PERIÓDICO TCHÊ QUÍMICA
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DETERMINAÇÃO QUANTITATIVA DE RESÍDUOS DE TERBINAFINA NAS SUPERFÍCIES DE EQUIPAMENTOS DE FABRICAÇÃO FARMACÊUTICA

QUANTITATIVE DETERMINATION OF TERBINAFINE RESIDUES ON THE PHARMACEUTICAL MANUFACTURING EQUIPMENT SURFACES

ტერბინაფინის ნარჩენების რაოდენობრივი განსაზღვრა ფარმაცევტული საწარმოო დანადგარის ზედაპირებზე

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RESUMO

Introdução: A validação de limpeza é uma responsabilidade analítica crítica exigida pelo GMP (Good Manufacturing Practice), que confirma a eficácia do procedimento padrão de limpeza no processo de fabricação farmacêutica para todos os medicamentos produzidos em contato com o equipamento de fabricação farmacêutica compartilhado. A necessidade de realizar a validação da limpeza é especialmente importante quando o medicamento é o “pior caso” para o procedimento de limpeza quanto à solubilidade, potência terapêutica, toxicidade e aderência superficial dos ingredientes farmacêuticos ativos (API). A estimativa de resíduos de API requer um método seletivo e sensível capaz de determinar quantitativamente os vestígios de API remanescentes na superfície do equipamento de fabricação após o procedimento de limpeza. **Objetivo**: O presente estudo demonstra a adequação e a aplicabilidade do método proposto obtido com uma combinação de um HPLC analítico sensível, seletivo e específico e procedimentos eficazes de amostragem de swab para estimativa quantitativa de resíduos de terbinafina em amostras coletadas de superfícies de equipamentos de fabricação farmacêutica e a eficiência do procedimento padrão desenvolvido para limpeza do equipamento de fabricação compartilhado em apoio à validação de limpeza. **Métodos**: O procedimento de amostragem por swab foi desenvolvido para obter uma recuperação adequada (>80%). A superfície (área de amostragem – 25 cm²) foi limpa com um swab umedecido com metanol. O procedimento analítico foi desenvolvido utilizando o sistema de HPLC “Agilent 1260 Infinity II” e coluna BDS Hypersil C18 250×4,6 mm, 5 µm com eluição isocrática de fase móvel. **Resultados e Discussão**: O método combinado foi validado quanto ao teste de adequação do sistema cromatográfico, especificidade, faixa de linearidade, exatidão, precisão, limite de detecção (LOD) e quantificação (LOQ). A estabilidade das soluções de terbinafina HCl em metanol, o material do *swab* e a compatibilidade do filtro de membrana também foram estudados. A curva de calibração foi linear (R^2=0,99999) em uma faixa de concentração de 0,00003-0,025 mg/mL; LOD – 0,000015 mg/mL e LOQ – 0,00003 mg/mL; As concentrações determinadas de resíduos de terbinafina nas soluções de teste não foram superiores a 0,00090 mg/mL que estão abaixo do limite de contaminação cruzada do seguinte medicamento. **Conclusões**: O estudo analítico realizado usando o método desenvolvido com uma combinação de um HPLC analítico e procedimentos de amostragem de swab para estimativa de resíduos de terbinafina nas superfícies do equipamento farmacêutico usado durante a fabricação de comprimidos não revestidos de terbinafina HCl 250 mg demonstra a validade do procedimento de limpeza . Outros laboratórios de controle de qualidade podem aplicar o método proposto para realizar a validação de limpeza em várias formulações de medicamentos de terbinafina HCl.

Palavras-chave: : *HPLC, Terbinafina, amostragem por swab, validação.*

ABSTRACT

Background: Cleaning validation is a critical analytical responsibility required by GMP (Good Manufacturing Practice), which confirms the effectiveness of the cleaning standard procedure in the pharmaceutical

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manufacturing process for all the produced drug products contacting the shared pharmaceutical manufacturing equipment. The need to carry out cleaning validation is especially important when the drug product is the “worst-case” for the cleaning procedure regarding solubility, therapeutic potency, toxicity, and surface adherence of active pharmaceutical ingredients (API). Estimation of API residues requires a selective and sensitive method capable of quantitative determination of API traces remaining over the surface of manufacturing equipment after the cleaning procedure. **Aim:** The present study demonstrates the suitability and the applicability of the proposed method obtained with a combination of a sensitive, selective, and specific analytical HPLC and effective swab wipe sampling procedures for quantitative estimation of terbinafine residues in samples collected from pharmaceutical manufacturing equipment surfaces and the efficiency of the developed standard procedure for cleaning of the shared manufacturing equipment in support of cleaning validation. **Methods:** The swab sampling procedure was developed to obtain a suitable recovery (>80 %). The surface (sampling area – 25 cm^2) was wiped with one swab moistened with methanol. The analytical procedure was developed using the HPLC system “Agilent 1260 Infinity II” and BDS Hypersil C18 250×4.6 mm, 5 µm column with an isocratic elution of mobile phase. **Results and Discussion:** The developed combined method was validated concerning chromatographic system suitability test, specificity, linearity range, accuracy, precision, the limit of detection (LOD), and quantitation (LOQ). The stability of terbinafine HCl solutions in methanol, the swab material, and membrane filter compatibility was also studied. The calibration curve was linear (R^2=0.99999) over a concentration range 0.00003-0.025 mg/mL; LOD – 0.000015 mg/mL, and LOQ – 0.00003 mg/mL; The determined concentrations of terbinafine residues in test solutions were not more than 0.00090 mg/mL which are below the limit of cross-contamination of the next drug product. **Conclusions:** The performed analytical study using the developed method with a combination of an analytical HPLC and swab sampling procedures for estimation of terbinafine residues on surfaces of the pharmaceutical equipment used during the manufacture of Terbinafine HCl 250 mg uncoated tablets demonstrates the validity of the cleaning procedure. Other quality control laboratories can apply the proposed method to carry out cleaning validation on various drug formulations of terbinafine HCl.

Keywords: *HPLC, Terbinafine, Swab sampling, validation*

რეზიუმე

შესავალი: დასუფთავების ვალიდაცია არის კრიტიკული ანალიზური პასუხისმგებლობა, რომელიც წარმოადგენს GMP-ის (კარგი საწარმოო პრაქტიკა) მოთხოვნას დაადასტურებს ფარმაცევტულ საწარმოო პროცესში გამოყენებული დასუფთავების პროცედურის ეფექტურობას ყველა წარმოებული ფარმაცევტული პროდუქტისთვის, რომელიც შეხებაშია საერთო გამოყენების საწარმოო დანადგართან. დასუფთავების ვალიდაციის ჩატარების საჭიროება განსაკუთრებით მნიშვნელოვანია, როდესაც დასუფთავების პროცედურისთვის ფარმაცევტული პროდუქტი წარმოადგენს „უარეს შემთხვევას“ აქტიური ფარმაცევტული ინგრედიენტის (აფი) ხსნადობის, თერაპიული მოქმედების, ტოქსიკურობისა და ზედაპირზე ადჰეზიის მიხედვით. აფი-ის ნარჩენების შეფასება საჭიროებს სელექტურ და მგრძნობიარე მეთოდს, რომლის საშუალებით საწარმოო დანადგარის ზედაპირზე შესაძლებელია დასუფთავების პროცედურის ჩატარების შემდეგ აფი-ის ნარჩენების რაოდენობრივი განსაზღვრა განხორციელდეს. **მიზანი:** წინამდებარე კვლევა აჩვენებს დასუფთავების ვალიდაციისთვის საერთო გამოყენების ფარმაცევტული საწარმოო დანადგარის ზედაპირებიდან შეგროვებულ ნიმუშებში ტერბინაფინის ნარჩენების რაოდენობრივი შეფასების მგრძნობიარე, სელექტური და სპეციფიკური ანალიზური მაღალეფექტური სითხური ქრომატოგრაფული (მესქ) და სვაბით სინჯის აღების ეფექტური პროცედურების გაერთიანებით მიღებული მეთოდის, ასევე შემუშავებული დასუფთავების სტანდარტული პროცედურის გამოსადეგობასა და გამოყენებადობას. **მეთოდები:** კარგი აღდგენის (>80 %) მიღების მიზნით შემუშავებული იქნა სვაბით სინჯის აღების პროცედურა. ზედაპირიდან სინჯის აღება განხორციელდა (სინჯის ასაღები ფართობი – 25 $სმ^2$) მეთანოლში დასველებული სვაბის გამოყენებით. ანალიზური პროცედურა შემუშავებული იქნა მოძრავი ფაზის ელუირების იზოკრატულ პირობებში მესქ სისტემის “Agilent 1260 Infinity II” და სვეტის BDS Hypersil C18 250×4.6 მმ, 5 მკმ გამოყენებით. **შედეგები და განსჯა:** შემუშავებული მეთოდი ვალიდირებული იქნა ქრომატოგრაფიული სისტემის ვარგისობის, სპეციფიკურობის, სწორხაზოვნებისა და დიაპაზონის, სისწორის, სიზუსტის, აღმოჩენის ზღვრისა (LOD) და რაოდენობრივი განსაზღვრის ზღვრის (LOQ) მიხედვით. შესწავლილი იქნა ტერბინაფინის ჰიდროქლორიდის მეთანოლიანი ხსნარების სტაბილურობა, სვაბის მასალისა და მემბრანული ფილტრის შესაბამისობა. საკალიბრო მრუდი აჩვენებდა სწორხაზოვან ბუნებას (R^2=0.99999) კონცენტრაციების 0.00003-0.025 მგ/მლ დიაპაზონში; LOD – 0.000015 მგ/მლ და LOQ – 0.00003 მგ/მლ. ტერბინაფინის ნარჩენების განსაზღვრული კონცენტრაციები საკვლევ ხსნარებში არ აღემატებოდა 0.00090 მგ/მლ-ს, რომელიც ნაკლებია შემდგომი პროდუქტის ჯვარედინი კონტამინაციის ზღვარზე. **დასკვნები:** ტერბინაფინის ჰიდროქლორიდის 250 მგ შემოუგარსავი ტაბლეტების წარმოების დროს გამოყენებული ფარმაცევტული საწარმოო დანადგარის ზედაპირებზე ტერბინაფინის ნარჩენების

რაოდენობრივი განსაზღვრის მესქ და სვაბით სინჯის აღების კომბინაციის გზით მიღებული მეთოდის გამოყენებით ჩატარებული ანალიზური კვლევა აჩვენებს დასუფთავების პროცედურის ვალიდურობას. შემოთავაზებული მეთოდი შესაძლებელია გამოყენებული იქნეს ხარისხის კონტროლის სხვა ლაბორატორიების მიერ ტერბინაფინის ჰიდროქლორიდის შემცველი სხვადასხვა წამლის ფორმებზე დასუფთავების ვალიდაციის ჩასატარებლად.

საკვანძო სიტყვები: მაღალეფექტური სითხური ქრომატოგრაფია, ტებინაფინი, სვაბით სინჯის აღება, ვალიდაცია

1. INTRODUCTION

Cleaning validation is a critical analytical responsibility required by GMP (Good Manufacturing Practice), which confirms the effectiveness of the cleaning standard procedure in the pharmaceutical manufacturing process for all the produced drug products contacting the shared pharmaceutical manufacturing equipment. The event-based scientific experiment series establishes documented evidence with a high assurance that the cleaning procedure effectively removes chemical residues of the previous product's active pharmaceutical ingredient (API) from the manufacturing equipment's surfaces below the scientifically predetermined acceptable level. The need to carry out cleaning validation is especially important when the drug product is the "worst-case" for the cleaning procedure regarding solubility, therapeutic potency, toxicity, and surface adherence of API. In addition, estimation of API residues requires a selective and sensitive method capable of quantitative determination of API traces remaining over the surface of manufacturing equipment after the cleaning procedure (Food and Drug Administration, 2014; Rubashvili *et al.,* 2018).

Terbinafine hydrochloride ($C_{21}H_{25}N{\cdot}HCl$) (MW=327.9) namely (2E)-N,6,6-Trimethyl-N-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine hydrochloride [CAS number: 78628-80-5] is an antifungal medication that fights infections caused by fungus. Terbinafine comes as a cream, gel or spray, liquid (solution), and tablets used to treat infections caused by a fungus that affect the fingernails or toenails at the scientifically predetermined acceptable level. The Terbinafine HCl is a white, almost white powder, slightly soluble in water and freely soluble in ethanol and methanol. The chemical structure of this compound is shown in Figure 1 (Kanakapura and Penmarta, 2016; drug bank online, 2022).

This study aimed to estimate a trace amount of residues of terbinafine quantitatively by the combined method of a swab sampling procedure concerning a very high recovery rate and a selective, specific, and sensitive analytical HPLC procedure in cleaning control samples collected from manufacturing equipment surfaces after manufacturing of Terbinafine HCl 250 mg uncoated tablets. This study demonstrates the efficiency and the removability of the established cleaning procedure used in the pharmaceutical manufacturing process. The need to develop this method arose because a number of analytical methods available in the literature were not appropriate for our analytical purposes, and none of the available HPLC methods combined with the sampling procedures was not found (Kanakapura and Penmatsa, 2016; Florea *et al.,* 2009; Kassem and Almardini, 2013; Domadiya *et al.,* 2012; Ji *et al.,* 1997; Blessy *et al.,* 2014; Goswami, 2013; Patel *et al.,* 2012; Raju *et al.,* 2011). The proposed method fully responds to the complex analytical tasks for conducting cleaning validation on drug dosage forms such as Terbinafine HCl uncoated tablets. The suitability and the applicability of the method were evaluated based on the validation study concerning chromatography system suitability test (SST), specificity, linearity range, the limit of detection (LOD), quantitation (LOQ), accuracy, precision, and robustness.

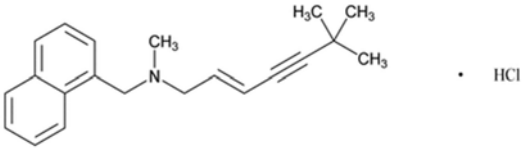

Figure 1. Chemical structure of terbinafine hydrochloride

2. MATERIALS AND METHODS

2.1 Materials

The United States Pharmacopeia (USA) supplied the reference standard of terbinafine HCl. The HPLC grade methanol, acetonitrile, analytical grade triethylamine, orthophosphoric acid, potassium dihydrogen phosphate, orthophosphoric acid were purchased from Sigma-Aldrich (Germany). The swabs, namely

polyester micro swabs (3×2.5×10 mm), Teflon template holder, screw cap vials for sampling, were purchased from ITW Texwipe (USA). Three stainless steel plates were used as a representative surface. The Durapore® polyvinylidene difluoride (PVDF) membrane filters were used in this study. The cleaning procedure was performed using Microbac Forte 1% solution as a disinfectant and cleaning agent, purchased from BodeChemie (Germany). The ultrapure grade water was prepared using Milli Q Adventage A10 purification system (Millipore, France).

2.2 Methods

The HPLC analysis was performed using Ag 1260 Infinity II system (Agilent Technologies) and the column - BDS Hypersil C18 250×4.6 mm, 5 µm (Thermo Scientific) with an isocratic elution of mobile phase containing a mixture of buffer solution (1.3 mL 85 % orthophosphoric acid and 1.7 mL triethylamine diluted to 1000 mL with water), acetonitrile and methanol (40/20/40 v/v); the flow rate of mobile phase was 1.0 mL/min; the diode-array detection was performed at 275 nm; the injected volume was 20 µL; the column temperature was maintained at 25°C. The output signal was monitored and processed using Chemstation software. SONOREX™ Digital 102P Ultrasonic bath DK102 (Germany) was used for sample preparation. All the measuring equipment was qualified. Statistical assessment of the obatiend analytical data was performed using Microsoft Excel.

Terbinafine HCl reference standard diluted in methanol was used as a standard solution at 0.01 mg/mL and 0.025 mg/mL concentrations calculated on terbinafine concentration. 11.3 /28.2 mg of terbinafine HCl reference standard corresponding to 10/25 mg terbinafine was weighed, transferred accurately in a 100 mL volumetric flask, and 80 mL of methanol was added, sonicated until it became completely dissolved and diluted to volume with the same diluent, mixed well. Then it was filtered through a 0.45 µm membrane filter, discarding the first 5 ml of the filtrate. Finally, 1 mL of this solution was transferred to a 10 mL volumetric flask, diluted to volume with diluent – methanol, and mixed well.

For the preparation of the placebo solution, all the excipients according to the drug formula were weighted and transferred accurately in a 100 mL volumetric flask, and 80 mL of methanol was added, sonicated for 20 minutes, and then diluted to volume with the same diluent, mixed well. Then it was filtered through a 0.45 µm membrane filter, discarding the first 5 ml of the filtrate.

The swabbing was used as a sampling procedure, a subjective manual process involving physical interaction between the swab and the equipment surface. The sampling procedure involved moistening the swab with methanol and swabbing the area (5×5=25 cm^2) to be sampled in an overlapping zigzag pattern. Excess solution was squeezed to avoid unnecessary dilution of the drug substance. First, the surface area was wiped horizontally from one side to the other (back and forth), then, after rotating the swab, vertically (up and down). Next, the swabs were placed in the 5 mL screw-cap test tubes containing 1 mL of methanol, then the tubes were placed in an ultrasonic bath for 2-3 minutes, and HPLC analyzed the solutions. The selected material - stainless steel, was previously cleaned by using disinfectant/detergent and dried before the experiment (Rubashvili *et al.,* 2018; Rubashvili *et al.*, 2015; Rubashvili *et al.,* 2020).

In order to study the accuracy parameter, the percentage recovery rate (Rec, %) (three individual determinations in two different days – two analyses with three determinations) of the combined swab sampling and analytical procedures were checked. First, the selected surface area (25 cm^2) of the plate was sprayed with 0.1 mL of standard solution at two different concentration levels (0.01 mg/mL and 0.025 mg/mL) using a micropipette, and the solvent was allowed to evaporate. Then sampling was performed according to the swab wipe procedure as described above. Next, the swab samples were diluted with the same diluent to 1 mL to obtain the swab spike solution. Finally, the negative control solution (blank) was prepared in the same manner using the placebo solution of Terbinafine 250 mg tablets instead of the standard solution.

The Rec, % was calculated by Equation 1 (Rubashvili *et al.,* 2020):

$$Rec, \% = A_{rec} \times 100 / A_{sp} \quad \text{(Eq. 1)}$$

Where, A_{rec} is the peak area of terbinafine obtained from test solution (recovered amount); A_{sp} is the peak area of terbinafine obtained from swab spiked solution (amount added).

The concentration (mg/mL) of terbinafine residues (X) in the test solution was calculated by Equation 2:

$$X = A_u \times W \times D \times 291.4 \times P / A_{st} \times 327.9 \times 100 \quad \text{(Eq. 2)}$$

Where A_u is the peak area of terbinafine obtained

from the chromatogram of test solution; A_{st} is the peak area of terbinafine obtained from the chromatogram of standard solution; W is the standard mass, mg; D is the dilution factor; P is the purity of the terbinafine HCl standard, %; 291.4 – MW of terbinafine; 327.9 – MW of terbinafine HCl.

The determined concentration of terbinafine residues in test solutions was estimated against the acceptance limit - AL (0.0094 mg/mL) for the smallest batch size of the next drug product. The AL was established based on the different pharmacological, toxicity evaluation, and health-based exposure approaches. Therefore, the obtained strictest limit was considered the AL (Food and Drug Administration, 2014; Rubashvili *et al.,* 2018; Rubashvili *et al*., 2015; Rubashvili *et al.,* 2020).

The analytical HPLC procedure was checked for the following validation parameters: robustness – standard solution stability, a membrane filter, swab material compatibility tests, SST, specificity, linearity-range, LOD, and LOQ according to ICH guideline and the appropriate methodologies (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005; Rubashvili *et al*., 2019).

3. RESULTS AND DISCUSSION:

3.1 Results

The SST parameters were checked using the data obtained with the chromatograms of the standard solution. The relative standard deviation - RSD, % of peak areas (Ar) (n=6), the RSD, % of the retention times (RT), the tailing factor (Tf), and the number of theoretical plates (N) were measured. The SST was checked by two standard solutionswo standard solutions checked the SST at the same concentration, and the similarity factor between the peak areas of terbinafine obtained with two standard solutions was calculated. The similarity factor should be within 0.98-1.02. The results are summarized in Tables 1, 2, 3.

The specificity of the method was checked by injecting the standard solution, spiked swab solution, swab test solution, and the negative control solution (blank). The terbinafine peak was pure (purity factor 999.2>purity threshold 990.0).

To study the linearity range of the method from the standard solution of terbinafine, working solutions were prepared at eight different concentration levels ranging from 0.00003 mg/mL to 0.025 mg/mL. Six replicate injections (n=6) were performed at each concentration level. The linearity was checked by the square of the correlation coefficient (acceptance criteria - AC: R^2>0.99). The calibration (linearity) curve was constructed by plotting the response peak area (Ar) against the corresponding concentration of the injected working solutions. The calibration curve is shown in Figure 2. The results are summarized in Table 4.

Table 1. *The characteristics of terbinafine peak*

Parameter	Result	AC
Tf	0.81-0.86	0.8-1.5
N	8340-8645	≥2000

Table 2. *The values of RSD for peak areas and retention times of standard solution at 0.025 mg/mL with six replicate injections*

№	Ar	RT (min)
1	571.46936	14.443
2	570.54785	14.350
3	570.90216	14.296
4	570.91272	14.248
5	570.96143	14.222
6	570.36621	14.192
Average	**570.85996**	**14.292**
RSD, %	**0.067**	**0.649**
AC, %	**≤2**	**≤1**

Table 3. *The values of RSD for peak areas and retention times of standard solution at 0.01 mg/mL with six replicate injections*

№	Ar	RT (min)
1	227.25635	14.152
2	228.12552	14.152
3	227.74524	14.148
4	229.37147	14.154
5	228.22284	14.182
6	227.96542	14.190
Average	**228.11447**	14.163
RSD, %	**0.309**	**0.128**
AC, %	**≤2**	**≤1**

The LOD is the minimal concentration of terbinafine that can be detected but not accurately and precisely quantified in the sample. The LOQ is the lowest concentration of the targeted drug compound, which can be quantitatively determined under the experimental conditions

prescribed, which included inside the acceptance limits over the concentration range investigated. The signal-to-noise ratio (s/N) and the RSD % of peak areas were adopted for establishing the LOQ and the LOD. The LOQ is estimated to be ten times the s/N ratio and the RSD, % of peak areas ≤10 %; The LOD is estimated to be three times of s/N ratio. The results of the LOQ and the LOD are shown in Table 5.

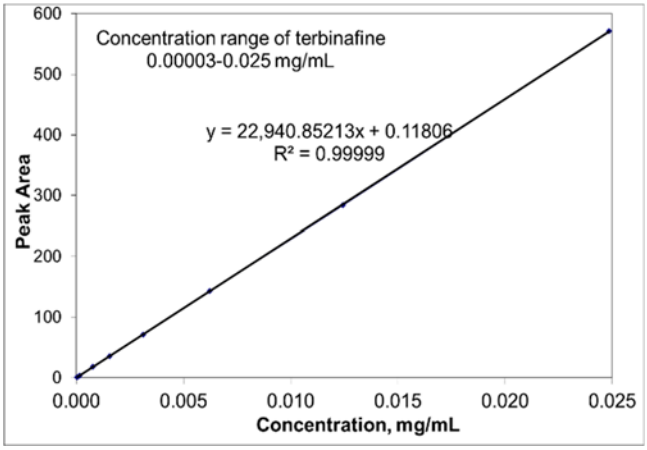

Figure 2. *The linearity curve*

Table 4. *The Results of linearity-range*

Concentr. Level	Concentr. mg/mL	Average Ar
I	0.0249173	571.17952
II	0.0124309	283.61106
III	0.0062154	143.15639
IV	0.0031077	71.51938
V	0.0015539	35.64462
VI	0.0007769	18.22605
VII	0.0001554	3.80000
VIII	0.0000311	0.96000

Table 5. *The LOQ and the LOD of the analytical procedure*

Parameter	Value
The LOQ, mg/mL	0.00003
The LOD, mg/mL	0.000015
RSD, % for the LOQ (n=6)	8.75
RSD, % for the LOD (n=6)	21.65
s/N for the LOQ	17.07
s/N for the LOD	3.90

The stability of the standard solution at room temperature for 24 hours was evaluated against the freshly prepared standard solution. The bias % between peak areas of standard solutions stored and freshly prepared was 1.1 % (AC: ≤3 %).

The accuracy of the method was checked by comparing the analyte amount determined versus the known amount spiked at two different concentration levels (0.001 mg/mL and 0.0025 mg/mL) by three individual determinations on two different days. The accuracy is expressed as the percentage of standard compound recovered from swab spiked solution with corresponding RSD, %. The main Rec % of swab sampling from stainless steel surfaces should be ≥80 %, and the RSD % of each recovery ≤5.0% (AC). The main Rec % was 86.72% (The average Rec is 85.99 % at the concentration - 0.001 mg/mL and 87.44 % at the concentration – 0.0025 mg/mL).

Table 6. *The precision test results at the concentration of 0.001 mg/mL*

№	Rec, %
1	83.84
2	82.36
3	84.43
4	89.21
5	88.00
6	88.09
Average	**85.99**
RSD, %	**3.250**
AC, %	**≤5**

Table 7. *The precision test results at the concentration of 0.0025 mg/mL*

№	Rec, %
1	86.34
2	87.72
3	86.55
4	87.25
5	88.47
6	88.33
Average	87.44
RSD, %	**1.019**
AC, %	**≤5**

The RSD of recovery rates evaluated the precision parameter for six individual determinations at two different concentration levels (0.001 mg/mL and 0.0025 mg/mL) carried out within the accuracy parameter. The results are given in Tables 6, and 7.

To estimate the compatibility of the used

swab material - polyester, standard solution, and the standard solution added swabs of the same concentration were prepared and injected. The compatibility of swab material was evaluated quantitatively by the calculated percentage difference between peak areas obtained from the standard solution and the standard solution added swab, which should be ≤3.0% (AC). The result was 0.18 %.

The PVDF membrane filter compatibility was evaluated using the standard solution and calculated the percentage difference between peak areas of filtered and non-filtered. The result was 0.35 % (AC: ≤0.5%), which gives the confidence that analyte adsorption does not occur on the used filter.

After manufacturing of Terbinafine 250 mg uncoated tablets and equipment cleaning samples were collected from different sampling points of equipment surface area hardest to clean. Swab samples were analyzed immediately for the estimation of terbinafine residues. The determined concentration of terbinafine residues was <LOQ=0.00003 mg/mL, and in several samples, it was varied from 0.00003 to 0.00090 mg/mL. Figure 3 shows typical chromatograms obtained from blank, test, spike, and standard solutions.

3.2 Discussions

The final chromatographic conditions were established by optimizing the system operation parameters: the wavelength for detection, the composition of the mobile phase, the flow rate, the nature of stationary phase, the injection volume, and checking the chromatographic peak parameters: theoretical plates, tailing factor and peak purity. The established optimal conditions of the chromatographic system with the isocratic elution ensures the suitability of the HPLC analytical procedure. The specificity study has shown no interference from the blank and the extraction solvent - methanol at the retention time of the analyte peak. A value closer to the unit of the square of the correlation coefficient (R^2) and the linear curve indicates a good linearity of the analytical procedure. The obtained values of the LOQ and LOD indicate that the method is very sensitive with lower noise of the baseline. The result of the stability study of standard solution gives the confidence that terbinafine residues are stable and the concentration does not change during cleaning validation analyses. The main value of the recovery rate (>80 %) confirms the combination of analytical HPLC and swab sampling procedures have a good accuracy. The precision checked at two different concentration levels of terbinafine HCl indicates that the analytical procedure has a good repeatability. The filter compatibility test confirms the existence of full desorption of terbinafine residues from the swab material, and it does not affect the determination of the API mentioned above residues. Hence, the developed HPLC method gives a precise, accurate, linear and robust analytical results.

The determined concentrations of the terbinafine residues in the samples were well below the AL (<0.0094 mg/mL). Even though the pharmaceutical formulation Terbinafine HCl 250 mg uncoated tablet contains very slightly soluble and slightly toxic (oral LD50=2000 mg/kg) API, the cleaning procedure provides enough efficacy to remove terbinafine residues from the equipment surfaces and completely excludes the risk of cross-contamination of the next drug product after manufacturing the drug mentioned above product.

4. CONCLUSIONS:

The performed analytical study using the developed method combined with an analytical HPLC and swab sampling procedures for estimation of terbinafine residues on surfaces of the pharmaceutical equipment used during the manufacture of Terbinafine HCl 250 mg uncoated tablets demonstrates the validity of the cleaning procedure routinely used in pharmaceutical manufacture. This work confirms that the cleaning procedure using Microbac Forte 1 % solution ensures removing residues from equipment surfaces well below the limit of cross-contamination. Other pharmaceutical quality control laboratories may successfully use sampling and HPLC analytical procedures to sustain cleaning validation procedures for terbinafine residues after manufacturing various drug formulations.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the sample size observed.

5.2. Acknowledgements

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5.3. Funding source

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5.4. Competing Interests

No potential conflict of interest is in this publication.

5.5. Open Access

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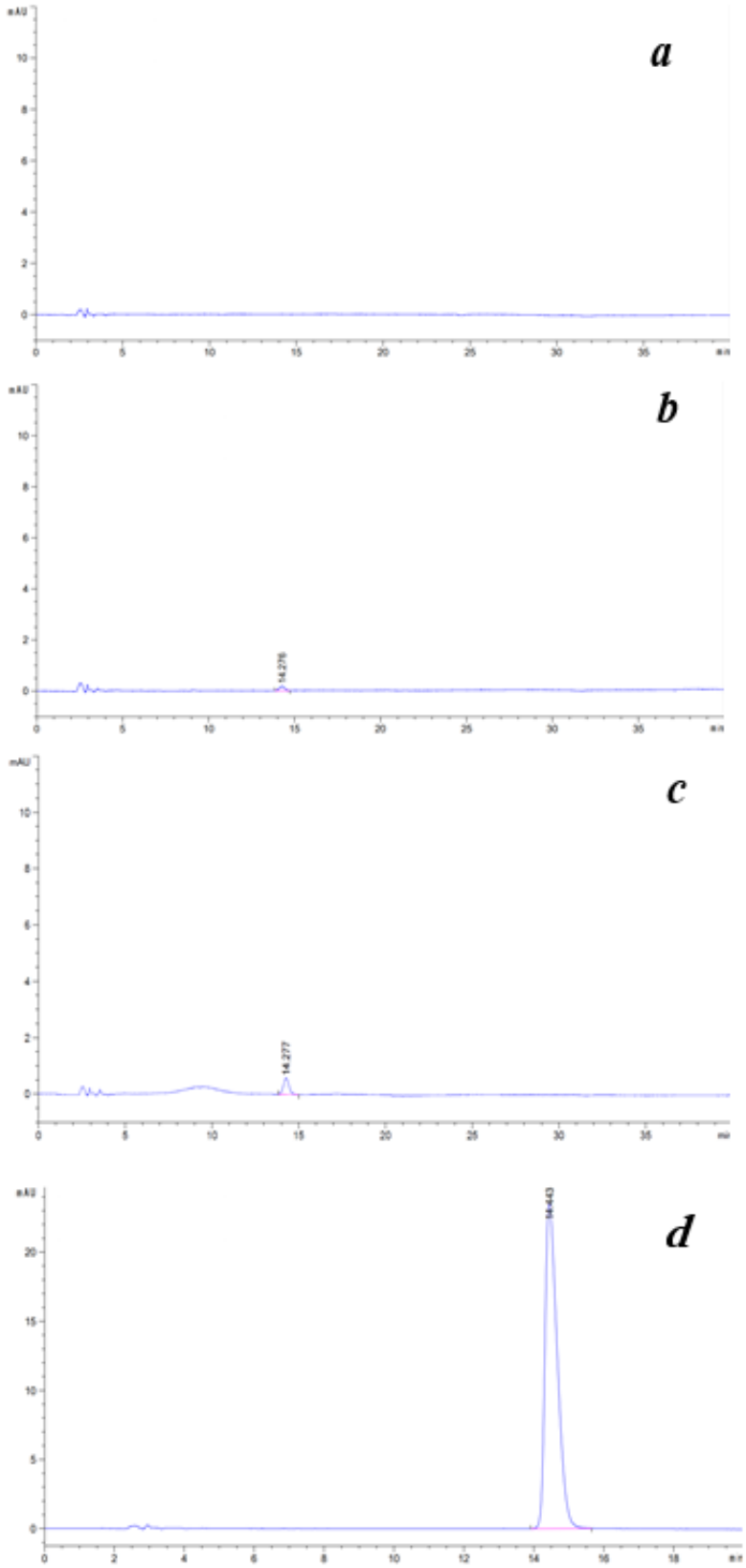

Figure 3. *The chromatograms of blank (a), swab test solution (b), swab spiked solution (c), and standard solution (d)*