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ORIGINAL ARTICLE

HPLC and TLC chromatographic methods for simultaneous determination of binary mixture of isoconazole nitrate and diflucortolone valerate

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Abstract HPLC and TLC-densitometric methods were used to determine a binary mixture of isoconazole (ISO) and diflucortolone (DIF). For HPLC method a rapid separation could be achieved on a C18 column using mobile phase of 80% acetonitrile–20% methanol. The components were monitored at 230 nm over a concentration range of 10–90 μg mL^{-1} for ISO and 2–18 μg mL^{-1} for DIF with mean percentage recoveries 99.95 ± 0.866 and 99.98 ± 0.744, respectively. The second method is TLC-densitometric, where ISO and DIF are separated on silica gel plates using ethyl acetate: chloroform: toluene (60:10:10 by volume) as a developing system and scanning of the separated bands at 215 nm over a concentration range of 0.1–4 μg $spot^{-1}$ for ISO and scanning of the separated bands at 237 nm over a concentration range of 0.1–1.4 μg $spot^{-1}$ for DIF with mean percentage recoveries 100.19 ± 0.956 and 100.1 ± 0.689 for ISO and DIF, respectively. The suggested methods were used to determine both drugs binary mixture in pure form and dosage form. The validity of the proposed methods was further assessed by applying standard addition technique. The obtained results were statistically compared with official HPLC method, showing no significant difference with respect to accuracy and precision.

1. Introduction

Isoconazole nitrate (ISO) (Fig. 1a), is a broad-spectrum imidazole derivative topical antifungal drug. It is chemically designated as 1-[(2RS)-2-[(2,6-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole. It is used for the treatment of Candida species and dermatophytes.[1] Different HPLC methods have been reported for the determination of isocnazole.[2–4] Diflucortolone valerate (Fig. 1b), 6α,9α-difluoro-3,20-dioxo-11β-hydroxy-16α-methylpregna-1,4-dien-21-yl valerate is an anti-inflammatory corticosteroid.[1] A HPLC method for determination of diflucortolone valerate is reported in literature.[5] Binary mixture of these drugs is used for the treatment of eczematous mycoses.[6] Only two methods were reported for the simultaneous determination of these components in

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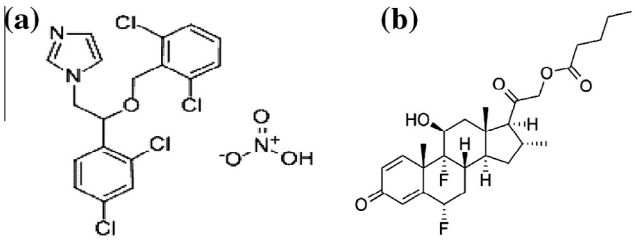

Figure 1 Structures of isoconazole nitrate (a) and diflucortolone valerate (b).

their pharmaceutical formulations as HPLC and spectrophotometry.[7,8]

Both HPLC methods utilized different mobile phases which consist of methanol–water (95:5 v/v) on Phenomenex ODS (250 × 4.6 mm; 5 μm) for HPLC and methanol–water (69:31, v/v) for UPLC on Acquity HSS C18 (50 × 2.1 mm; 1.8 μm).

2. Experimental

2.1. Instruments

1. HPLC instrument
   - Agilent 1100 series, equipped with a variable wavelength detector and 20 μL volume injection loop using Column of ODS C18-Agilant HC.C18 (2)-5 μm (4.6 × 250 mm I.D.), U.S.A.

2. TLC-densitometric instrument
   - Camag TLC scanner 3 S/N 130319 operated with winCATS software.
   - Linomat IV with 100 μL syringe (Camag, Muttenz, Switzerland).

   The following requirements are taken into consideration:
   i. Slit dimensions: 6 × 0.3 mm.
   ii. Scanning speed: 20 mm s^{-1}.
   iii. Spraying rate: 10 s μL^{-1}.
   iv. Data resolution: 100 μm $step^{-1}$.
   v. Band width: 5 mm.
   vi. Result output: Chromatogram and integrated peak area.

3. TLC plates precoated with silica gel 60 F254 (20 × 20), 0.2 mm thickness (E. Merck), Darmstadt, Germany.
4. UV short wavelength (254 nm) lamp. (Desaga, Germany).
5. Ultrasonic bath, Soniclean pty. Ltd 160T, HF-Frequency 50/60 Hz (10 L capacity), Australia.

2.2. Reagents and solvents

All reagents used throughout this work were of analytical pure grade, and solvents were of HPLC grade: methanol, acetonitrile, chloroform, ethyl acetate and toluene (HPLC grade) (Merck).

2.3. Samples

- Pure samples were kindly supplied by Misr Company for pharmaceutical industries. The percentage purity was found to be 99.56 ± 0.649% and 99.96 ± 0.767 for ISO and DIF, respectively according to the official method.[1]
- Travodermal cream, Batch No. 906023, manufactured by Misr Company for pharmaceutical industries and were purchased from a local market.

2.4. Standard stock and working solutions

A stock standard solution of ISO and DIF (1 mg/mL) were prepared by dissolving separately ISO and DIF in methanol then completing to 100 mL measuring flask with the same solvent. Aliquot of the prepared stock solution was further diluted with methanol to get working solution with final concentration (100 μg mL^{-1}).

2.5. Procedures

For HPLC method:

Chromatographic conditions:

Chromatographic separation was carried out using isocratic mode on a Agilant ODS-C18 (4.6 × 250 mm I.D.) column with a mobile phase consisting of 80% acetonitrile: 20% methanol. (The mobile phase was filtered using 0.45 m membrane filter and degassed by ultrasonic vibrations for 10 min) with a flow rate of 1 mL/min and the eluate was scanned at 230 nm at room temperature. All the injections were run in three replicates and the injection volume was 20 μL. The run time was 6 min and the total peak areas were used to quantify the studied components.

2.5.1. Construction of calibration graph for the determination of the binary mixture by HPLC method

Aliquots of ISO and DIF working standard solution (100 μg mL^{-1}) equivalent to 100–900 μg and 20–180 μg, respectively were accurately transferred into a two series of 10-mL volumetric flasks, the volume was completed to the mark with the mobile phase. Analyze the prepared samples using the previously mentioned chromatographic conditions and record the peak area then construct a calibration curve correlating the peak areas of ISO and DIF to the corresponding concentrations. Compute the corresponding regression equations.

2.5.2. Construction of calibration graph for the determination of ISO and DIF by TLC-densitometric method

Aliquots equivalent to 0.05–2 mg, 0.05–0.7 mg of ISO and DIF, respectively from stock standard solution (1 mg mL^{-1}) were transferred into 10-mL volumetric flasks and the volume was completed with methanol. 20 μL was applied to thin layer chromatographic plates (20 × 20) using applicator. Spots were spaced 2 cm apart from each other and 1.5 cm from the bottom edge of the plate. The plates were developed in the chromatographic tank previously saturated with the developing mobile phase, ethyl acetate: chloroform: toluene (60:10:10 by volume), for at least 20 min. The plates were developed by