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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TERBINAFINE HCl IN FORMULATED PRODUCT USING REVERSE PHASE ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (RP-UPLC)

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ABSTRACT
Terbinafine HCl is an antifungal agent used for the treatment of mycosessince as oral as well as a topical pharmaceutical dosage form. The UPLC method developed and validated for determination of Terbinafine hydrochloride is rapid, simple, sensitive and accurate. The purpose of the method development is to work on green chemistry by reducing the use of solvent and run time. The method developed is speedy, high resolution, solvent consumption is low and low cost of analysis with respect to HPLC method. Waters Acquity UPLC BEH C18 column (2.1 × 30mm, 1.7µm) column was used as stationary phase using by using mobile phase i.e. ACN: H_2O (50:50). Method was developed in gradient mode with 2.5 minutes runtime, at flow rate of 0.6 ml/minute. The UV detection wavelength selected for this method is 222nm. The method recovery was found to be 97.66% - 98.53%. The method developed on UPLC was validated with respect to linearity, specificity, accuracy, precision, ruggedness (reproducibility), robustness and stability. Hence it can be apply for routine analysis of Terbinafine HCl in bulk and pharmaceutical formulation.

KEYWORDS: Terbinafine HCl, UPLC, Accuracy, Precision, Robustness.

INTRODUCTION
Terbinafine HCl (TBF), (E)-N,6,6-trimethyl-N-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1 amine hydrochloride, Fig. 1, having empirical formula C21H25N·HCl and molecular weight 327.89084 g/mol. The salt form of terbinafine with log *P* of 3.3[1] is an allylamine/ benzylamine derivative together with other antifungal agents butenafine and naftitine. TBF salt is preferably used instead of its base form since the salt form gives a higher stability and aqueous solubility.[2] After being recrystallized from 2-propanol the melting point of TBF ranges from 204-208°C.[3,4] TBF solubility in several pharmaceutical-related solvents is listed in **Table 1**. TBF has a rather limited aqueous solubility despite its salt form and its fairly high solubilities in alcohols.[5] **Terbinafine HCl** is available in the market with the brand names Atmofine, Lamifine, Lamisil, Terbinaforce, etc. Various methods have been reported in the literature for the determination of Terbinafine HCl, in particular those using chromatography.[6-14] The earlier reported study of this drug was mainly performed by RP-HPLC methods on long columns with higher particle size, which were more time consuming. Even though the method was using a complex mobile phase mixture with high flow rates, the analysis was lacking sensitivity and peak symmetry. The purpose of the present study is to develop a simple, sensitive, accurate, precise, rugged and time saving method for the determination of Terbinafine HCl in formulated product. However, there was some report available on the estimation of Terbinafine HCl by UPLC method.[15] The method developed gives more accurate result in shorter run time i.e 2.5 minute which is attained by selecting more advance technique of Waters Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7µm) which. The developed method has been validated by following several parameters as mentioned in ICH guideline[16-18] i.e. linearity, specificity, accuracy, precision, robustness, ruggedness, limit of detection, etc.

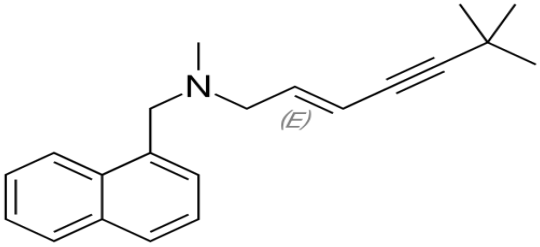


Fig. 1 Chemical structure of Terbinafine HCl

Table 1. TBF solubility in several pharmaceutical-related solvents

Solvent	Solubility [g/L]
Anhydrous ethanol	Freely soluble
Methanol	Freely soluble
Isopropyl alcohol	120
Isobutyl alcohol	21
Water	8
Acetone	Less than 0.5

MATERIAL AND METHOD

Materials

Apparatus

Chemicals used in this study included gradient grade Acetonitrile (Sigma-Aldrich, USA) and HPLC grade trifluoro acetic acid (TFA) (Sigma-Aldrich, USA). Water used for UPLC analysis was purified using Millipore Milli-Q Plus water purification system (Millipore SAS, France).

Reagents and Chemicals

A well-characterized working standard of Terbinafine HCl was procured from Curetech Skincare, Baddi, H.P. Commercially available Terbinaforce (Terbinafine HCl Tablet) purchased from local pharmacy (Allahabad, India) having batch number F1CGO088 manufactured by Penta Biotech, Haridwar, Uttarakhand, India.

Methods

Solubility

From the literature review as well as self performed Terbinafine HCl is soluble in Acetonitrile and Water.

Selection of chromatographic method

On the basis of the sample nature (ionic /ionisable/ neutral molecule), its molecular weight and solubility the proper selection of the method development depends. The drug selected i.e. Terbinafine HCl for the study is highly polar in nature and hence reversed or ion-pair or ion exchange chromatography method may be used. The reversed phase UPLC was selected for the separation because of its simplicity and suitability.

Selection of wavelength

The sensitivity of the any LC method which uses UV detection depends upon the proper selection of wavelength. Selection of the ideal wave length gives good response for all the drug have been detected. During conditions optimization and from literature review we found that 222 nm is the appropriate wavelength for this analysis.

Chromatographic conditions

Waters Acquity UPLC with photodiode array detector was used to perform the chromatography separation. The output signal was monitored and processed using MassLynx software. The chromatographic column is Water Acquity BEH C18 column (50 × 2.1 mm, 1.7µm). The mobile phase of 0.1% TFA and acetonitrile in the ratio 80:20 v/v at a flow rate of 0.6 ml/min. The injection volume was 1.0µL and the chromatographic run time of 2.5 min was used. A linear gradient elution method was applied as follows:

Table 2. Mobile Phase Ratio with flow rates

Time (min)	0.2	1.30	1.90	2.00	2.40	2.50
Flow (ml/min	0.6	0.6	0.6	0.6	0.6	0.6
A%	90	90	55	35	10	99
B%	10	10	45	65	90	1

A- trifluro acetic acid
B- Acetonitrile.

PREPARATION OF SOLUTIONS

Preparation of Buffer

Pipette out 1 ml of Tri Fluoro Acetic Acid in 1000 ml of milli-Q water and sonicate for 1min and filter through 0.2µ 6, 6 Nylon membrane filter paper.

Preparation of Diluents

Mixed well Milli-Q water: Acetonitrile in a ratio (50:50) sonicated and degassed and filter through 0.2µ 6, 6 Nylon membrane filter paper.

Preparation of Standard Solution

Weighed accurately and transferred about 99.52 mg of Terbinafine HCl standard in a 100 ml volumetric flask. Pipette out 10 ml of this solution and volume made up to 20 ml, then further pipette out 10 ml of this solution and volume made up to 20 ml with diluent to dissolve sonicated and degassed.

Preparation of Sample

Weighed and transferred drug substance 163.4 mg (equivalent to 100 mg of standard) in 100 ml volumetric flask. Pipette out 10 ml of this solution and volume made up to 20 ml, then further pipette out 10 ml of this solution and volume made upto 20 ml with diluent to dissolve, sonicated and degassed.

Assay procedure

Inject 1µL of the standard and sample solutions into the UPLC system and the chromatograms were recorded and measured the areas for the Terbinafine HCl peak and calculate the % Assay by using following formula.

% Assay = (At/As) x (Ws/Ds) x (Dt/Wt) x (P/100) x(Avg. weight/Label Claim) x 100

Where,
At = average area counts of sample preparation,
As = average area counts of standard preparation

Ws= Weight of working standard taken in mg,
Wt =Weight of sample taken in mg
Dt = sample dilution
DS= standard dilution
P= Purity of Standard

In RP-UPLC method, chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds with shorter run time, less consumptions of solvent and mass compatible for further studies.

Validation of developed UPLC method

Different chromatographic conditions such as mobile phase, wavelength, column and column temperature were experimented to achieve efficiency of the chromatographic system. Different gradients of buffer and solvents were checked in order to attain optimum retention of the API. Minimizations of run time and cost were the major tasks while developing the method.

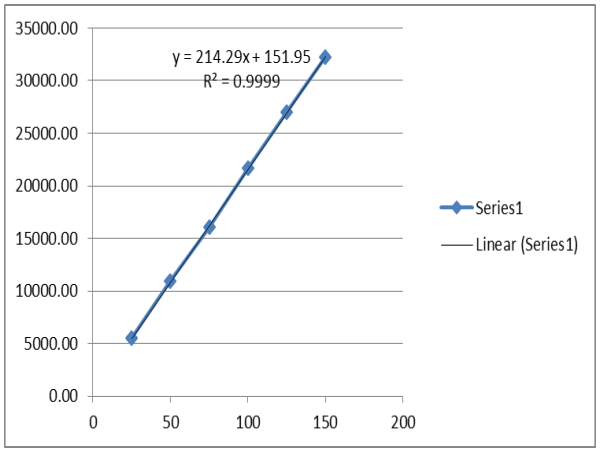
Based on International Conference on Harmonization (ICH) guidelines, the method was validated with regard to precision, specificity, reproducibility, accuracy, linearity, stability of solution, robustness, limit of detection and quantification.

Linearity

Linearity was assessed in the range of 25%, 50%, 75%, 100%, 125% and 150% of working concentration. Injections of all concentrations were carried out in replicate. Calibration curve was constructed by plotting the mean peak area versus concentration which was observed to be linear. The Linearity co-efficient of mean response which was plotted against respective concentration, was calculated. The results are summarized in Table-3 and Fig. 2.

Table 3. linearity data

Concentration (in %)	Peak Area		
	Injection-1	Injection-2	Average
25	5492.78	5473.02	5482.90
50	10892.15	10912.96	10902.56
75	16086.33	16145.32	16115.83
100	21781.52	21660.82	21721.17
125	27039.83	26904.81	26972.32
150	32069.71	32373.28	32221.50



Peak Area v/s Conc.

Fig. 2 Calibration curve of Terbinafine HCl

ACCURACY

Recovery of the assay method for Terbinafine HCl was established by three determinations of test sample using Tablets at 50%, 100% and 150% concentration. Each solution was injected thrice (n=3) into UPLC system and the average peak area was calculated to obtain percentage recoveries. All the individual recoveries were found to be between 97.66% to 98.53%. All individual recovery levels were found to be within 0.17 to 1.06 (%RSD). The results are summarized in Table-4.

Table 4. Recovery studies of Terbinafine HCl

Conc.	Sample area	Avg. area	Sample Wt. (mg)	Amt. added (µg)	Amt. recovered (µg)	% Recovery	Avg. % recovery	SD	% RSD
50%	10900.45	10786	85.60	50	49.35	98.69	97.66	1.04	1.06
	10788.16				48.84	97.67			
	10670.79				48.31	96.61			
100%	21537.68	21759	171.19	100	97.50	97.51	98.51	1.00	1.02
	21760.26				98.51	98.51			
	21979.99				99.50	99.51			
150%	32582.75	32646	256.79	150	147.50	98.34	98.53	0.17	0.17
	32682.25				147.95	98.64			
	32673.14				147.91	98.61			

Precision (system and method)

The precision of the system was evaluated by carrying out six independent injection of standard. The % RSD of peak area of the standard was found to be **0.85.** The results are summarized in Table 5.

Table 5 Result of system precision

S.No	Standard	RT	Standard Area
1	Injection1	1.66	21503.73
2	Injection 2	1.66	21625.45
3	Injection 3	1.66	21234.59
4	Injection 4	1.66	21628.54
5	Injection 5	1.66	21677.48
6	Injection 6	1.66	21321.96
	Average	**1.66**	**21498.36**
	SD	**0.0**	**182.16**
	%RSD	**0.0**	**0.85**

The precision of the method was evaluated by carrying out six independent injection of test sample against a qualified reference standard. The % RSD of peak area of the standard was found to be **0.94.** The results are summarized in Table 6.

Table 6 Result of method precision

NAME	INJECTION NO.	AREA	Avg.Area	Retention Time	%Assay
Sample-1	1	21523.93	21688.68	1.66	100
	2	21853.42			
Sample-2	1	21565.07	21627.76	1.66	100
	2	21690.45			
Sample-3	1	21079.78	21277.21	1.65	100
	2	21474.63			
Sample-4	1	21703.13	21635.80	1.66	100
	2	21568.47			
Sample-5	1	21791.54	21544.39	1.66	100
	2	21297.23			
Sample-6	1	21865.52	21899.00	1.66	100
	2	21932.48			
Mean			21612.14	1.66	100
SD			202.84	0.25	00
%RSD			0.94		

Reproducibility (Intermediate Precision)

An assay was performed by analyzing six samples of Terbinafine HCl against a qualified reference standard. The %RSD obtained from these samples was observed as **0.68** and %RSD of peak area of reference standard was observed as **0.85**. The results are summarized in Table 7.

Table 7. Result of intermediate precision

S. No.	Name	Std. Area	TEST AREA		Avg. Test Area
			Inj. 1	Inj. 2	
1	Int.precision 1	21503.73	22668.48	22553.18	22610.83
2	Int.precision 2	21625.45	22689.25	22895.19	22792.22
3	Int.precision 3	21234.59	22260.99	22730.23	22495.61
4	Int.precision 4	21628.54	21945.10	22710.89	22328.00
5	Int.precision 5	21677.48	22126.51	22908.64	22517.58
6	Int.precision 6	21321.96	22668.48	22553.18	22610.83
	Average	**21498.63**			**22559.18**
	SD	**182.16**			**154.18**
	%RSD	**0.85**			**0.68**

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the sample containing Terbinafine HCl standard stock with those of the test sample. The specificity study reveals the absence of interference of impurities with the drug, since no extra

peak appeared at the same retention time. The RSD for six replicate measurements of peak area of standard preparation was found to be **0.73**. The results are summarized in Table 8.

Table 8 Result of specificity

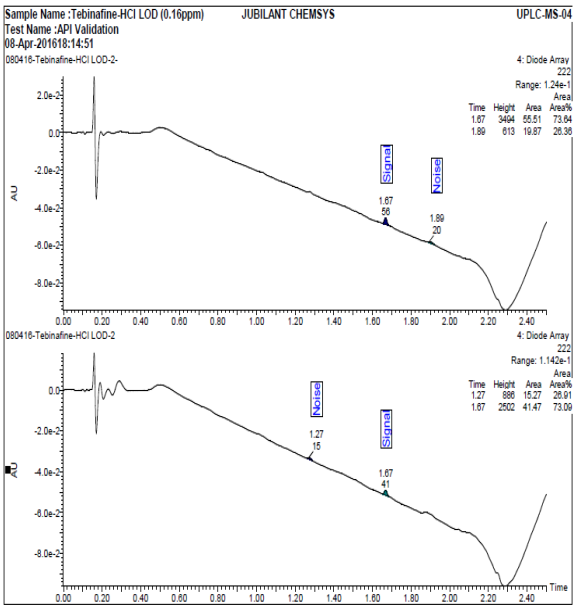
S. No.	R.T	Std. Area	Test Area
1	1.66	21517.68	22402.11
2	1.66	21937.92	22578.80
3	1.66	21514.04	
4	1.66	21688.63	
5	1.66	21716.17	
6	1.66	21744.24	
Mean		**21686.45**	**22490.455**
SD		**158.54**	**124.94**
%RSD		**0.73**	**0.56**

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (±0.10 mL/min), buffer concentration (±10%), column temperature (±5 ºC). In all the above varied conditions, the component of mobile phase was held constant, but no marked changes were observed in the chromatograms, which confirmed that the developed UPLC method is robust.

Limit of detection (LOD)

Limit of detection is the lowest amount of an analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected was determined by visual examination of signal to noise ratio which should be 3:1 with respect to height.



CONCLUSION

A new method has developed to determine Terbinafine HCl efficiently and accurately within a relatively short period by using Reverse phase UPLCMS method. It showed a good precision (RSD<2%) and recovery (97.66% - 98.53%). and proved to be simple, linear, precise, accurate, robust, rugged and rapid. It gives faster elution, maintaining good separation more than that achieved with conventional HPLC. Short run time allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. This method can be directly used for LC-MS analysis on need basis.

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