Cleaning Method: Residue Determination of Terbinafine hydrochloride on the Surface of Manufacturing Equipment by RP-UPLC

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Received 12 February 2013; accepted 25 February 2013

Abstract
Cleaning validation is required in the pharmaceutical field to avoid potential clinically significant synergistic interactions between pharmacologically active chemicals. A rapid, simple, sensitive and accurate UPLC method has been developed and validated for determination of Terbinafine hydrochloride residues on the surface of manufacturing equipment. Aquity UPLC-BEH C-18 column (100 X 2.1 mm, 1.7 μm) column was used as stationary phase by using mobile phase (pH 7.5 of 0.1% tri-ethyl amine: Acetonitrile (15: 85), % v/v).Method was developed in isocratic mode with 3 minutes runtime, at flow rate of 0.4 ml/minute. Terbinafine hydrochloride was detected at 224 nm. Method was linear in the concentration range of 0.02–20 μg/ml. RSD of the six replicates results (Precision) was found to be 0.11%. The swabbing procedure was optimized to achieve suitable recovery of Terbinafine hydrochloride from stainless steel surfaces. Recovery of the method was found > 90%. This method can be used to determine trace levels of Terbinafine hydrochloride residues in production equipment area to confirm the efficiency of the cleaning procedure in pharmaceutical industries to avoid cross contamination.

Keywords: Cleaning validation, Terbinafine hydrochloride, Residue analysis, UPLC

Introduction:
Cleaning validation is required in the pharmaceutical field to avoid potential clinically significant synergistic interactions between pharmacologically active chemicals. The prevention of cross-contamination of drugs in pharmaceutical production must be avoided; therefore the cleaning of the manufacturing equipment is an important aspect of good manufacturing practice. The cleaning validation consists therefore in two separate activities: the first is the development and validation of the cleaning procedure that is used to remove drug residues from the manufacturing equipment surfaces and the second is application of the method to quantify residuals from surfaces that are used in the manufacturing environment.

To monitor the effectiveness of the cleaning operation, the analytical method should be selective for the substance considered and must be of sufficient sensitivity, because residue concentrations are usually low. According to the FDA, the limit should be based on logical criteria involving the risk associated with residues of the product determined. Calculation of an acceptable residue limit and maximum allowable carryover for active products in production equipment should be based on therapeutic doses, toxicological index and a general limit (10 ppm). According to the food and drug administration (FDA), two different method of sampling are generally admitted for performing a cleaning control: the direct surface sampling, using the swabbing technique and the indirect sampling based on the analysis of solutions used for rinsing the equipment [1-6].

Terbinafine hydrochloride is a synthetic allylamine antifungal. It is highly lipophilic in nature and tends to accumulate in skin, nails, fatty tissues, bacteria of the duodenum and viral infections of the stomach. Terbinafine Hydrochloride is an allylamine antifungal agent and acts by inhibiting squalene epoxidase, thus blocking the biosynthesis of ergosterol, an essential component of fungal cell membranes. Terbinafine is mainly effective on the dermatophytes group of fungi. Like other allylamines, terbinafine inhibits ergosterol synthesis by inhibiting squalene epoxidase, an enzyme that is part of the fungal cell membrane synthesis pathway. Because terbinafine...
prevents conversion of squalene to lanosterol, ergosterol cannot be synthesized. This is thought to change cell membrane permeability, causing fungal cell lysis. It is available in dosage forms such as tablets, cream, gel, ointment etc. Structure of Terbinafine hydrochloride is shown in fig. 1

![Fig-1: Chemical Structure of Terbinafine hydrochloride.](image)

Literature survey reveals that numerous methods have been published for quantitative analysis of Terbinafine hydrochloride alone and in combination with other drugs such as Spectrophotometric [10], HPLC [11-14] etc. A literature revealed that no validated cleaning method is available for Terbinafine hydrochloride residue analysis in pharmaceutical equipment. Taking this consideration into account that the aim of this study was to develop and validate a rapid and sensitive analytical method enabling determination of trace levels of Terbinafine hydrochloride residues in production area equipment to confirm the efficiency of the cleaning procedure. The analytical method was validated in the terms of selectivity, linearity, accuracy, precision and limit of detection and limit of quantification and solution stability. This method would be helpful in pharmaceutical industry for determination of residues on the surface of manufacturing area and equipment to avoid any potential cross contamination.

**Materials and Methods**

**Instruments and apparatus**

Waters Aquity uplc system connected with PDA detector and Empower 2 software for data acquisition, XS205 Dual range balance (Make: Mettler Toledo), Bandelin Sonorex sonicator, Heraeus Biofuge Stratos Centrifuge and Stainless steel plates (4 cm × 4 cm) were used during development study. Class A Volumetric flasks, pipettes, beakers, measuring cylinders and centrifuge tubes of Borosil glass were used.

**Chemicals and reagents**

Terbinafine hydrochloride standard was provided by Dr. Reddy’s Laboratories, Hyderabad, India. Methanol-HPLC grade (SDFCL, India), Acetonitrile (Rankem, India), Triethylamine, Orthophosphoric acid (Merck, India), Water-HPLC grade and 0.22μ PVDF membrane filters were used.

**Method**

**Chromatographic parameters**

All chromatographic experiments were performed in the isocratic mode. Separation was achieved on column Acquity BEH C18, 100 × 2.1 mm, 1.7 μ by using waters Acquity UPLC with PDA detector and controlled by Empower 2 software. Mobile phase was filtered and degassed mixture of (pH 7.5 of 0.1% tri-ethyl amine: Acetonitrile (15: 85), % v/v). Other parameters such as flow rate of 0.4 ml/minute, detection at 224 nm, column temperature of 45°C, injection volume of 2 μl and run time of 3 minutes were finalized during development. Diluent was used as Methanol.

**Standard solution preparation**

Standard stock solution was prepared by weighing about 50.0 mg of Terbinafine hydrochloride standard into 50 ml of volumetric flask, added 40 ml of diluent followed by sonication for 5 minutes in ultrasonic bath to dissolve it. Then cooled at room temperature and made up to volume with diluent and mix (Standard stock of Terbinafine hydrochloride: 1000 μg/ml). Transferred 5.0 ml of this stock solution into 50 ml of volumetric flask and made up to volume with diluent. Further dilute 5.0 ml of this solution to 50 ml with diluent. (Standard solution of Terbinafine hydrochloride: 10 μg/ml). The standard stock solution was subsequently diluted with diluent to furnish calibration curve (Linearity) in the range of 0.02–20 μg/ml.

**Sample preparation:**

Selected stainless-steel surfaces (4 cm × 4 cm), previously cleaned and dried, were sprayed with 0.1 ml Terbinafine hydrochloride standard stock solution for positive swab control and then solvent was allowed to evaporate (approximate time was 15 minutes). The surfaces were wiped with the first cotton swab soaked with methanol, passing it in various directions to remove the residues from the stainless steel. The other dry cotton swab was used to wipe the wet surfaces. The swabs were placed in a 25-ml screw cap test tube containing 10 ml of diluent. The negative swab control was prepared in the same way as the sample, using swabs, which had not been in contact with the test surface. Subsequently, the tubes were shaken for 10 minutes on rotary shaker followed by sonication for 10 minutes in ultrasonic bath. Squeezed the swabs and filtered the solutions through 0.22μm PVDF filter and solutions were analyzed by UPLC.

**Rinse method**

Selected stainless-steel surfaces (4 cm × 4 cm), previously cleaned and dried, were sprayed with 0.1 mL Terbinafine hydrochloride standard stock solution for positive swab control and then solvent was allowed to evaporate (approximate time was 15 minutes). Then rinsed effective area of the stainless steel plate with 10mL of diluent and collected the rinsing solution into beaker. Sonicated the beaker for 10 minutes in ultrasonic bath and filtered the solutions through 0.22μm PVDF filter and solutions were analyzed by UPLC.

**Swab samples from different locations within the Manufacturing equipment:**

Swab samples from different locations within the manufacturing equipment and relevant area were submitted to the laboratory for analysis of Terbinafine hydrochloride residues. These samples were prepared and analyzed as described above.

**Results and Discussion**

**Acceptance limit calculation:**

The maximum allowable carryover (MACO) is the acceptable maximum allowed concentration of previous substance to next batch. The MACO is determined based on the therapeutic dose, toxicity and generally 10 ppm criteria. Based on determined maximum allowable residual limit in subsequent product, the next step was the
determination of the residue limit in terms of the contamination level of active ingredient per surface area of equipment. The total surface area of the equipment in direct contact with the product was accounted for in the calculations.

The limit for the previous product in the subsequent product is determined by use of the equation:

\[ \text{MACO}_{\text{PPm}} (\text{mg}) = \frac{\text{MB}_{\text{next}} (\text{mg})}{1000000} \]

Where MACO_{PPm} is the acceptable amount transferred to the next product, calculated from the general 10 ppm limit, MB next is the minimum batch size for the next product, and 10/1000000 denotes 10 ppm. When the maximum allowable residue limit in the subsequent product has been determined, the next step is to determine the residue limit as the level of active ingredient contamination per unit surface area of the equipment.

The equation used for determining the limit per surface area are:

\[ \text{LSA} (\text{mg/cm}^2) = \frac{\text{MACO}_{\text{PPm}} (\text{mg})}{\text{SA} (\text{cm}^2)} \]

\[ \text{LSA} (\text{mg/16 cm}^2) = \text{LSA} (\text{mg/cm}^2) \times S (\text{cm}^2) \]

Where LSA is the limit per unit surface area, calculated on the basis of equipment surface area and the most stringent MACO, SA is the equipment surface area in common between one product and the subsequent product, expressed in cm², and S is the swab area (16 cm²). Based on contact surface area for equipment items included in production of Terbinafine hydrochloride cream, the calculated limit per surface area (LSA) in the case of Terbinafine hydrochloride was 100 μg/swab for a 16 cm² area (appropriate concentration 10 μg/ml).

Optimization of chromatographic conditions

To find the best chromatographic conditions, wavelength for detection, column and mobile phase were adequately selected. The main objective was to develop an UPLC method enabling determination of Terbinafine hydrochloride residues collected by swabs without interference from excipients or impurities present in formulation or active pharmaceutical ingredient.

Wavelength for detection was selected by scanned known concentration of Terbinafine hydrochloride solution in UV Visible spectrophotometer. Terbinafine hydrochloride spectra showed wavelength maxima at 223.4 nm. See Terbinafine hydrochloride spectra in fig 2.

Optimization of sample treatment

Cotton swabs were spiked with different quantities of Terbinafine hydrochloride and placed in glass tubes. After addition of different solvents and their mixtures (water, methanol and Acetonitrile), the tubes were sonicated for different times (5, 10, 15 and 30 minutes) and the solutions were analyzed by UPLC. The optimum conditions were achieved with Methanol as diluent and a sonication time of
Table I: Results obtained from analysis of Terbinafine hydrochloride in swab samples collected from different locations within the equipment area.

<table>
<thead>
<tr>
<th>Equipment swabbed</th>
<th>Location swabbed</th>
<th>Terbinafine hydrochloride detected (μg per swab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material dispensing scoops</td>
<td>Internal surface</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td></td>
<td>External surface</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td>Weighing balance</td>
<td>Weighing surface</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td>Manufacturing vessel</td>
<td>Top surface</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td></td>
<td>Middle surface</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td></td>
<td>Bottom surface</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td></td>
<td>Line end point</td>
<td>&lt; LOQ</td>
</tr>
</tbody>
</table>

Table II: Results of System suitability, LOD /LOQ, Linearity and Method precision.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Terbinafine hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>System suitability</td>
<td></td>
</tr>
<tr>
<td>a) USP tailing factor (&lt;2.0)</td>
<td>1.1</td>
</tr>
<tr>
<td>b) USP plate count (&gt;5000)</td>
<td>12330</td>
</tr>
<tr>
<td>c) % RSD of five standard injections (&lt;2.0%)</td>
<td>0.08</td>
</tr>
<tr>
<td>LOD and LOQ Values</td>
<td></td>
</tr>
<tr>
<td>a) LOD Value</td>
<td>0.007 μg/mL</td>
</tr>
<tr>
<td>b) LOQ Value</td>
<td>0.02 μg/mL</td>
</tr>
<tr>
<td>Linearity</td>
<td></td>
</tr>
<tr>
<td>a) Correlation coefficient (NLT 0.999)</td>
<td>0.99999</td>
</tr>
<tr>
<td>b) Linearity equation</td>
<td>y = 320,101.00799x + 4,640,78551</td>
</tr>
<tr>
<td>c) Bias at 100% response(NMT 2.0)</td>
<td>0.19</td>
</tr>
<tr>
<td>Method precision (Repeatability)</td>
<td></td>
</tr>
<tr>
<td>a) Mean % Recovery (n=6) (&gt;90%)</td>
<td>95.6 %</td>
</tr>
<tr>
<td>b) % of RSD (n=6) (NMT 5.0%)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table III: Results of Accuracy

<table>
<thead>
<tr>
<th>Amount added μg/mL</th>
<th>Amount found μg/mL</th>
<th>Recovery (%) (&gt;90%)</th>
<th>% RSD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.98</td>
<td>4.82</td>
<td>96.7</td>
<td>0.12</td>
</tr>
<tr>
<td>9.87</td>
<td>9.76</td>
<td>98.8</td>
<td>0.09</td>
</tr>
<tr>
<td>14.95</td>
<td>14.89</td>
<td>99.5</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table IV: Results of solution stability

<table>
<thead>
<tr>
<th>Standard</th>
<th>Time</th>
<th>% Assay</th>
<th>% Assay difference (3.0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>100.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>100.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Test sample</td>
<td>Initial</td>
<td>98.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Day 2</td>
<td>98.5</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

Validation of analytical method
Once the chromatographic conditions had been optimized, the method was validated for linearity, precision, accuracy, LOD and LOQ, selectivity, solution stability.

System suitability test
System suitability test is essential for the assurance of the quality performance of a chromatographic system. Standard of 10 μg/ml was injected five times into UPLC as per test method. RSD of area of Terbinafine hydrochloride peak from five injections was found 0.08%. Theoretical plates of Terbinafine hydrochloride peak was found to be 12330. USP tailing of Clobetasol Propionate peak was found to be 1.1. The results are summarized in table II.

Selectivity
The selectivity of the method was checked by injecting Terbinafine hydrochloride standard solution, background control sample, Positive swab control sample and negative swab control sample into UPLC with PDA detector as per 5 minutes. In all the cases, the best results were obtained using two cotton swabs (the first wetted with diluent and the second dry). Hence this technique was applied in the subsequent work. Some of the results obtained for swab samples from different locations within the manufacturing equipment are summarized in table I.

Fig-6: Linearity plot for Terbinafine hydrochloride.
test method. There was no interference at retention time of Terbinafine hydrochloride due to back ground control sample and the negative swab control samples. Peak purity of Terbinafine hydrochloride peak was passed in standard and positive swab control sample.

**LOD and LOQ**

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve at low concentration levels according to ICH guidelines. The LOD and LOQ for Terbinafine hydrochloride were found to be 0.007 µg/ml and 0.02 µg/ml respectively. The results are summarized in table II.

**Linearity**

Linearity of the test method was conducted by injecting Terbinafine hydrochloride solution in the concentration range of 0.02–20 µg/ml. Plotted a linearity graph of concentration versus area for Terbinafine hydrochloride. The correlation co-efficient, slope, intercept and bias at 100% response are summarized in table II and See fig 3 for linearity plot.

**Method precision**

The precision of the test method was performed by spiking Terbinafine hydrochloride solution on to stainless steel plate to achieve final solution containing 10 µg/ml. Performed swabbing and analyzed as per test method. This study was assessed by comparing the amount of analyte determined versus the known amount spiked for six replicates. The RSD of % recovery of six sample preparations was found 0.11%. The results are summarized in table II.

**Accuracy**

The recovery was assessed by comparing the amount of analyte determined versus the known amount spiked at three different concentration levels (5.0 µg/ml, 10 µg/ml and 15 µg/ml) with 3 replicates (n=3). The accuracy was determined by spiking Terbinafine hydrochloride on stainless steel plates, performed swabbing and analyzed as per test method. The recovery at three different concentrations was found more than 90%. The results are summarized in table III.

**Solution stability of standard and sample solution**

Prepared standard solution and positive swab control sample as per test method and kept on bench top at room temperature. Injected standard and sample solutions into UPLC as per test method at initial and after 2 days. Calculated % assay of standard and test solution against fresh standard. The assay of standard and sample was found satisfactory from initial to 2 days. This indicate that standard and sample preparation (Positive swab samples) are stable for 2 days at room temperature. The results are summarized in table IV.

**Conclusion**

A rapid, simple, sensitive and accurate isocratic UPLC method has been developed for determination of Terbinafine hydrochloride residues on the surface of manufacturing equipment. Validation of the method revealed that method is selective, precise, linear and accurate. Extraction of Terbinafine hydrochloride residues from surface by using two cotton swabs, the first wet with methanol and the second dry, is recommended. The recovery of Terbinafine hydrochloride obtained from swabs was found > 90% for three different concentrations. The solution stability data revealed that standard and swab samples are stable for 2 days at room temperature. The overall procedure can be used to determine trace levels of Terbinafine hydrochloride residues in production equipment area to confirm the efficiency of the cleaning procedure in pharmaceutical industries.

**Acknowledgement**

The authors are very thankful to Dr. Reddy’s Laboratories, Hyderabad, India for providing research facilities for this work. They are also very thankful to Dr. Madhusudan of R & D, Dermatology, Dr. Reddy’s laboratories, India for providing samples and materials for this research work.

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Source of support: Nil; Conflict of interest: None declared