



High Performance Liquid Chromatography Method for the Determination of Terbinafine Hydrochloride in Semi Solids Dosage Form

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ABSTRACT

A reversed-phase high performance liquid chromatography (HPLC) method has been developed and validated to determine terbinafine hydrochloride in semi solids dosage forms (creams). The chromatography was performed on C18 column and a mobile phase consisting of methanol and acetonitrile (60:40 v/v) with (0,15% triethylamine and 0,15% phosphoric acid), eluents were monitored by UV photo diode array detector at wave length of 224nm. The method was statistically validated for linearity, specificity, accuracy, precision, limit of detection, limit of quantification and robustness.

Keywords: Liquid chromatography, Terbinafine hydrochloride, semi solid dosage form.

INTRODUCTION

Terbinafine hydrochloride is a synthetic antimycotic agent from a new class of compounds, the allylamines (fig 1). It selectively inhibits fungal squaline epoxidase causing a fungicidal action due to the intracellular accumulation of the toxic sterol squaline, it also exerts a fungistatic action by depletion of ergosterol.^{1,2}

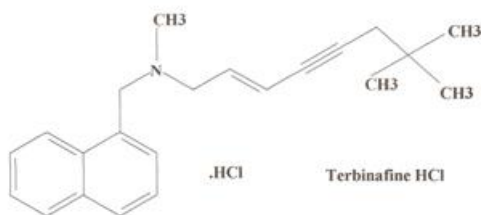


Figure 1: Chemical structure of terbinafine hydrochloride.

Terbinafine hydrochloride now is the drug of choice in dermatophyte nail and skin infections because of its fungicidal mode of action over a short treatment duration.³

There are few methods to determine terbinafine hydrochloride in dosage forms and biological fluids, these methods include microbiology^{4,5}, electrochemistry⁶, spectrophotometry⁷⁻¹¹, titrimetry⁸, high performance thin layer chromatography (HPTLC)^{12,9}, high performance liquid chromatography (HPLC)^{13,21} and gas chromatography (GC)^{22,23}, therefore the aim of our study was to develop a simple and sensitive reversed -phase (HPLC) method for determination of terbinafine hydrochloride in semi solids dosage forms (creams).

MATERIALS AND METHODS

Materials

Reference terbinafine (assigned purity 99, 9%) was kindly supplied by al Phares Company (local private

pharmaceutical company, Damascus - Syria) which offered also samples of terbinafine hydrochloride cream and all solvents and reagents which were needed to the study. THE creams were claimed to contain 1% of terbinafine hydrochloride.

HPLC apparatus VWR Hitachi La Chrom Elite model with auto sampler and diode array detector.

Standard solution preparation

Approximately (10) mg of terbinafine hydrochloride (RS) accurately weighed was transferred to (100) ml volumetric flask and diluted with methanol to the volume. Then (10) mg of this solution was transferred to (100) ml volumetric flask and diluted to the volume with methanol. The final concentration of this solution was considered to be (0, 01) mg of terbinafine hydrochloride.

Sample preparation

A quantity of the cream containing (1) mg of terbinafine hydrochloride was extracted with (25) methanol with the help of sonicating, then this solution was transferred quantitatively to (100) ml volumetric flask and methanol was added to make up to volume. After sonicating for (5) minutes a portion of the solution was filtered through a filter having a porosity of 0, 45 nm. The final concentration of terbinafine hydrochloride was considered to be (0, 01) mg/ml.

Methods

Chromatographic conditions

The chromatographic conditions which were used in the method are displayed in table 1.

Preparation of solutions for validation study

Solutions for linearity: Five sequential concentrations were prepared containing 50%, 75%, 100%, 125% and 150%, respectively of the standard solution.

Table 1: Chromatographic conditions of HPLC method.

Column	Intersil : L1ODS(4,6mm.15cm) particle size 5 µg
Mobile phase	Methanol - acetonitrile (60:40 v/v) with (0,15% triethylamine and 0,15% phosphoric acid) PH=7,68
Detection and wave length	Photo diode array detector monitored at 224 nm
Flow rate	0,4 ml/minute
Injection volume	10 µl
Oven temperature	25°C

Solutions for selectivity: A drug -free sample (placebo) was prepared from the excipients. Six samples were prepared also, three of them contain placebo and the others do not contain placebo.

Solutions for accuracy: Cream excipients were individually spiked with the standard solutions to obtain analysis samples. Nine samples were divided into three groups containing respectively 50%, 100% and 150% of the corresponding standard solution.

Solutions for precision: Nine samples of terbinafine hydrochloride cream covering the working range 50% to 150% were prepared.

Solutions for robustness: Samples of terbinafine hydrochloride cream containing 100% of standard solution were prepared.

RESULTS AND DISCUSSION

Chromatographic conditions

It was noticed that lipophilic compounds with alkylated nitrogen can easily be separated on reversed -phase column using acetonitrile as a mobile phase and if triethylamine is added, peak tailing due to analyte interaction with free silanol groups could be avoided. In our study a mobile phase consisting of water – acetonitrile (60:40 v/v) with (0,15% triethylamine and 0,15% phosphoric acid) was tested first and the chromatography was performed on C18 column and the eluents were monitored with photo- diode array UV detector at 224 nm wave length. By using these chromatographic conditions a peak of terbinafine hydrochloride appeared at a retention time of 4 minute but this peak was not suitable for analysis.

The composition of mobile phase was modified using methanol instead of water, then the peak of terbinafine hydrochloride appeared at a retention time of (8, 5) minute and this peak was suitable for analysis (fig 2).

Validation of analytical method

Validation was based on the requirements of the USP²⁴ by studying the following parameters: linearity, specificity, precision, accuracy and recovery, limit of detection, limit of quantification and robustness.

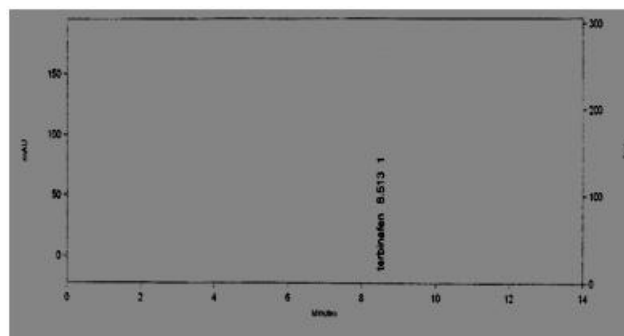


Figure 2: HPLC chromatogram for terbinafine hydrochloride by using C18 column and a mobile phase consisting of methanol – acetonitrile (60:40 v/v) with (0,15% triethylamine and 0,15% phosphoric acid) PH=7,68.

Range and linearity

A series of terbinafine hydrochloride concentrations containing 50%-150% of the standard concentration (0, 01 mg/ml) was injected in HPLC apparatus and the analyses were performed triplicate. By drawing the regression line for peak area versus the concentration linearity of the method was proved (fig 3).

The equation of the regression line was: $y = 1E + 0,9x - 594568$ and the correlation coefficient was 0, 9993.

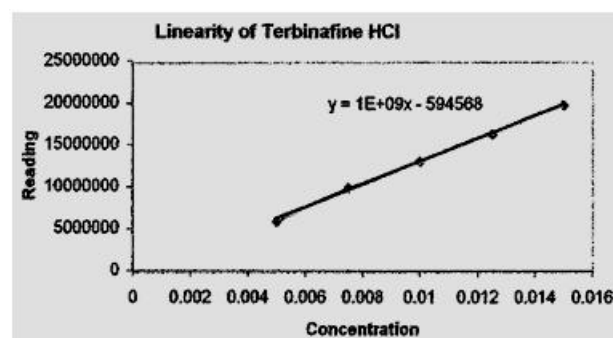


Figure 3: linearity of HPLC method.

Specificity

To prove that the method could determine accurately terbinafine hydrochloride in the presence of other components in a sample matrix a placebo sample was injected in the HPLC apparatus there was no response and no peak appeared at the standard retention time and also the bias between test results obtained by analysis of samples containing added placebo and the test results obtained by analysis of samples without placebo was calculated and the value did not exceed 2%.

The results of specificity are displayed in table (2).

Table 2: Specificity of the HPLC method

The average of test results with placebo	102,62%
The average of test results without placebo	102,43%
The bias	0,28%

Accuracy and recovery

Accuracy was determined by applying the method to samples of placebo to which known amounts of terbinafine hydrochloride were added covering the working range 50% to 150% of the standard solution, then the recovery was calculated from the test results as the percentage of terbinafine hydrochloride recovered by the assay and it was 100,43%. The results of recovery are displayed in table 3.

Table 3: Recovery of the HPLC method

Number of samples	9
Recovery	100,43%
Relative standard deviation RSD	1,63%

Precision

The precision of the method was determined by repeatability (intraday) and intermediate precision (intraday) studies.

Repeatability

Nine samples of terbinafine hydrochloride cream covering the working range 50% to 150% of the standard solution were injected in HPLC apparatus in the same day, then the RSD of the results was calculated and it did not exceed 2%.

Intermediate precision

Nine samples of terbinafine hydrochloride cream covering the working range 50% to 150% of the standard solution were injected in HPLC apparatus in the same day by different analysts, then the RSD of the results was calculated and it did not exceed 3%. The results of repeatability and intermediate precision are displayed in table 4.

Table 4: Repeatability and intermediate precision of HPLC method.

	Repeatability	Intermediate precision
Number of samples	9	9
Average of test results	105,90%	102,75%
Relative standard deviation	1,99%	2,6%

Limit of detection

Limit of detection which is the lowest concentration of terbinafine hydrochloride that can be detected but not necessary quantified by the method was (0, 1) µg/ml.

Limit of quantification

Limit of quantification which is the lowest concentration of terbinafine hydrochloride that can be determined with acceptable precision and accuracy by the method was (0, 2) µg /ml.

Robustness

Robustness is the capacity of the method to remain unaffected by small and deliberate variations in method

parameters. In our study flow rate was increased from 0, 4 ml / minute to 0, 6 ml / minute and this small change in flow rate did not cause important differences on assay value and relative retention time. The results are displayed in table 5.

Table 5: Robustness of HPLC method.

	Flow rate 0,4 ml / minute	Flow rate 0,6 ml / minute
Assay value	99,66%	100,85%
Relative retention time	1	1,001

CONCLUSION

The HPLC method developed in this study is sensitive, specific and rapid, more over the method is simple and uses simple reagents with minimum sample preparation procedures encouraging its application in routine analysis of terbinafine hydrochloride in creams.

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