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Micro-liquid chromatography method for the determination of ciclopiroxolamine after pre-column derivatization in topical formulations

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ABSTRACT

Ciclopiroxolamine is a broad-spectrum antimycotic drug. Neither the free acid (ciclopirox) nor its salt with ethanolamine (ciclopiroxolamine) can directly be quantified by liquid chromatography (LC) on both normal and reversed phases. This is due to the chelating function of the N-hydroxypyridone group that interacts strongly with stationary phases. Derivatization by alkylation forms a 1-alkyloxypirydone with regular chromatographic behaviour. A micro-LC method based on an isocratic elution reversed-phase system for quantification of ciclopiroxolamine in topical formulations is described. Chromatography was carried out using an LC Packings fused-silica capillary column (15 cm × 330 μm I.D.; Delta Pak, RP-18, 5 μm, 300 Å) coupled to a Kontron 433 UV capillary detector. Data with respect to the derivatization reaction, recovery, reproducibility and limits of detection of the proposed method are reported and discussed.

INTRODUCTION

Ciclopiroxolamine, the 2-aminoethanol salt of 6-cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone (Fig. 1), is a synthetic antifungal agent with a broad spectrum of *in vitro* activity against most pathogenic fungi, including dermatophytes, *Candida albicans* and numerous non-pathogenic fungi [1–4]. Like many other antimycotic drugs, ciclopiroxolamine possesses antibacterial properties. Its activity against *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and relevant *Staphylococcus* and *Streptococcus* species has also been demonstrated [5].

Good penetration through hornified skin layers shows that ciclopiroxolamine is a useful drug in onychomycosis therapy [6]. Its antifungal action differs from other antimycotic drugs, in that ciclopiroxolamine exerts its activity not directly on the

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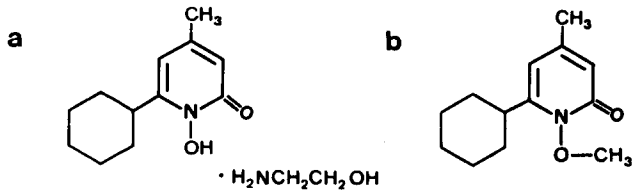

Fig. 1. Chemical structure of (a) ciclopiroxolamine and (b) 1-methoxyciclopiroxolamine

fungal plasma membrane but by intracellular depletion of some essential substrates and/or ions caused by inhibition of their uptake [7].

Ciclopiroxolamine is used, at relatively low concentration (1%), in different pharmaceutical formulations (creams, powders, lotions). Spectrophotometric [8] and high-performance thin-layer chromatography (HPTLC) [9] methods have been proposed for the quality control of various pharmaceutical dosage forms containing ciclopiroxolamine. The spectrophotometric method does not seem sufficiently quantitative when ciclopiroxolamine is incorporated into a complex matrix such as cream. Furthermore it involves preliminary extraction procedures that are laborious and time consuming.

In addition, HPTLC methods lack the sensitivity required for analysis of pharmaceutical preparations: the free acid and its salt with aminoethanol migrate as a uniform spot and severe tailing was also observed.

An alternative approach to quantitative determination of ciclopiroxolamine is liquid chromatography (LC). However, with the use of silica-based material two problems are encountered: irreversible absorption of small amounts of ciclopiroxolamine and tailing peaks. The strong absorption of ciclopiroxolamine onto the packing material observed is presumably due to the chelating function of the N-hydroxipyridone group. This interaction results in non-linear calibration curves. Methylation of the weak acidic N-hydroxyl group of the ciclopiroxolamine gave the 1-methoxy derivative that shows a normal chromatographic behaviour [10].

The present paper describes a micro-LC method based on an isocratic elution reversed-phase system for direct quantitation of the 1-methoxy derivative of ciclopiroxolamine in commercial dosage forms (foam and powder).

EXPERIMENTAL

Reagents

Acetonitrile was of HPLC grade (Fluka, Buchs, Switzerland); distilled water used was purified by a Water I system (Gelman, MI, U.S.A.). All other reagents were of analytical-reagent grade and were used without further purification.

Samples

Pharmaceutical dosage forms, foam and powder, containing 1% ciclopiroxolamine were obtained from Laboratori Delalande (Italy).

Sample preparation: foam. A sample equivalent to about 10 mg of the antimycotic drug was weighed exactly in a 50-ml calibrated flask. For derivatization, 2 ml

of 1 *M* sodium hydroxide solution and 30 μl of methyl iodide were added. After vortex-mixing, the flask was kept in an ice-bath for 10 min. Methyl iodide excess was destroyed by adding 30 μl of 25% ammonium hydroxide solution to the mixture. It was then dissolved and diluted to volume with acetonitrile–water (1:1, v/v). An aliquot of 200 nl was injected into the chromatographic system.

Sample preparation: powder. A sample equivalent to about 2 mg of the antimycotic drug was weighed exactly in a 10-ml PTFE-lined screw-capped glass tube, then 2 ml of acetonitrile–water (1:1, v/v), 1 ml of 1 *M* sodium hydroxide solution and 20 μl of methyl iodide were added. After vortex-mixing, the tube was kept in a ice-bath for 10 min. Methyl iodide excess was destroyed by adding 20 μl of 25% ammonium hydroxide solution to the mixture.

The mixture was vortexed and centrifuged (10 min, 3000 *g*). The liquid layer was carefully transferred into a 50-ml volumetric flask. The solid residue was resuspended in 5 ml of acetonitrile–water (1:1, v/v) and again extracted. The liquid phase was carefully transferred directly into the previous volumetric flask; this was repeated twice. The volume was then adjusted to 50 ml with the extraction mixture. An aliquot of 200 nl was injected into the chromatographic system.

Standard

Ciclopiroxolamine standard of know purity was provided by Sibefat (Milan, Italy) and was used without further purification.

Calibration procedures. The calibration curves of ciclopiroxolamine in foam and powder were constructed from replicate samples over the concentration range 0–12 mg/g. Samples of control (drug free) foam and powder were mixed with appropriate amounts of ciclopiroxolamine standard, weighed exactly on a Cahn model G2 electrobalance, to give concentrations of 0, 2, 4, 6, 8, 10 and 12 mg/g. All the samples were derivatized according to the procedure described above. The peak areas were then compared to those obtained from derivatized ciclopiroxolamine standard solutions with comparable concentrations.

Accuracy

The accuracy of the method was checked by means of recovery experiments carried out on representative pharmaceutical preparations (foam and powder) spiked with known quantities of the drug to give three series of 10 samples with concentrations of 8, 10 or 12 mg of the drug per g of foam and powder.

Apparatus

The LC system consisted of the following components: Carlo Erba (Milan, Italy) Phoenix 20 CU micro-pump; Kontron (Zurich, Switzerland) 433 UV capillary detector, equiped with an ultrasensitive UV flow-cell (total volume 90 nl, optical path length 20 mm); Valco (Houston, TX, U.S.A.) injector Model C14W with a 200-nl internal loop. Peak areas were measured with a Perkin-Elmer (Norwalk, CT, U.S.A.) LCI-100 laboratory computing integrator.

Chromatographic conditions. A Fusica column (LC Packings, Amsterdam, The Netherlands) (15 cm × 330 μm I.D.; Delta Pak, RP-18, 5 μm, 300 Å) was used. The column was connected directly the flow-cell via a small piece of PTFE tubing (tubing kit TF-K1, LC Packings).

The separations reported were achieved under the following conditions: mobile phase, acetonitrile–water (1:1, v/v); flow-rate 10 μl/min; chart speed, 0.5 cm/min; temperature, 20°C; wavelength, 300 nm. The quantity of ciclopiroxolamine was calculated by applying the external standard method.

RESULTS AND DISCUSSION

The irreversible absorption of ciclopiroxolamine on reversed-phase which appears in non-linear response to the quantity injected and the peak area or height, in different sequential injections of the same amount, is assumed to be the result of the chelating properties of the analyte with residual silanol groups on the surface of the stationary phase. In agreement with reported data, methylation of ciclopiroxolamine gave a stable derivative that shows a single non-tailing peak [10].

Derivatization of ciclopiroxolamine by means of reaction with methyl iodide

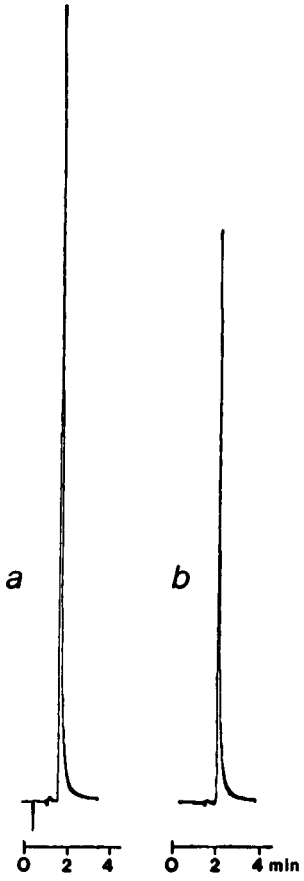

Fig. 2. Chromatograms of ciclopiroxolamine in (a) dermal foam and (b) in topical powder. Column, Fusica (15 cm × 330 μm I.D.; Delta Pak, RP-18, 5 μm, 300 Å); mobile phase, acetonitrile–water (1:1, v/v); flow-rate 10 μl/min; chart speed, 0.5/min; temperature, 20°C; wavelength, 300 nm.

and dimethyl sulphate was investigated. The methyl iodide reagent was selected due to its ability to react more rapidly.

Examples of chromatograms resulting from the LC assay of ciclopiroxolamine in foam and powder are shown in Fig. 2. With the chromatographic solvent system used, the Fusica column was found to give sharp, symmetrical peaks and good selectivity. No interference at the retention time of the analyte peaks due to other active or inactive substances present in the pharmaceutical forms was observed.

The overall precision of the retention time was studied with respect to run-to-run and day-to-day precision. The run-to-run precision for 30 runs within a single day averaged 1.21% relative standard deviation (R.S.D.), while a day-to-day precision over a 4-week period with the same column averaged slightly more, *ca.* 1.97% R.S.D.

The calibration graphs for the measurement of ciclopiroxolamine in foam and powder were constructed by analyzing spiked samples over drug concentration ranges 0–12 mg/g. The response was linear over the concentration range examined for both dosage forms. The linear least-squares regression relationships for ciclopiroxolamine in foam and powder were $y = 1.012\,x + 0.054$ and $y = 1.120\,x + 0.8753$, respectively.

The assay recoveries of ciclopiroxolamine from spiked foam and powder samples were 98.1% with 1.5% R.S.D. and 96.1% with 2.4% R.S.D., respectively. The detection limit of ciclopiroxolamine (signal-to-noise ratio 3) was 10 ng.

In conclusion, the proposed method offers high selectivity and sensitivity for the determination of ciclopiroxolamine in commercial dosage form.

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