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Short communication

Chromatographic approach to study β-cyclodextrin as a promoter of the penetration of bifonazole into keratinic tissue

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Abstract

A high-performance liquid chromatographic method for the determination of bifonazole in dimethyl sulfoxide solvent was developed to study its penetration into sheephoof. The analytical method was linear over the concentration range studied, i.e., from 0.1 mg/ml to 1 mg/ml. The relative standard deviation was less than 2%. The data obtained showed that complex forming with β-cyclodextrin greatly improved the penetration of bifonazole.

Keywords: Cyclodextrins; Bifonazole

1. Introduction

Bifonazole is used for the treatment of onychomycosis [1–3]. Its inclusion in the apolar cyclodextrin cavity [4–10] could improve its penetration into human nails or sheephoof. Capillary electrophoresis (CE) has already been used for the qualitative and quantitative determination of imidazole derivatives [6,11]. In this study the efficiency of penetration into sheephoof of bifonazole in its free form and its complex form with β-cyclodextrin (β-CD) was assessed by using a high-performance liquid chromatography (HPLC) method.

2. Experimental

2.1. Apparatus

The reversed-phase liquid chromatography (RPLC) system consisted of a Waters RPLC pump 501 (Saint Quentin, Yvelines, France), an Interchim Rheodyne injection valve, Model 7125 (Montluçon, France), fitted with a 20-μl sample loop, a Shimadzu SPD-10A (Touzart-Matignon, Vitry-Sur-Seine, France) and a variable-wavelength UV spectrophotometer detector (Nogent Sur Marne, France). A LiChrocart 125 mm×4 mm I.D. RP18 column (5 μm particle size) (Merck, Darmstadt, Germany) was used with a controlled temperature (in an Interchim oven, TM No. 701) for high temperatures and an Osi Julabo FT 200 cryoimmerser (Elancourt, France) for low temperatures. The mobile phase flow-rate was kept at 1 ml/min and the wavelength at 230 nm.

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2.2. Solvents and samples

RPLC-grade methanol (Carlo Erba, Val-de-Reuil, France) was used without further purification. The mobile phase for these studies was a methanol–phosphate buffer (85:15, v/v) adjusted to pH 7. The phosphate buffer was composed of 0.01 *M* diammonium hydrogenphosphate, 0.02 *M* ammonium dihydrogenphosphate and 0.005 *M* *n*-nonylamine to avoid peak tailing. The racemate form of bifonazole was purchased from Sigma (Saint Quentin, Fallavier, France). β-CD was a gift from the Roquette Labs. (Lestrem, France).

2.3. Bifonazole penetration study

A co-precipitation method was used to prepare the inclusion compound [6–10]. $3.6 \cdot 10^{-3}$ mmol of β-CD for the first complex (CI) or $14.4 \cdot 10^{-3}$ mmol for the second complex (CII) was dissolved in 1 ml of water for CI or 4 ml for CII and mixed at 65°C. 0.032 mmol of bifonazole was dissolved in 25 ml of acetone and added to the mixture. The proportions of bifonazole in CI and CII were, respectively, 50% and 20%. The specimen was heated and stirred for 2 h. The inclusion compound crystallization was obtained by cooling. The crystals were separated by filtration using 0.2 μm cellulose acetate filters, dried and stocked at ambient temperature. Three solutions were prepared using dimethylsulfoxide (DMSO) as solvent. In S1 S2 and S3, bifonazole was in its ''free'' form, and in its complex forms CI and CII, respectively. In each solution, the bifonazole concentration was the same, i.e., 1 mg/ml. A 200-mg amount of a calibrated sheephoof (substrate) strip was added to 10 ml of DMSO (blank sample) and to 10 ml of each of the three solutions S_i. The determination of the bifonazole concentrations in the different solutions was carried out on day *x* after the addition of the substrate and at the same time for each solution S_i, i.e., D_0, D_2, D_4, D_6, D_8, D_{14}, D_{21}, D_{24}, D_{28}.

3. Results and discussion

The chromatograms of blank sample, S1, S2 and S3 are shown in Fig. 1.

No chromatographic interference from the blank samples was found. Obviously, the bifonazole retention time ≈3.6 min was similar in the four chromatograms as β-CD and bifonazole in the complex form were in rapid equilibrium in the mobile phase [12]. The calibration curve was linear in the concentration range studied, i.e., from 0.1 mg/ml to 1 mg/ml. This equation was determined from five different concentrations (experiments determined twice) was as follows $y=1541x+123$ ($r^2=0.98$) where *y* is the peak area of bifonazole, *x* is the concentration of bifonazole in mg/ml and *r* is the correlation coefficient. The within day relative standard deviation (RSD) was less than 2% ($n=6$). This result indicates good precision for the assay. Table 1 gives the detailed intra-day precision and accuracy results. The concentration of bifonazole which re-

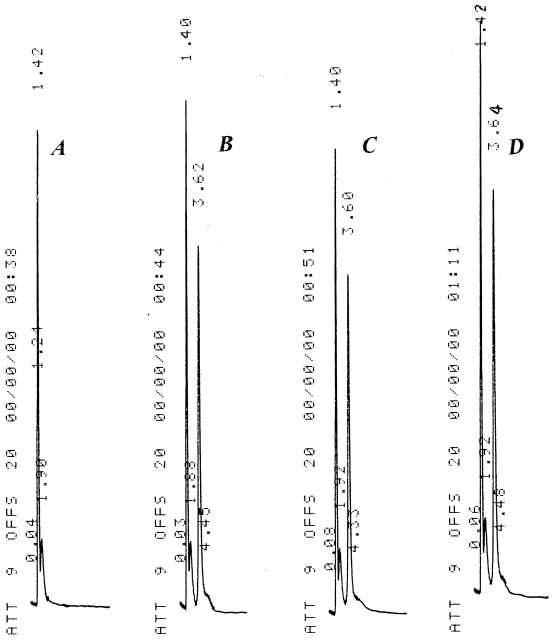

Fig. 1. Chromatograms of (A) blank sample (B) S1 (C) S2 (D) S3. The retention times are indicated in min.

Table 1
Precision and accuracy ($n=6$)

Spiked concentration (mg/ml)	RSD (%)	Accuracy (%)
0.3	3.2	99.4
0.5	2.7	101.2
0.7	1.9	98.9

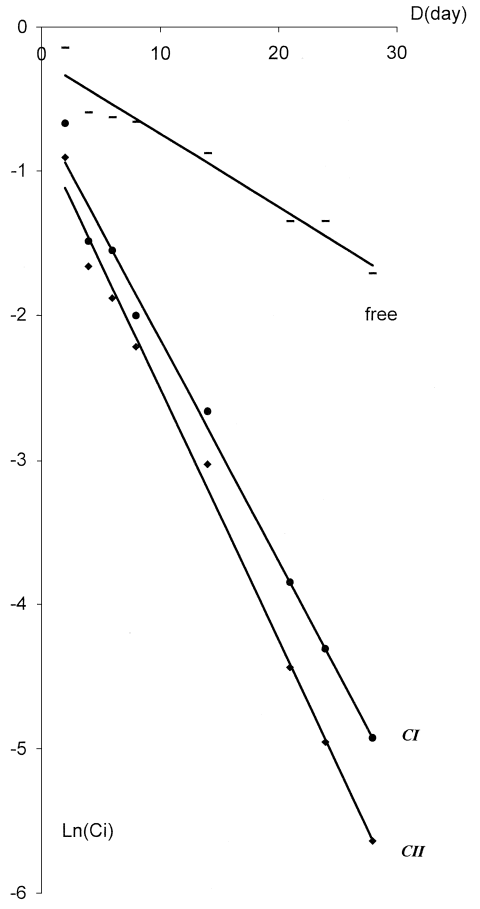

Fig. 2. Ln C_i versus D plots for ''free'' bifonazole and its complex forms CI and CII.

mained in solution after D days can be therefore determined. Using the calibration curve, the three plots of ln C_i vs. ln D calculated for each of the three solutions were drawn. The correlation coefficient for the linear fits was at least equal to 0.972 (Fig. 2). The typical standard deviations of slope and intercept were, respectively, 0.009 and 0.07. This linear behavior demonstrates that for each solution, the variations in C_i in the solution S_i with D can be related by the equation $C_i = A \exp(-kD)$ where A is a preexponentiel factor and k a constant. The half reaction time which corresponded to the number of days for which $C_i = A/2$ is given by $D_{1/2} = \ln 2/k$.

Table 2
Values of A and $D_{1/2}$ with standard deviation in parentheses obtained when bifonazole was in its free form and in its CI and CII complex forms

Bifonazole	A	D
Free	0.82 (0.07)	12.72 (0.10)
CI	0.53 (0.04)	4.53 (0.02)
CII	0.42 (0.05)	4.05 (0.04)

The values obtained for $D_{1/2}$ and A are summarized in Table 2. In summary, the data obtained showed that the complex greatly improved the penetration of bifonazole into sheephoof. This penetration can be objectivized by a great affinity of the hydrophilic complex form with hydrophilic keratins constituting the sheephoof [13]. The bioavailability of the complex forms with the free form must be similar according to previous works [14].

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