Novel stability indicating HPLC method for determination of Terbinafine tablet for the treatment of Anti-fungal infection

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Abstract—**Terbinafine Hydrochloride (TH) is a new potent antifungal agent. Several HPTLC, non-aqueous voltametric, spectrometric methods, ion-pair RP chromatography and Stability-indicating HPTLC methods have been published till now. The aim of the present study is to develop and validate simple, precise, specific and sensitive stability indicating reversed-phase HPLC (RP-HPLC) method for analysis of Terbinafine Hydrochloride in bulk and in tablet dosage form. Based on the TBH peak limit, initially there was no fixed and steady retention time and consequently in order to maintain the column saturated with high concentration of TBH (1 mg/ml) solution, the column was initially run with the solution at the start of a batch and also at every specified time in the series to determine the stillness of the column. TBH was subjected to acidic, basic and neutral (with water) hydrolysis, oxidative, thermal and photodegradation and it was found that drug was degraded in acidic and photolytic condition. Statistical analysis proved that the developed method was accurate, precise, reproducible, specific and sensitive and can be used for routine analysis. Results also indicate that HPLC method employed in the study can detect any degradation of the product and any degradation impurities formed thereby making it a proper stability indicating method.**

Index Terms—**Terbinafine hydrochloride, reproducible, degradation, stability indicating method.**

I. INTRODUCTION

The stability of pharmaceutical formulations is a critical aspect of ensuring their efficacy and safety throughout their shelf life. Terbinafine, a widely used antifungal agent, is often formulated into oral dosage forms, such as tablets, to treat various fungal infections. As with any pharmaceutical product, it is crucial to monitor the presence of related substances, such as impurities and degradation products, in the formulation to ensure that the drug remains stable and free from potentially harmful substances. The tablets of Terbinafine can like other pharmaceutical materials suffer degradation caused by, among other factors, light, temperature and humidity. These associated substances may change the therapeutic value and become a safety hazard to a patient. Hence, to assure the drug product quality during shelf life it is needed to develop a stability-indicating method, which will determine and quantitate these substances.

Importance of detecting related substances

Detecting related substances in pharmaceutical products is of paramount importance product. Related substances, including impurities, degradation products, and by-products, during storage, or due to chemical instability substances in pharmaceutical formulations can significantly affect the therapeutic outcome, potentially cause harmful side effects or reduce the effectiveness of the drug.

Role of extrude base technology in drug formulation

Extrude base technology formulation of pharmaceutical products, particularly in the development of controlled-release oral dosage forms. The extrude base process involves the creation of a matrix or base that serves as a foundation for the active pharmaceutical ingredient (API), ensuring a controlled and sustained manner over an extended period.

ICH guidelines for stability testing and related substances

The International Council for Harmonization (ICH) provides comprehensive guidelines for stability testing and the evaluation of related substances in pharmaceutical products. These guidelines, primarily outlined in the ICH Q1 series, emphasize the importance of conducting stability studies to ensure the long-term safety, efficacy, and quality of pharmaceutical products.

Anti-Fungal

An antifungal medication, also known as an antimycotic medication, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Such drugs are usually obtained by a doctor's prescription, but a few are available over the counter (OTC). The evolution of antifungal resistance is a growing threat to health globally.

Challenges in analyzing Terbinafine in extrude base matrices

Analyzing Terbinafine in extrude base matrices presents several challenges due to the complex nature of the formulation and the properties of the drug itself. The extrude base formulation, which typically involves the use of polymers and excipients to create a controlled-release matrix, can impact the ability to isolate and quantify the active pharmaceutical ingredient (API) and its related substances effectively. One of the key challenges in analyzing Terbinafine in such matrices is the potential for interference from the excipients and polymers used in the extruded base.

Importance of sensitivity, specificity, and robustness in impurity profiling

In impurity profiling, the sensitivity, specificity, and robustness are critical factors that ensure the accurate detection and, such as degradation products, impurities, and by-products. Sensitivity is especially important in the context of stability-indicating methods because pharmaceutical formulations, such as Terbinafine tablets, may contain very low concentrations of related substances that arise due to degradation over time or from manufacturing processes.

II. APPLICATION OF THE METHOD

The application stability studies and quality control is critical to ensuring that pharmaceutical products. In primary objectives is to evaluate how a drug formulation, such as Terbinafine tablets, responds to various environmental conditions, including temperature, humidity, and light, which can accelerate degradation.

Scope of the research

The scope of this research of a novel stability-indicating HPLC method for the detection and quantification of related substances in extrude base Terbinafine tablets. The primary aim of this research is to create a reliable and sensitive analytical method that can accurately monitor the stability of Terbinafine throughout its shelf life, identifying any degradation products, impurities, or by-products that may arise during storage or manufacturing processes.

HPLC method

High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify specific components in mixtures. The mixtures can originate from food, chemicals, pharmaceuticals, biological, environmental and agriculture, etc., which have been dissolved into liquid solutions.

III. AIM AND OBJECTIVES OF THE STUDY

Literature survey reveals spectrophotometric, HPLC methods for the estimation of Terbinafine tablet for the treatment of fungal infection. Similarly various analytical methods have been reported for estimation of Terbinafine tablet such as HPLC. Terbinafine tablet with other drugs has also been estimated spectrophotometrically. No analytical method has yet been reported for the determination of Terbinafine tablet. The aim of the research was to explore the possibility of techniques using HPLC spectrophotometer for estimation of Terbinafine tablet for fungal treatment. Thus, the purpose of the present study was to establish and validate as per International Conference on Harmonization (ICH) guidelines, an accurate, rapid, economical and precise procedure for quantitative analysis of Terbinafine tablet.

IV. OBJECTIVE

Following are the method validation parameters which are to be study and check; develop a novel and robust HPLC method for detecting related substances in extrude base Terbinafine tablets, establish the method's ability to indicate stability under various stress conditions, validate the method as per ICH guidelines for specificity, accuracy, precision, and sensitivity, identify and quantify potential degradation products and impurities in the formulation.

V. MATERIAL AND METHOD

Materials: - Reference standard (RS) of terbinafine hydrochloride (assigned purity 99.9 %) was provided by the pharmaceutical Company (India), and the samples of terbinafine hydrochloride bulk drug, and all solvents and reagents required in the course of the research were supplied as well. Methanol and acetonitrile of HPLC grade were performed by Merck, Germany whereas the triethylamine and phosphoric acid 85% was of pure and analytical grade. HPLC system VWR Hitachi La Chrom Elite range which is with auto sampler and diode array detector.

Common preparation of solutions
An amount of (10) mg terbinafine hydrochloride (RS) was weighed accurately and measured into (100) ml volumetric flask and made up to volume with methanol. Then (10) ml of this solution was added (100) ml volumetric flask and was diluted to the mark in the flask with methanol to obtain final concentration of (0.01) mg/ml of terbinafine hydrochloride.

Sample solution preparation: The previous steps were repeated using terbinafine hydrochloride bulk drug sample instead of (RS) sample.

Stock solution preparation for stress stability study: Steps mentioned in the previous section were repeated by taking (100) mg of terbinafine hydrochloride.

Methods: -Conditions chromatographic

Table 01: Chromatographic condition of HPLC method

Column	Intersil: L1ODS (150*4.6mm) Particle
Mobile Phase	Methanol-acetonitrile (60:40v/v) with (0.15% triethylamine and 0.15% phosphoric acid) pH=7.68
Detection	Photodiode array detector monitored
Wavelength	224 nm
Flow rate	4 ml/minute
Injection volume	10 ul
Oven temperature	25°C

Preparation of solutions for validation study:
Linear-solving methods:
Five consecutive concentrations were made-up of 50, 75, 100, 125, and 150 percent of the standard solution.

Specificity solution
Methanolic solution of impurities A, B, C and D containing terbinafine hydrochloride standard solution was prepared (his impurities were identified in the BP)
Sides to the problem of accuracy
Three levels of terbinafine hydrochloride bulk substance with a working range of 50 per cent to 150 per cent were prepared in nine samples.
Precisions of solutions to be used: Nine standard samples of terbinafine hydrochloride bulk substance within the working range of 50% to 150% were generated.
Resistance to solution: Samples of terbinafine hydrochloride bulk solutions with 100 percent of standard solution were prepared.

Solutions to be used in stress stability studies
Acidic hydrolysis:
Acid hydrolysis was done by adding 10 ml of stock solution with 0.5N HCl and placed in a water bath for an hour. Then the solution was cooled and adjusted to pH=7 using 0.5N NaOH and the concentration adjusted to 0.01mg/ml.

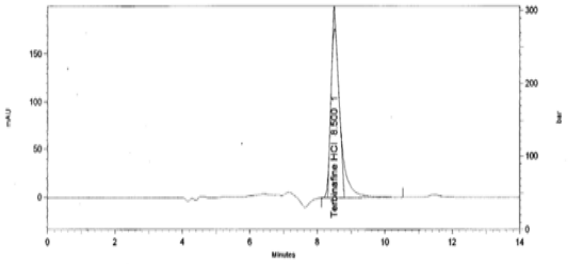
Basic hydrolysis type:
(10) ml of the stock solution was neutralized with 0.5N NaOH and kept in water bath for an hour. The solution was then left to cool and made neutral at pH=7 using 0.5N HCL and concentration adjusted to 0.01mg/ml.
Oxidative degradation technique: 10ml stock solution was added into H 2O 2 10 % and was stirred thoroughly to half an hour and the concentration adjusted to 0.01mg/ml.

Thermal degradation solution: (10) ml of stock solution was kept at 60degrees Celsius temperature over a period of one week after which the concentration was adjusted to (0.01) mg/ml.
To solve photodegradation: (10) ml stock solution was left to evolve under the influence of white light, during the duration of one week at a temperature of 25ºC and adjustment of concentration made to (0.01) mg/ml.

Results: -Chromatographic condition: - Lipophilic compounds in which the nitrogen would be alkylated, were said to be fairly easy to separate on reversed -phase column with acetonitrile as the mobile phase and addition of triethylamine to avoid peak tailing caused by interaction of the analyte and free silanol groups.

In our work a mobile phase was tried as water: acetonitrile (60:40, v/v) and water: acetonitrile (60:40, v/v) with (0.15%) triethylamine and phosphoric acid and the chromatography was done on C18 column and the eluents monitored by photodiode array UV detector at wavelength 224 nm.

Degradation condition	Result of degradation
Acidic hydrolysis	27%
Basic hydrolysis	44%
Oxidation	50%
Thermal degradation	20%
Photo degradation	No degradation

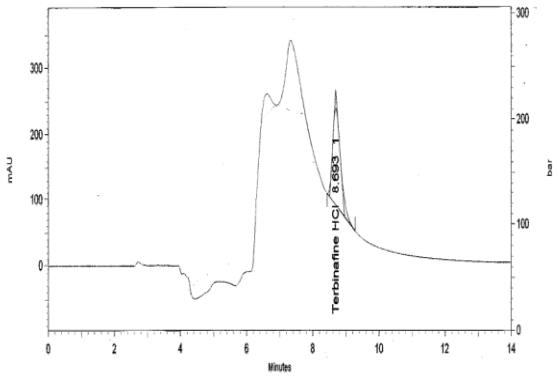


HPLC chromatogram for terbinafine hydrochloride

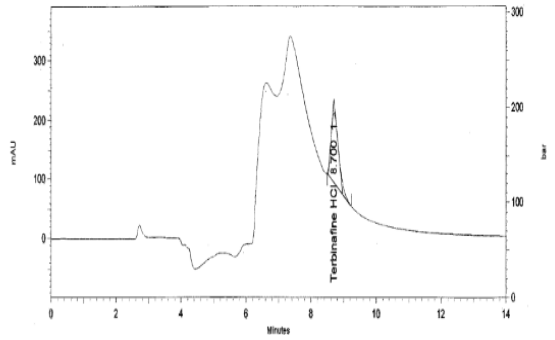
Mobile phase was adjusted by changing the composition with methanol since it was used as a substitute to the water then the peak of terbinafine hydro chromium was obtained which occurred at the retention time of 8.5 minute and this peak was adequately acceptable for analysis, Fig (1).
The RSD of five consecutive injections of the standard were used to calculate the column efficiency. The theoretical plates were 4729, more than 2500 and the tailing factor was 1.65, which did not exceed 3.0 and RSD of 5 consecutive injections of the standard was 0.65, which did not exceed 1.5 percent.

Forced degradation studies:
To prove stability indicating property of the method the terbinafine hydrochloride was used. The degradation study of terbinafine hydrochloride was studied in various stress conditions that are acidic hydrolysis, basic hydrolysis, oxidation, thermal and photo-degradation.

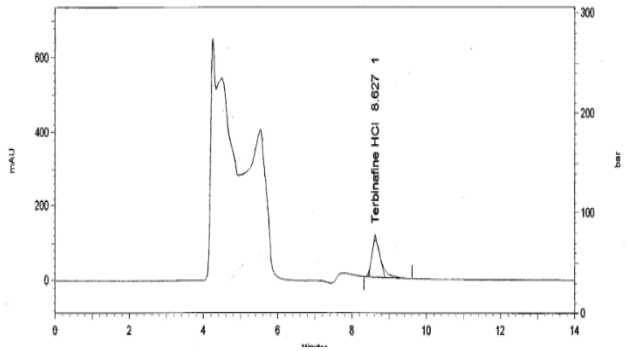
2: Results of degradation studies on terbinafine hydrochloride



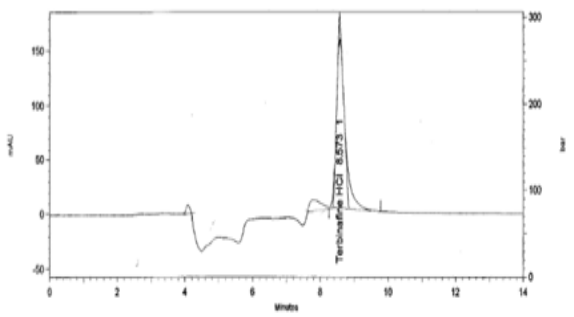
Chromatogram of TH subjected to acidic hydrolysis



chromatogram to basic hydrolysis



Chromatogram to oxidative degradation



Chromatogram to thermal degradation.

Demonstration of method of analysis

It was validated according to the demands of the USP with the investigation of such parameters as the linearity, the specificity, the precision, the accuracy and recovery, the limit of detection, the limit of quantification, and the robustness. The relation of regression was: y= 1367706200x -594568 and correlation factor 0.9993.

Table :3 Calibration curve terbinafine hydrochloride

S. No	Concentration. (ug/ml)	Peak Area
1	20	5000
2	40	10000
3	60	15000
4	80	20000
5	100	25000

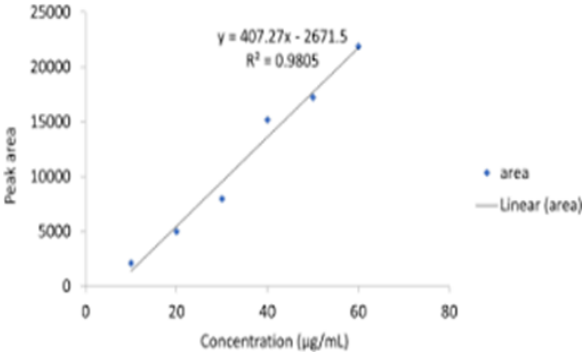


Fig 8 Calibration curve of Terbinafine HCL

Specificity: Specificity is how the method can use to quantify the analyte response in the presence of interference such impurities and degradation products. The specificity of the generated method was executed in the presence of its impurities as identified by the BP as A, B, C and D. The findings are in the figure (7) and they indicated no interference and fine resolution between the impurity's peaks and the drug one.

Also applying forced degradation studies on terbinafine hydrochloride demonstrated that the method could differentiate between terbinafine hydrochloride and this confirm.

Accuracy: There were nine terbinafine hydrochloride bulk substance samples or working range of 50-150 % which were injected into the HPLC devices. The practical then the percentage of every sample was found out using the formula: the value of the expression percentage = (practical concentration / theoretical concentration).100 then the formula of relative error (RE) was:RE % = (A - P) / P .100

- Where A is the average of the percentages of the nine samples,
- P is the purity of reference standard and it did not exceed 2%. The results of accuracy are displayed in table (3).

Table :4 Accuracy of HPLC method

The average of the percentages	101.90%
Purity of reference standard	99.96%
RE%	1.95%

Precision: The precision of the method was determined with respect to both repeatability (intraday) and intermediate precision (intraday) studies.

Repeatability: On the same day, nine batches of terbinafine hydrochloride bulk substance within the working range of 50 to 150 of the standard solution have been injected in HPLC apparatus. RSD of the results was determined and it was not more than 2% meaning that there was a good repeatability.

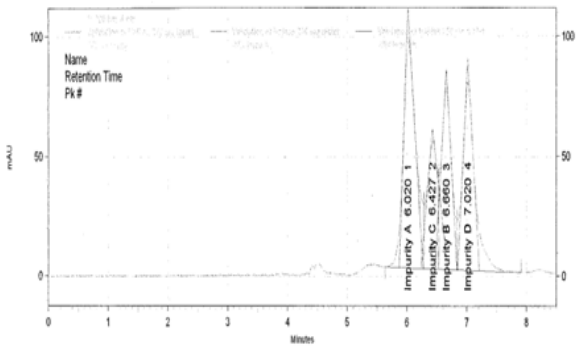


Fig 9 chromatogram of a solution contains terbinafine hydrochloride spiked with A, B, C and D impurities.

Intermediate precision: Terbinafine hydrochloride samples of bulk substance that covered the working range of 50 to 150 percent of the standard solution were injected into HPLC apparatus by different analysts on the same day. The results were computed in RSD, which was not more than 3%. Table 05 shows the results of repeatability and intermediate precision

Table:5 Repeatability and intermediate precision of HPLC

	Repeatability	intermediate precision
Number of samples	9	9
Average of test results	98.61%	98.66%
Relative standard deviation RSD	1.98%	1.76%

Limit of detection and limit of quantification: The LOD and the LOQ was calculated using signal to noise ratios as an analysis response to 3 and 10 times the background noises respectively. It was established that (LOD) was (0.1) 10 -6/ml and (LOQ) of 0.3/10-6 10-6.

Robustness: Robustness is the ability of the method to be unaltered by small and intentional changes in the method parameters. Our experience is that flow rate was raised to 0.6 ml/min instead of 0.4 ml/min and this slight alteration in the flow rate did not result in significant changes in assay value and relative retention time. The table (6) represents the results.

Table:6 Robutness of HPLC method

	Flow rate 0.4ml/min	Flow rate 0.4ml/min
Assay value	99.66%	100.85%
Relative retention time	1	1.001

Assay of Formulation

The 1.75 g of the 1 % Lamisil 1 % (GlaxoSmithKline) that contains 17.5 mg TBH was weighed into a 100 ml round bottomed flask and about 30 ml of methanol was added to the flask. The mixture was then warmed in a copper water bath, in a hot plate (Fried Electric, Haifa, Israel), containing 40 C and with a light agitation, till the cream liquefied and there might be an extraction of TBH in the methanol, approximately 10 min. The solution was cooled to room temperature, where the solution was transferred quantitatively to a 50 ml volumetric flask, and methanol was added to make the solution up to volume in order to obtain a final concentration of TBH as 350 0g/ml. The resulting HPLC was determined by HPLC chromatogram as shown in figure 10.

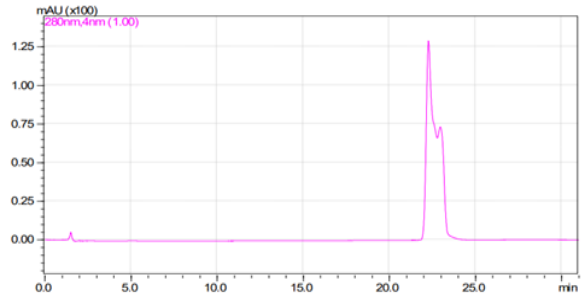


Figure 10: HPLC chromatogram for the assay of Lamisil® cream

Chromatogram of the Lamisil cream assay only showed a peak as a result of TBH that had a retention time of 22.3min. The peak of TBH was recognized by the LC Solutions software (Shimadzu, version 1.25, Japan) as two peaks, whereas the standard TBH sample that was run at the start of the batch had the same shape, and, in fact, was also recognized by the software as two peaks. The software did not find additional peaks and the profile of peak purity of both parts of the TBH peak was the same and the peak purity calculated peak purity index was 0.999999, which means that there were not any other co-eluting peaks (Figure 11).

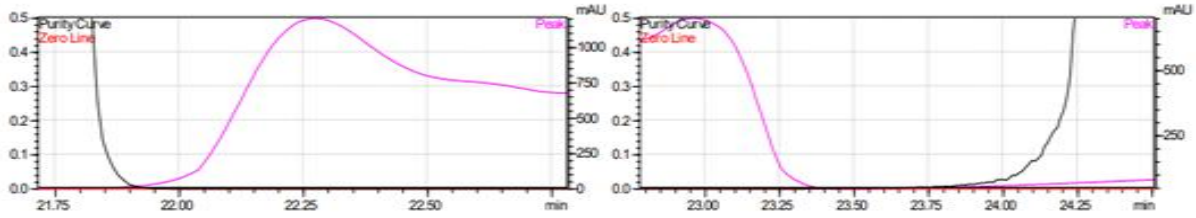


Figure 11: Peak purity profiles for TBH

It had been calculated the peak with the PDA data (190 - 800 nm) of a 10ul injection of 350g/ml TBH in methanol, extracted off Lamisil cream. Pink and black represent peak and purity curve accordingly. The peak purity index of each part of the TBH peak is 0.999999; threshold of each point is 0.999951.

Table 7: Calculation of TBH recovery from assay of Lamisil® cream, where conc. = concentration.

Peak area responses	Mean Peak area	Recovered TBH conc.(ug/ml)	Mass of cream weighted off (g)	Expected TBH conc.(ug/ml)	% TBH Recovery
5580128.3	5580840.5	350.07	1.76	351.86	99.49
5580602.3					
5581790.9					

The assay of the Lamisil cream revealed 99.49 % recovery of TBH that made it well within the specifications of a TBH containing cream as stated by the JP. According to the JP, a cream that contains TBH is not less than 95.0 % and not more than 105.0 % of the amount of TBH set in the label should be present in the TBH cream (Japanese Pharmacopoeia, 2011).

VI. STABILITY OF SOLUTIONS CONTAINING TBH

Investigations on the stability of TBH solutions in different solvents (especially in methanol) revealed that when kept under appropriate conditions (controlled environment; in this case, refrigerated at 4°C), the compound demonstrates greater stability over long term storage without much degradation. As an example, HPLC conditions were optimized around a 350 ug/ml solution of TBH in methanol, and 10 days of refrigerated storage provided an exceptional chemical stability as indicated by constant retention times and no secondary peaks in HPLC chromatograms. These indicate that low temperature storage is efficient in retarding or rather oxidation and other degradation pathways.

These results emphasize the need of adequate storage procedures and formulation considerations particularly in quality control, in the research and studies field, and in shelf-life of products where the product efficiency of an antioxidant should not be impaired in any way during its service life.

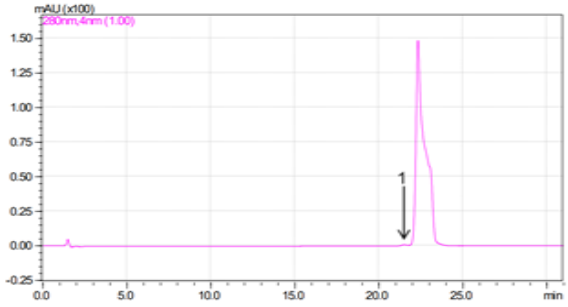


Figure 12: HPLC chromatogram.

The TBH solution stored in the refrigerator did not show signs of instability until after 10 days' storage, at which point a small extra peak was detected at 21.5 min by the LC Solutions software (Shimadzu, version 1.25, Kyoto, Japan), in addition to the TBH peak at 22.4 min (Figure 12).

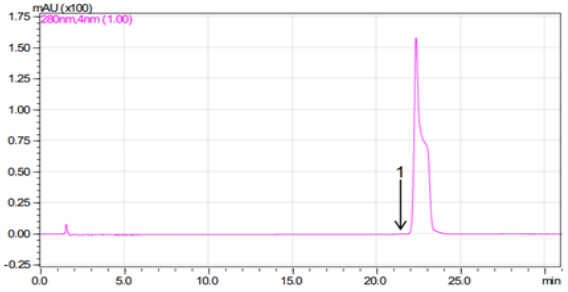


Figure 13: HPLC chromatogram

Figure 13 shows HPLC chromatogram of a 350 mug/excitation of the ter t-butylhydroquinone (TBH) distributed in methanol and stored at set temperature of 4 level Celsius in a conventional refrigerator throughout 10 days. There is a clear and narrow peak in the chromatogram at TBH reflecting that the

compound does not lose its structural integrity when stored refrigerated at the tested time. Retention time was comparable to that of fresh standard solution of TBH indicating little or no degradation.

VII. CONCLUSION

The current analysis was able to produce and confirm an effective reverse-phase high-performance liquid chromatography (RP-HPLC) technique to quantitatively determine the terbinafine hydrochloride of bulk drug substances. The technique is specific, sensitive, accurate, precise and robust and most importantly it is stability-indicating. It is very useful in differentiating terbinafine hydrochloride with its degradation products, which is crucial in the attainment of stability studies as well as establishment of the shelf-life. In general, the technique proves very useful both in the development of pharmaceuticals and in the regular QC setting in terms of the regulatory requirements of the validation process of an analytical method and as an effective method of studying stability and assay of terbinafine hydrochloride in topical and bulk forms of pharmaceutical products.

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