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.

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-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1H-pyrazol-5(4H)-one and (E)-N,6,6-trimethyl-N-(naphthalen-1-ylmethyl)-2-

methylenecyclohexane-1-carboxylic acid, 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.

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% 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 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.
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.

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

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**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

**Table 5: % recovery results for itraconazole**

| Level | % recovery | Average | SD  | % RSD |
|-------|------------|---------|-----|-------|
| 50%   | 99.0       | 99.7    | 0.70| 0.70  |
| 100%  | 99.9       | 99.9    | 0.25| 0.25  |
| 150%  | 99.5       | 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       | 99.4    | 0.49| 0.49  |
| 100%  | 99.9       | 99.9    | 0.06| 0.06  |
| 150%  | 100.4      | 99.3    | 1.01| 1.01  |

SD: Standard deviation, RSD: Relative standard deviation

**Table 6: % recovery results for terbinafine HCl**

| Concentration (ppm) | Area | Itraconazole | Terbinafine HCl | Itraconazole | Terbinafine HCl |
|---------------------|------|--------------|-----------------|--------------|-----------------|
| 20                  | 50   | 331,698      | 4,371,822       |
| 32                  | 80   | 530,320      | 7,422,916       |
| 40                  | 100  | 652,900      | 9,245,345       |
| 52                  | 120  | 833,091      | 11,074,374      |
| 60                  | 150  | 984,345      | 13,763,467      |

**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      | 7,422,916       |
| 40                  | 100  | 652,900      | 9,245,345       |
| 52                  | 120  | 833,091      | 11,074,374      |
| 60                  | 150  | 984,345      | 13,763,467      |
| 20                  | 50   | 331,698      | 4,371,822       |
| 32                  | 80   | 530,320      | 7,422,916       |
| 40                  | 100  | 652,900      | 9,245,345       |
| 52                  | 120  | 833,091      | 11,074,374      |
| 60                  | 150  | 984,345      | 13,763,467      |

**Fig. 6: Linearity graph of itraconazole**

y = 16064x + 11040

$R^2 = 0.9989$
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. The quantity of powder containing 80 mg of (itraconazole + terbinafine HCl) was transferred to 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).

**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).

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**Table 8: Robustness results for itraconazole and terbinafine HCl**

| Parameters            | Itraconazole | Terbinafine HCl |
|-----------------------|--------------|-----------------|
| Minus flow (1.0 ml/min) | 4.145        | 101.1           |
| Plus flow (1.4 ml/min) | 2.985        | 101.0           |
| Minus temperature (28°C) | 3.485        | 101.4           |
| Plus temperature (32°C) | 3.483        | 100.7           |
| Minus wavelength (223 nm) | 3.489        | 101.7           |
| Plus wavelength (227 nm) | 3.487        | 100.4           |

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 | 100.5   |
|                       | Terbinafine HCl | 99.8    |
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            |

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% H₂O₂), 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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