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Original Article

DEVELOPMENT AND VALIDATION OF RP-UFLC METHOD FOR THE ESTIMATION OF TERBINAFINE HCl IN PHARMACEUTICAL NANOEMULSION GEL

V.V.S.NARAYANA REDDY K*[1], SURESH KUMAR R[1], HEMNATH E[2], VENKATA SIDDHARTHA T[1], KARTHIK YAMJALA[3], RAJKUMAR MALAYANDI[4]

[1]Department of Pharmaceutics, JSS College of Pharmacy (A constituent college of JSS University, Mysore), Rocklands, Udhagamandalam, Tamilnadu, India. [4]COE-BIO, Clinical pharmacology and pharmacokinetics, IPDO, Dr.Reddys Laboratories Innovation plaza, Ranga Reddy District, Andhra Pradesh, India.
Email: ksnreddy87@gmail.com

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ABSTRACT

Objective: To develop a simple, accurate, sensitive and rapid isocratic reverse phase ultra-force liquid chromatographic (RP-UFLC) method for the quantitative and qualitative estimation of terbinafine HCl in the bulk and nanoemulsion gel formulation.

Methods: The chromatographic separation was achieved on stationary phase Phenomenex C18 column (250mm x 4.6mm, 5µ) by using mobile phase of methanol and 25mM of phosphate buffer (pH 4.0) in 80:20 ratio and detection was carried out at 222nm. Various validation parameters such as specificity, linearity, accuracy, precision, ruggedness and robustness were performed.

Results: The developed method provided coefficient correlation equal to 0.997 in the range of 10µg/ml to 500µg/ml indicating good linearity. Recovery studies showed that the results obtained were within the limits indicating the accuracy of the method. The intra-day and inter day variability were represented in percentage relative standard deviation (RSD) which showed a variation of less than 1.7. The presence of polymers and other components did not affect the results indicating the selectivity of the developed method.

Conclusion: The developed RP-UFLC method was validated as per ICH guidelines and can be used for the qualitative and quantitative estimation of terbinafine HCl in bulk, nanoemulsion gel formulation as well as other pharmaceutical formulation.

Keywords: Terbinafine HCl, RP-UFLC, ICH, Nanoemulsion gel.

INTRODUCTION

Terbinafine HCl chemically, [(2E)-6, 6-dimethylhept-2-en-4-yn-1-yl] (methyl) (naphthalen-1-ylmethyl) amine (Figure. 1) is an allylamine derivative having broad spectrum of antifungal activity. It is used to treat superficial skin infections such as Onychomycosis, athlete's foot (Tinea pedis), ringworm (Tinea corporis) and jock itch (Tinea cruris) [1, 2].

Literature survey reveals various methods for the estimation of terbinafine in different matrices. Schatz F et al have reported the determination of terbinafine and its metabolites in human plasma, milk and urine [3]. Zehender H et al have developed the simultaneous determination of terbinafine (Lamisil) and five metabolites in human plasma and urine by high-performance liquid chromatography using on-line solid-phase extraction technique [4]. Denouël Jet al. has determined the terbinafine and its desmethyl metabolite in human plasma by high-performance liquid chromatography [5]. Dykes PJ et al determined the terbinafine in nail samples during systemic treatment for Onychomycosis [6]. Cardoso SG et al have reported an HPLC assay method for terbinafine hydrochloride in tablets and creams [7]. Cardoso SG et al reported an UV spectrophotometry and non-aqueous determination methods for the terbinafine hydrochloride in various dosage forms [8]. L. Matysova et al have separated and determined terbinafine and its four impurities of similar structure using simple RP-HPLC method [9]. There is no reported mechanical method available for the preparation of terbinafine nanoemulsion gel formulation and also its estimation by reverse phase ultra force liquid chromatographic method, hence determined to develop a validated analytical method for terbinafine.

MATERIALS AND METHODS

Materials

Terbinafine HCl was purchased from SDFCL, Mumbai. Methanol HPLC grade, potassium dihydrogen orthophosphate, triethyl amine and orthophosphoric acid were procured from Merck, Mumbai. Triple distilled water was obtained from Milli Q RO system.

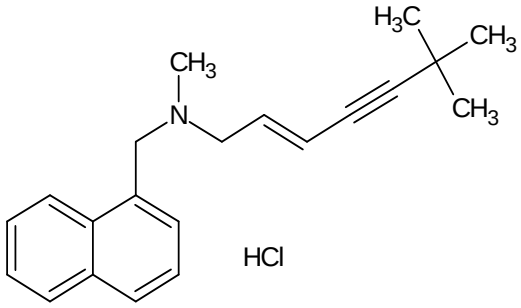

Fig. 1: Chemical Structure of Terbinafine HCl

Instrumentation

The Ultra-Force Liquid Chromatography (UFLC) equipped with Shimadzu LC-20AD solvent delivery system (pump), Photodiode Array Detector (PDA), 7725i rheodyne injector with 20µl loop volume and the date station used was LC Solutions. Chromatographic separation was achieved using Phenomenex C18 column (250mm x 4.6mm; 5µ particle size) and the mobile phase consisting of 25mM potassium dihydrogen orthophosphate pH 4.0 adjusted with orthophosphoric acid and methanol in the ratio of 20:80.

Selection of wavelength

100µg/ml standard solution of terbinafine HCl was prepared and used for scanning in the UV region of 200 – 400nm. At 222nm terbinafine Hydrochloride showed maximum absorption.

Preparation of Terbinafine HCl standard solution

10mg of terbinafine HCl was weighed accurately and dissolved in 10ml Methanol. The prepared solution was further diluted with Methanol to produce 100µg/ml.

Preparation of terbinafine HCl Nanoemulsion gel [10, 11]

The Nanoemulsion gel of terbinafine HCl was prepared by using high speed homogenization technique, which is a mechanical process. In this process two phases of an emulsion i.e., aqueous and oil phases were prepared separately and then, the oil phase (dispersed phase) was added drop wise to the aqueous phase (continuous phase) under high speed homogenization. The aqueous phase was prepared by adding sodium acetate, disodium edentate, vitamin E tpgs, glycerine and polysorbate 80 to 5-10% purified water. Terbinafine HCl was added to specified quantity of liquid paraffin (oil phase) and dispersed. The oil phase was then slowly transferred in to aqueous phase during high speed homogenization at 5000 rpm for 1 hr. After homogenization the resultant Nanoemulsion was incorporated in to the Carbopol gel base which was previously prepared by adding 1.2 gm of Carbopol 934 to 20-30% of water under stirring. Finally the pH of prepared nanoemulsion gel of terbinafine HCl was adjusted to 7.0 using 2N NaOH which is isotonic to the skin pH.

Assay of Terbinafine HCl nanoemulsion gel by RP-UFLC

100mg of nanoemulsion gel containing 1 mg of terbinafine HCl was taken and was dissolved in 10ml of methanol and sonicated to get a concentration of 100µg/ml. The sample solution was filtered through 0.22µm membrane filter to obtain a clear solution and analysed at 222nm by the developed RP-UFLC method.

Validation of the method

The proposed UFLC method was validated as per ICH guidelines [12, 13] for linearity, range, accuracy, precision, sensitivity and robustness.

Linearity and Range

1mg/ml stock solution of terbinafine HCl was prepared and it was further diluted to obtain standard solutions of 10-500µg/ml. These solutions were injected in triplicate into the UFLC system and the chromatograms were recorded with the optimized chromatographic conditions.

Accuracy

The accuracy of the method, it is generally expressed in terms of recovery studies and was performed by standard addition method by adding known amount of drug (75, 100 and 150µg/ml) to the real samples.

Precision studies

The precision of the method was determined by six independent injections of three different concentrations (25, 50 and 100µg/ml) were injected on the same day (intra-day precision) and the values of % RSD were calculated. The same concentration solutions were injected into the system on different days (inter-day precision).

Specificity

A method is specific when it produces a response only for a single analyte in the presence of other interferences. It was determined by comparing the chromatograms of terbinafine HCl loaded nanoemulsion gel and placebo nanoemulsion gel (without terbinafine HCl).

Sensitivity

The method is said to be sensitive if it detects very low levels of the drug. It is based on the limit of detection (LOD) and limit of quantification (LOQ) values and was determined at a signal-to-noise (S/N) ratio of 3:1 and 10:1 respectively.

Robustness

Robustness of the method was checked by injecting the standard solution with slight variations in the optimized chromatographic conditions such as flow rate, pH of the buffer and composition of organic phase.

RESULTS AND DISCUSSION

Method development and validation

Optimized chromatographic conditions

The method was finally optimized with the following conditions, mobile phase consisting of Methanol and 25mM Potassium dihydrogen orthophosphate buffer pH 4.0 in the ratio 80:20 v/v and Phenomenex C18 column (250mm x 4.6mm, 5µ) column as stationary phase. The analysis was carried out in an isocratic elution mode using a flow rate of 1.0 ml/min, injection volume of 20µl at room temperature and the detection of analyte was recorded at 222nm. The mobile phase solvents were filtered through 0.45µm Polytetrafluoroethylene filter before delivering into the UFLC system. The chromatogram was recorded using LC solution software.

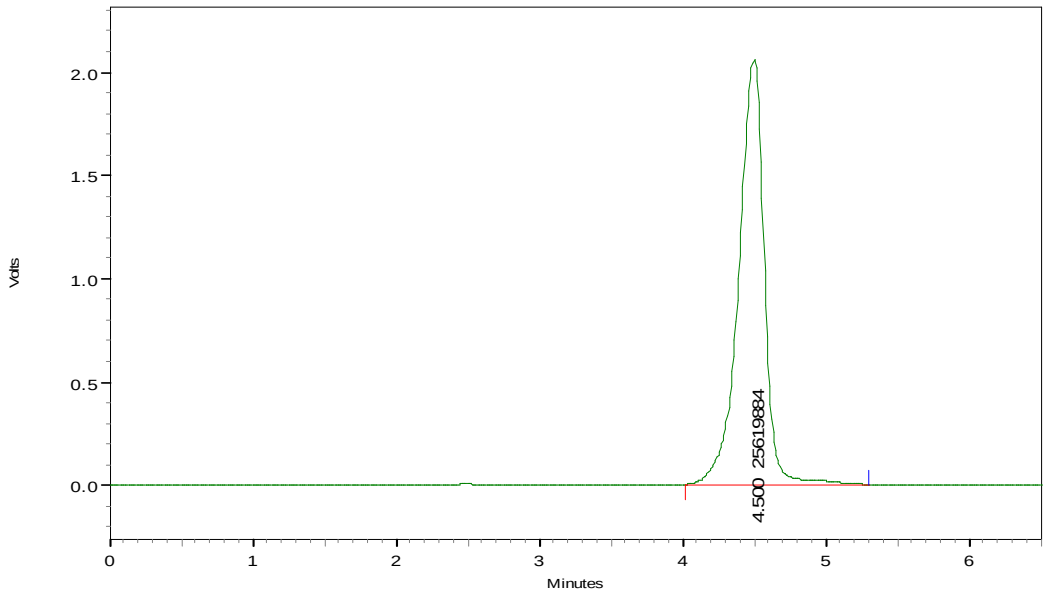

Fig. 2: Typical chromatogram of Terbinafine HCl

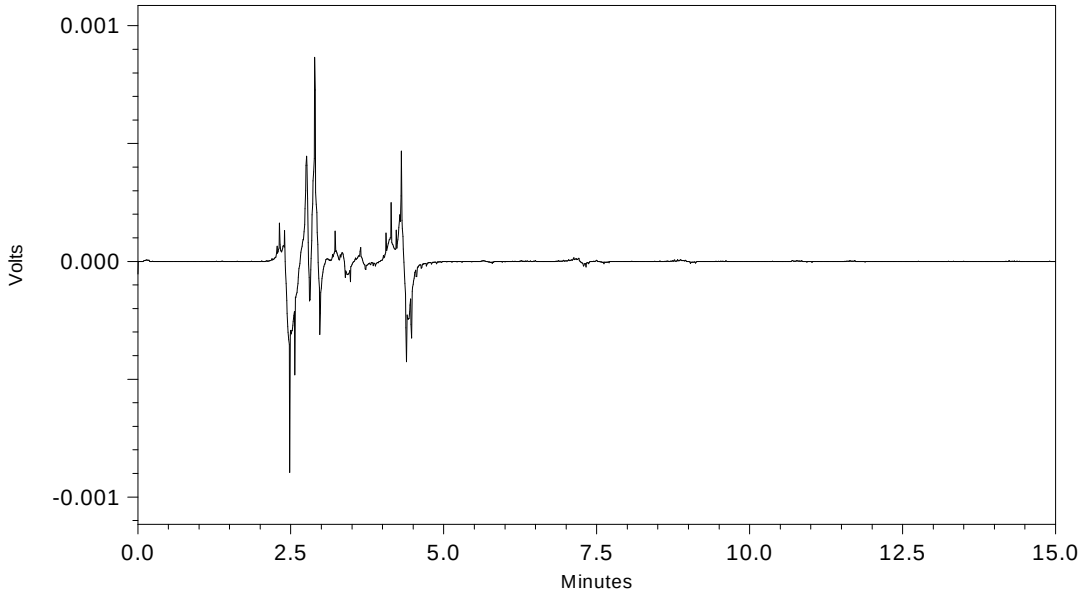

Fig. 3: Chromatogram of Placebo

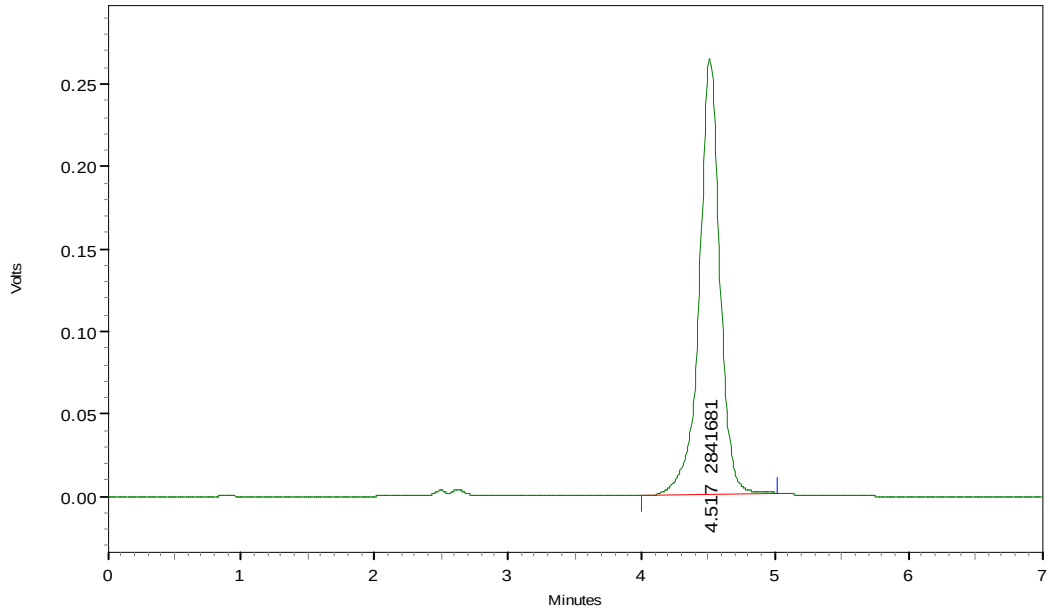

Fig. 4: Chromatogram of Terbinafine HCl in nanoemulsion gel formulation

Validation of the developed UFLC method

Selectivity

The selectivity of the method was performed by injecting the mobile phase and placebo nanoemulsion for any co-eluting peaks, at retention time of the terbinafine drug (4.50 min). The chromatograms of standard terbinafine HCl, placebo and terbinafine HCl loaded nanoemulsion gel are shown in (Figure. 2, 3 and 4)

Accuracy and precision

The accuracy of the method was measured in terms of recovery studies and it is carried at three different concentrations by standard addition method and the accuracy was between 98.84 to 99.55%.The accuracy results were represented in (Table. 1). The developed UFLC method was applied for the estimation of terbinafine HCl in in-house nanoemulsion gel formulation. The acquired result for terbinafine was comparable with a corresponding label claim (Table. 2). The intra and inter-day precision studies showed a % RSD of <1.580% and <1.687% respectively which evidenced the method was adequately precise (Table. 3).

Linearity

The calibration curve was plotted between 10 – 500µg/ml. The co-efficient of correlation was (r^2) 0.997 with the slope 10702x and y-intercept value of 46723. The linearity curve is represented in (Figure.5)

Limit of detection and Quantification

The LOD and LOQ were found to be 5ng/ml and 15ng/ml respectively which indicate that the developed method was sensitive.

Table 1: Accuracy

Actual concentration (µg/ml)	Recovered concentration (µg/ml)±S.D.; R.S.D % (n=3)	Percentage Recovered
75	74.18±0.891; 1.201	98.90
100	99.55± 1.518; 1.525	99.55
150	148.27± 0.950; 0.641	98.84

Table 2: Assay results of Terbinafine HCl nanoemulsion gel formulation

Sample	Label Claim	Amount Present (mg)±S.D.; %R.S.D (n=3)
Formulation - I	1mg	0.994±0.016; 1.612

Table 3: Precision studies

Actual concentration (µg/ml)	Intra-day calculated concentration (µg/ml)± S.D.; R.S.D % (n=6)	Inter-day calculated concentration (µg/ml) ± S.D.; R.S.D % (n=3)
25	24.67± 0.390; 1.580	24.64 ± 0.415; 1.687
50	49.02± 0.395; 0.806	48.79 ± 0.144; 0.295
100	99.82±0.141; 0.142	99.75± 0.0901; 0.090

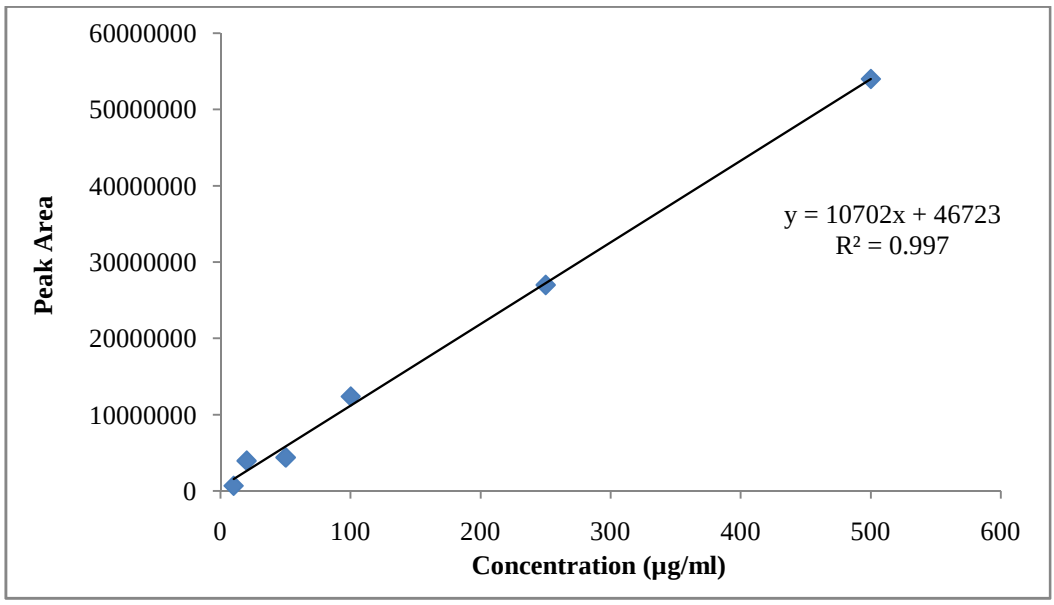

Fig. 5: Linearity of Terbinafine HCl

Table 4: Robustness studies

Parameter	Retention Time (R_t)
Flow rate (ml/min)	
0.9	4.66
1.0	4.50
1.1	4.34
Potassium dihydrogen orthophosphate pH 4.0: Methanol (v/v)	
22:78	4.54
20:80	4.50
18:82	4.45
pH of buffer solution	
3.9	4.42
4.0	4.50
4.1	4.58

Robustness

Robustness was performed by minor deliberate changes in optimized chromatographic conditions such as flow rate (±0.1ml min^{-1}), pH (±0.2), and organic phase composition (±2%). Upon changing these conditions, the method was proven to be robust as there was no greater deviation in the retention time of the analyte (Table. 4).System suitability studies were an integral part of the developed analytical method. The number of theoretical plates (N) was found to be 8754 per meter and Asymmetric factor (As) of 1.03. Tailing factor (T_f) was calculated according to USP and was found to be 1.09.

CONCLUSION

The developed UFLC method was found to be accurate, precise, simple, sensitive, and selective, with good system suitability and validated as per ICH guidelines. The method can be used for the qualitative and quantitative estimation of terbinafine HCl in both bulk drug and nanoemulsion gel formulation.

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