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Short communication

High-performance liquid chromatographic assay of terbinafine hydrochloride in tablets and creams

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1. Introduction

Terbinafine hydrochloride (TH) (Fig. 1) is a new potent antifungal agent of the allylamine class that selectively inhibits fungal squalene epoxidase. This drug is indicated for both oral and topical treatment of mycoses [1,2]. Chemically it is (*E*)-*N*-(6,6-dimethyl-2-hepten-4-ynyl)-*N*-methyl-1-naphthalenemethanamine hydrochloride and is not yet official in any pharmacopoeia. Previously, the drug has been determined in biological fluids by HPLC [3–6] and in tablets by UV-spectrophotometric methods [7]. However, an HPLC method to determine TH in dosage forms has not been reported. Because of their selectivity, sensitivity and overall versatility, the development of reliable and validated HPLC methods has received considerable attention in the quality control of drugs. Therefore, a simple, rapid and reproducible reversed-phase HPLC method for the quantification of TH in raw materials, tablets and creams is presented here. This method can be applied to stability studies.

2. Experimental

2.1. Chemical and reagents

Reference TH (assigned purity 99.7%) was obtained from Galena (São Paulo) while pharmaceuticals containing terbinafine were obtained commercially. Terbinafine tablets were claimed to contain 125 mg (as base) of the drug and the cream 1% (as hydrochloric salt). Methanol was HPLC grade (Lichrosolv, Merck). Water was glass-distilled.

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2.2. Apparatus and chromatographic conditions

The HPLC apparatus consisted of a Shimadzu LC-10A system equipped with a model LC-10AS pump, an SPD-10A variable-wavelength detector (set at 254 nm), an SCL-10A system controller, a C-R6A integrator and a Rheodyne injection valve with a 20 μl loop (Shimadzu, Kyoto, Japan). A Shim-pack CLS-ODS (250 mm × 4 mm i.d., 5 μm particle size, 100 Å pore diameter, end-capped) was used with methanol–water (95:5 v/v), isocratic as the mobile phase, at a flow-rate of 1 ml min^{-1}; the sensitivity was 0.5 AUFS and the chart speed was 0.5 cm min^{-1}. The HPLC system was operated at ambient temperature (19 ± 1°C).

2.3. Calibrations curves

A stock solution of 1.0 mg ml^{-1} TH was prepared in a volumetric flask by dissolving 25.0 mg in 25.0 ml methanol. Appropriate amounts of the stock solutions were diluted with methanol yielding concentrations of 10.0, 12.0, 14.0, 16.0, 18.0 and 20.0 μg ml^{-1}. Triplicate injections of each were made.

2.4. Procedure for sample preparation

2.4.1. Tablets

The tablets were weighed and pulverized. An amount of powder equivalent to 100.0 mg TH, was transferred to 50.0 ml volumetric flask with 30 ml methanol and shaken for 10 min, followed by making up to volume with methanol. After filtration, the dilutions were made with methanol to give a final concentration of 16.0 μg ml^{-1}.

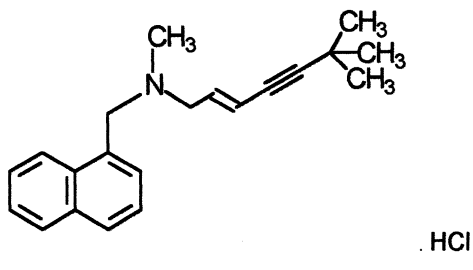

Fig. 1. The chemical structure of terbinafine hydrochloride.

2.4.2. Creams

A quantity of the cream containing 20.0 mg TH was extracted by warming with 30 ml methanol in a water-bath at 50°C for 10 min with occasional shaking. This solution was transferred to a 50.0 ml volumetric flask and methanol added to make up to volume. After centrifugation (5 min at 1500 rpm), the dilutions were made with methanol to give a final concentration of 16.0 μg ml^{-1}.

2.5. Calculation

The drug contents of the tablets and creams were determined by referring either to the calibration curve or by sample/equivalent reference substance direct matching.

2.6. Method validation

The method was validated by determination of the following operational characteristics: linearity, range, precision, accuracy, limit of detection and limit of quantitation.

3. Results and discussion

Drug analysis is undertaken during various phases of pharmaceutical development such as formulation and stability studies, quality control and pharmacological testing in animals and man. All these investigations require reliable and validated analytical methods in order to measure drugs in pharmaceutical formulations and biofluids [8]. There have been little or no reported methods for TH dosage forms. However, the aim of this study was to develop a simple and isocratic HPLC assay for the analysis of this drug in raw materials and pharmaceutical preparations. The choice of the method depends on factors such as the nature of the drug, the complexity of the sample and the intended use. For pharmacokinetic evaluation, HPLC methods have been developed for determination of terbinafine and its metabolites in biological fluids [3–6]. The chromatographic conditions were used to separate the TH and its metabolites in a complex media. For

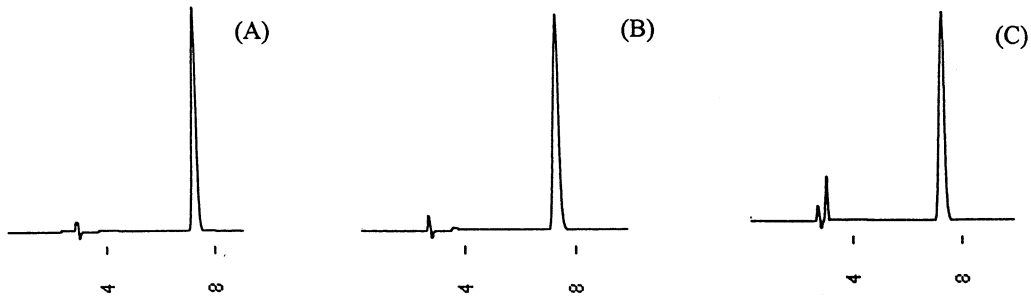

Fig. 2. Chromatograms of TH at 16.0 μg ml^{-1}: (A) reference substance; (B) tablets; and (C) creams. Chromatographic conditions: column, C18 CLS-ODS, end-capped (250 mm × 4.6 mm i.d., 5 μm, 100Å pore diameter); mobile-phase, methanol–water (95:5 v/v); flow-rate, 1.0 ml min^{-1}; chart-speed, 0.5 cm min^{-1}; detection, wavelength 254 nm (0.5 AUFS); retention time, 7.2 min.

drug analysis in quality control, the simplest and fastest procedures can be applied. In this study, the chromatographic conditions were influenced by the physico-chemical properties of TH, such as solubility, polarity and UV absorption. The optimum mobile phase was obtained with 95:5 (v/v) methanol–water. In general, it can be stated that the internal standard method clearly has the advantage of not being sensitive to complex sample preparation, or even to sample losses during sample cleanup and enrichment [9]. However, we used the external standard method, because it is simpler, fast and accurate for sample preparation. The UV absorption spectrum of TH shows an intense absorption at 224 nm, but the detection was monitored at 254 nm since it offered more selectivity. The retention time was about 7.2 min. It results were reproducible with the R.S.D. ranging from 0.29 to 1.26% for whithin-day and 0.42% for between-day studies. A typical chromatogram of pure TH is shown in Fig. 2A. The calibration curves for TH were constructed by plotting concentration versus peak area and showed good linearity in the 10.0–20.0 μg ml^{-1} range. The representative linear equation for TH was: $y = 7.957x + 637.6$ ($n = 6$, $r = 0.9997$, $r^2 = 0.9994$). The method was validated by evaluation of intra- and inter-day precision. In the range of 10–20 μg ml^{-1} the percent R.S.D. on the basis of peak area ratios for three replicate injections were found to be between 0.02 to 1.23%. The inter-day precision was evaluated by comparing the linear regressions of the four standard plots prepared on four different days, over a two month period. The average coefficient of correlation was $r = 0.9997$ and the R.S.D. of the slope of the four lines was 0.66%. Analysis of variance of the date indicated no significant difference in slopes of the four calibration curves ($P < 0.01$). The limit of quantitation, taken as the lowest concentration of analyte in a sample which can be determined with acceptable precision and accuracy under the stated experimental conditions, was 2.5 μg ml^{-1}. The detection limits, taken as the lowest absolute concentration of analyte in a sample which can be detected but not necessarily quantified under the stated experimental conditions, was found 0.16 μg ml^{-1}. The repetibility of the method was studied by assaying six samples of tablets and creams, at some concentration, during the some day under the some experimental conditions. The coefficient of variation was 0.62 and 0.98% for tablets and creams, respectively, as shown in Table 1. The recovery test and precision data for commercial tablets and creams are shown in Table 2. The mean absolute recovery, determined by adding known amounts of TH reference substance (2.0, 3.0, 6.0 and 10.0 μg ml^{-1}) to the samples at the beginning of the process, were found to be 99.25 (tablets) and 96.14% (creams). No interference from the sample solvent, impurities and dosage form excipients could be observed at the detection wavelength (254 nm), as shown in Fig. 2B and C. In preliminary studies conducted with light the method appeared to be stability-indicating. Confirmatory studies of photostability are been conducted in our laboratory.

Table 1
Data obtained from commercial sample analysis by HPLC.

Product	Theoretical amount (mg[a])	Experimental amount (mg[b]) ± S.E.	Purity (%)	R.S.D. (%)
Tablets	125	126.36 ± 0.32	101.15	0.62
Creams	10	9.67 ± 0.06	96.70	0.98

[a] Units: mg per tablet, mg per g of cream.
[b] Mean of six determinations.

Table 2
Recovery of drugs from samples with known concentrations.

Product	Amount of standard (μg ml^{-1})		Recovery[a] (%)
	Added	Found	
Tablets	2.00	1.95	97.50
	3.00	2.96	98.67
	6.00	5.98	99.83
	10.00	10.10	101.00
Creams	2.00	1.90	95.00
	3.00	2.90	96.67
	6.00	5.76	96.00
	10.00	9.69	96.90

[a] Mean of three replicate analysis.

4. Conclusion

The HPLC method developed in this study has the advantage of simplicity, precision, accuracy and convenience. Moreover, the method uses simple reagents, with minimum sample preparation procedures, encouraging its application in routine analysis.

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