD**

| Itraconazole | | |
|---|---|---|
| **Conc. in µg/ml** | **Absorbance at 225nm** | **Absorbance at 229nm** |
| 5 | 0.2347 | 0.3835 |
| 10 | 0.4246 | 0.5824 |
| 15 | 0.5643 | 0.7212 |
| 20 | 0.7459 | 0.8368 |
| 25 | 0.8674 | 0.9643 |

**TABLE 3: UV LINEARITY RANGE OF TERBINAFINE FOR ABSORBANCE RATIO METHOD**

| Terbinafine | | |
|---|---|---|
| **Conc. in µg/ml** | **Absorbance at 225nm** | **Absorbance at 229nm** |
| 5 | 0.4536 | 0.5436 |
| 10 | 0.6489 | 0.7683 |
| 15 | 0.7634 | 0.9356 |
| 20 | 0.8756 | 1.1462 |
| 25 | 1.1436 | 1.2863 |

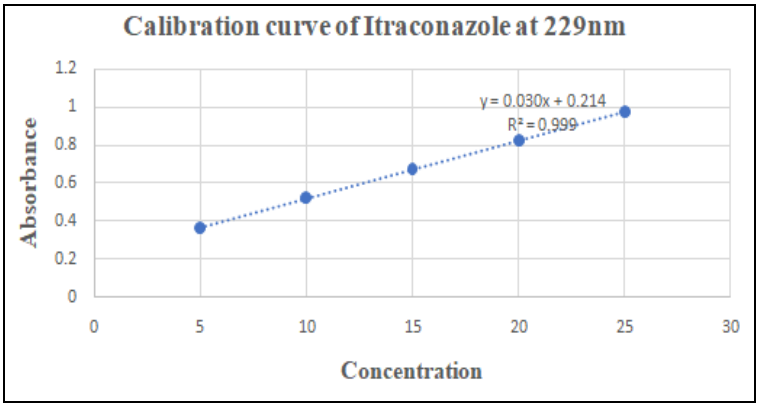


**FIG. 5: LINEARITY OF ITRACONAZOLE AT 229NM FOR SIMULTANEOUS EQUATION METHOD**

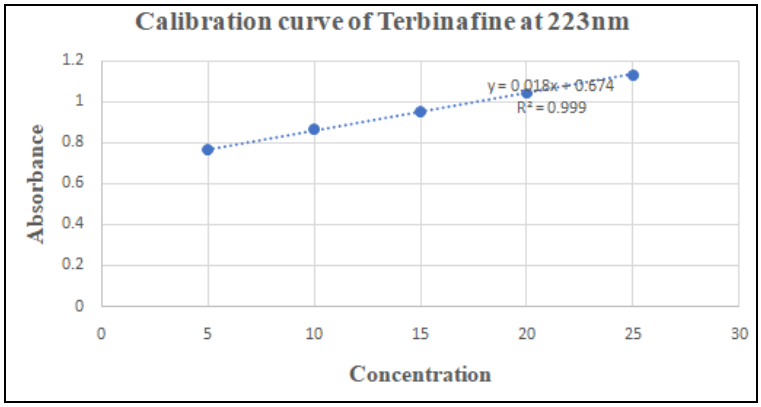


**FIG. 6: LINEARITY OF TERBINAFINE AT 223NM FOR SIMULTANEOUS EQUATION METHOD**

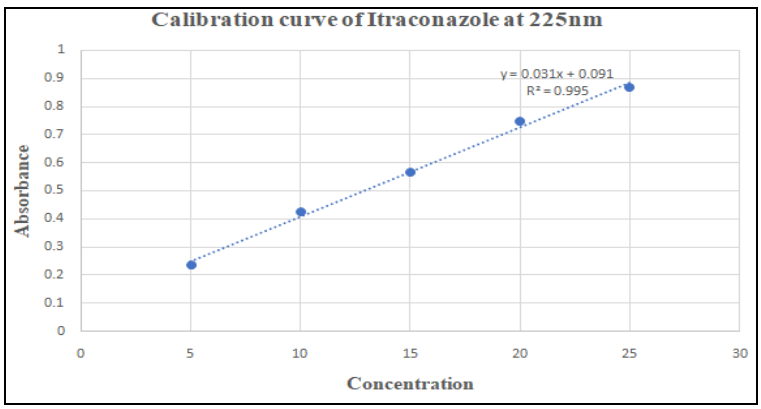


**FIG. 7: LINEARITY OF ITRACONAZOLE AT 225NM FOR ABSORBANCE RATIO METHOD**

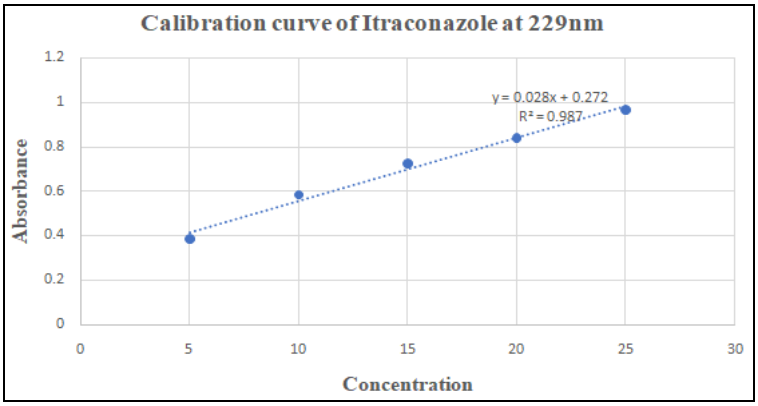


**FIG. 8: LINEARITY OF ITRACONAZOLE AT 229NM FOR ABSORBANCE RATIO METHOD**

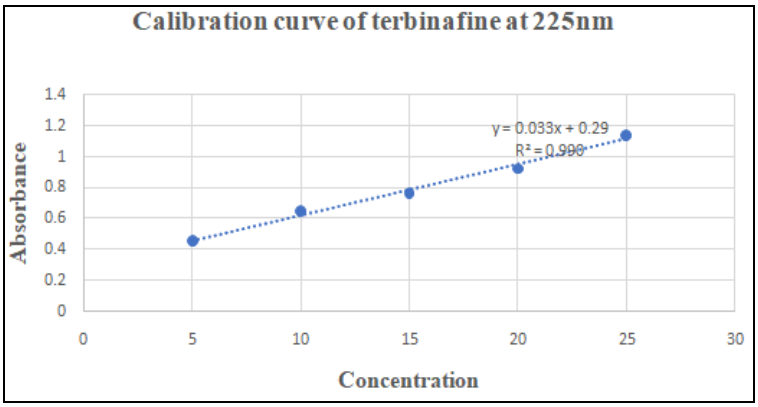


**FIG. 9: LINEARITY OF TERBINAFINE AT 225NM FOR ABSORBANCE RATIO METHOD**

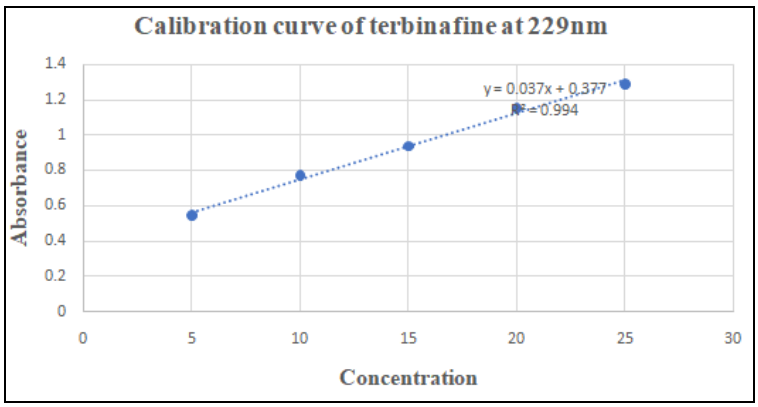


**FIG. 10: LINEARITY OF TERBINAFINE AT 229NM FOR ABSORBANCE RATIO METHOD**

**TABLE 4: LINEARITY DATA OF ITRACONAZOLE AND TERBINAFINE**

| Simultaneous equation method | | |
|---|---|---|
| **Parameter** | **Itraconazole at 229nm** | **Terbinafine at 223nm** |
| Linearity range | 5 -25µg/ml | 5 -25µg/ml |
| Regression equation | y=0.0340x+0.2144 | y=0.0184x+0.6742 |
| Correlation coefficient($R^2$) | 0.999 | 0.999 |
| Slope | 0.0340 | 0.2144 |
| Y intercept | 0.0184 | 0.6742 |

**TABLE 5: LINEARITY DATA OF ITRACONAZOLE**

| Absorbance ratio method | | |
|---|---|---|
| **Parameter** | **Itraconazole at 225nm** | **Itraconazole at 229nm** |
| Linearity range | 5 -25µg/ml | 5 -25µg/ml |
| Regression equation | y=0.0317x+0.0914 | y=0.0283x+0.2728 |
| Correlation coefficient($R^2$) | 0.9953 | 0.9877 |
| Slope | 0.0317 | 0.0283 |
| Y intercept | 0.0914 | 0.2728 |

**TABLE 6: LINEARITY DATA OF TERBINAFINE**

| Absorbance ratio method | | |
|---|---|---|
| **Parameter** | **Terbinafine at 225nm** | **Terbinafine at 229nm** |
| Linearity range | 5 -25µg/ml | 5 -25µg/ml |
| Regression equation | y=0.0331x+0.290 | y=0.0373x+0.377 |
| Correlation coefficient(R2) | 0.9903 | 0.9948 |
| Slope | 0.0331 | 0.0373 |
| Y intercept | 0.290 | 0.377 |

**Accuracy:** A sample solution of different concentrations were prepared that is 80%, 100%, 120% for determining the accuracy of the developed method. The amount of marketed formulation was constant, and the amount of pure drug was changed to 8gm, 10gm 12gm for 80%, 100%, and 120%, respectively. Two replicates were injected, the % recovery of itraconazole and terbinafine were calculated, and it was found within the 98 % to 101 % limit. The results of % recovery was shown in **Tables 7** to **12.**

**TABLE 7: % RECOVERY AND %RSD OF ITRACONAZOLE**

| For 80% | | | | | |
|---|---|---|---|---|---|
| **S. no.** | **Initial concentration (µg/ml)** | **Amount Added of the standard drug(µg/ml)** | **Amount Found** | **Amount Recovered** | **%Recovered** |
| 1 | 2 | 1.6 | 3.55 | 1.55 | 97.0% |
| 2 | 2 | 1.6 | 3.58 | 1.58 | 98.7% |
| | | Mean | 3.565 | 1.565 | 98.0% |
| | | SD | 0.0212132 | 0.0212132 | 0.01237437 |
| | | %RDS | 0.00595041 | 0.01355476 | 0.01264303 |

**TABLE 8: % RECOVERY AND % RSD OF ITRACONAZOLE**

| For 100% | | | | | |
|---|---|---|---|---|---|
| **S. no.** | **Initial concentration (µg/ml)** | **Amount Added of the standard drug (µg/ml)** | **Amount Found** | **Amount Recovered** | **%Recovered** |
| 1 | 2 | 2 | 3.95 | 1.95 | 98% |
| 2 | 2 | 2 | 3.94 | 1.94 | 97% |
| | | Mean | 3.945 | 1.945 | 98% |
| | | SD | 0.00707107 | 0.00707107 | 0.00353553 |
| | | %RDS | 0.00179241 | 0.00363551 | 0.00363551 |

**TABLE 9: % RECOVERY AND % RSD OF ITRACONAZOLE**

| For 120% | | | | | |
|---|---|---|---|---|---|
| **S. no.** | **Initial concentration (µg/ml)** | **Amount Added of the standard drug (µg/ml)** | **Amount Found** | **Amount Recovered** | **%Recovered** |
| 1 | 2 | 2.4 | 4.31 | 2.34 | 97.5% |
| 2 | 2 | 2.4 | 4.36 | 2.36 | 98.3% |
| | | Mean | 4.335 | 2.335 | 98.0% |
| | | SD | 0.03535534 | 0.01414214 | 0.00565685 |
| | | %RDS | 0.00815579 | 0.00601793 | 0.0057782 |

**TABLE 10: % RECOVERY AND % RSD OF TERBINAFINE**

| For 80% | | | | | |
|---|---|---|---|---|---|
| **S. no.** | **Initial concentration (µg/ml)** | **Amount Added of the standard drug (µg/ml)** | **Amount Found** | **Amount Recovered** | **%Recovered** |
| 1 | 5 | 4 | 8.91 | 3.91 | 97.75% |
| 2 | 5 | 4 | 8.9 | 3.9 | 97.50% |
| | | Mean | 8.90 | 3.90 | 98% |
| | | SD | 0.070711 | 0.070711 | 0.001768 |
| | | %RDS | 0.000794 | 0.001811 | 0.001811 |

**TABLE 11: % RECOVERY AND % RSD OF TERBINAFINE**

| For 100% | | | | | |
|---|---|---|---|---|---|
| **S. no.** | **Initial concentration (µg/ml)** | **Amount Added of the standard drug (µg/ml)** | **Amount Found** | **Amount Recovered** | **%Recovered** |
| 1 | 5 | 5 | 9.9 | 4.9 | 98% |
| 2 | 5 | 5 | 9.89 | 4.89 | 96% |
| | | Mean | 9.89 | 4.89 | 98% |
| | | SD | 0.007071 | 0.007071 | 0.001414 |
| | | %RDS | 0.000715 | 0.001445 | 0.001445 |

**TABLE 12: % RECOVERY AND % RSD OF TERBINAFINE**

| For 120% | | | | | |
|---|---|---|---|---|---|
| **S. no.** | **Initial concentration (µg/ml)** | **Amount Added of the standard drug (µg/ml)** | **Amount Found** | **Amount Recovered** | **%Recovered** |
| 1 | 5 | 6 | 10.90 | 5.9 | 98.5% |
| 2 | 5 | 6 | 10.79 | 5.79 | 96.5% |
| | | Mean | 10.84 | 5.845 | 98.0% |
| | | SD | 0.084853 | 0.077782 | 0.012728 |
| | | %RDS | 0.000849 | 1.33074 | 1.306768 |

**Precision:** Precision was determined by preparing three replicates of the same concentration of sample solution, and absorbance was noted. Intraday precision is determined by preparing three replicates of the same concentration of sample solution (6µg/ml, 8µg/ml, 10µg/ml). Absorbance was measured at different time intervals on the same day, and for the inter-day precision same procedure was followed at different days %RSD was calculated. The results of precision were shown in **Table 13 -16.**

**TABLE 13: INTRADAY PRECISION OF ITRACONAZOLE**

| S. no. | Con. (mcg/ml) | Absorbance I | Absorbance II | Absorbance III | Mean | Standard deviation | % RSD |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 0.4365 | 0.4397 | 0.4412 | 0.4391 | 0.0024 | 0.546759 |
| 2 | 8 | 0.5258 | 0.5227 | 0.5142 | 0.5209 | 0.0060 | 1.152972 |
| 3 | 10 | 0.7324 | 0.7386 | 0.7364 | 0.7358 | 0.0031 | 0.427188 |

Mean of three replicate determinations.

**TABLE 14: INTERDAY PRECISION OF ITRACONAZOLE**

| S. no. | Con. (mcg/ml) | Absorbance I | Absorbance II | Absorbance III | Mean | Standard deviation | % RSD |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 0.4387 | 0.4324 | 0.4465 | 0.4392 | 0.007063 | 1.60821 |
| 2 | 8 | 0.5269 | 0.5214 | 0.5123 | 0.5202 | 0.007374 | 1.41745 |
| 3 | 10 | 0.7345 | 0.7342 | 0.7332 | 0.733967 | 0.000681 | 0.09274 |

Mean of three replicate determinations.

**TABLE 15: INTRADAY PRECISION OF TERBINAFINE**

| S. no. | Con. (mcg/ml) | Absorbance I | Absorbance II | Absorbance III | Mean | Standard deviation | % RSD |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 0.6543 | 0.6523 | 0.6592 | 0.655267 | 0.00355 | 0.541782 |
| 2 | 8 | 0.7542 | 0.7595 | 0.7589 | 0.757533 | 0.002902 | 0.383125 |
| 3 | 10 | 0.8214 | 0.8287 | 0.8296 | 0.826567 | 0.004497 | 0.544062 |

Mean of three replicate determinations.

**TABLE 16: INTERDAY PRECISION OF TERBINAFINE**

| S. no. | Con. (mcg/ml) | Absorbance I | Absorbance II | Absorbance III | Mean | Standard deviation | % RSD |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 0.6529 | 0.6597 | 0.6575 | 0.6567 | 0.00347 | 0.52838 |
| 2 | 8 | 0.7563 | 0.7584 | 0.7556 | 0.756767 | 0.001457 | 0.192552 |
| 3 | 10 | 0.8235 | 0.8287 | 0.8254 | 0.825867 | 0.002631 | 0.318601 |

Mean of three replicate determinations.

**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 0.324 µg/ml and 0.935 µg/ml and for terbinafine is 0.647 µg/ml and 1.658 µg/ml.

**TABLE 17: (LOD) AND (LOQ) OF ITRACONAZOLE AND TERBINAFINE**

| S. no. | Drugs | LOD(µg/ml) | LOQ(µg/ml) |
|---|---|---|---|
| 1 | Itraconazole | 0.324 | 0.935 |
| 2 | Terbinafine | 0.647 | 1.658 |

**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. The result is shown in **Table 18.**

**TABLE 18: ROBUSTNESS OF ITRACONAZOLE AND TERBINAFINE**

| S. no. | Drugs | Wavelength | Absorbance | Mean absorbance | Standard deviation | %RSD |
|---|---|---|---|---|---|---|
| 1 | Itraconazole | 229nm | 0.84356<br>0.84562<br>0.84246 | 0.84388 | 0.001604 | 0.190089 |
| 2 | Itraconazole | 226nm | 0.86255<br>0.86352<br>086256 | 0.862877 | 0.000557 | 0.064571 |
| 3 | Itraconazole | 227nm | 0.82352<br>0.82428<br>0.82742 | 0.825073 | 0.002067 | 0.250583 |
| 4 | Terbinafine | 223nm | 0.75235<br>0.75423<br>0.75752 | 0.7547 | 0.002617 | 0.34674 |
| 5 | Terbinafine | 225nm | 0.74263<br>0.74653<br>0.74342 | 0.744193 | 0.002062 | 0.277052 |
| 6 | Terbinafine | 227nm | 0.79832<br>0.79632<br>0.79352 | 0.796053 | 0.002411 | 0.30288 |

**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. The result shown in **Table 19.**

**TABLE 19: RUGGEDNESS OF ITRACONAZOLE AND TERBINAFINE**

| | | | Analyst I | | | Analyst II | | |
|---|---|---|---|---|---|---|---|---|
| S. no. | Drug | Concentration | Mean absorbance | Standard deviation | %RSD | Mean absorbance | Standard deviation | %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 |

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