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Journal of Chromatography A, 871 (2000) 399–402

JOURNAL OF CHROMATOGRAPHY A

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Short communication

Capillary zone electrophoretic separation and determination of imidazolic antifungal drugs

Adela Arranz, Camilo Echevarría, Jose María Moreda, Adolfo Cid, Juan Francisco Arranz*

Departamento de Química Analítica, Facultad de Farmacia, Universidad del País Vasco/EHU, Apartado 450, E-01060 Vitoria, Spain

Abstract

Capillary zone electrophoresis (CZE) was adapted to the simultaneous determination of a mixture of three imidazolic antifungal drugs. Separation was achieved by using a fused-silica capillary column with an acetic acid–Tris buffer at pH 5.18 and UV detection at 196 nm. Several electrophoretic parameters were investigated: pH and buffer concentration, applied voltage, temperature and injection conditions. The optimized CZE method was applied to the individual determination of ketoconazole, clotrimazole and econazole in pharmaceutical forms, after a previous single extraction step in methanol, with recoveries of 98.00, 99.96 and 99.58% respectively. The antifungal drugs can be determined at a concentration level lower than $1.0 \cdot 10^{-7}$ *M*. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Pharmaceutical analysis; Ketoconazole; Clotrimazole; Econazole; Imidazolic compounds

1. Introduction

The incidence of mycoses continues to rise steadily. This increase is mainly due to the use of inmunosuppresant agents in cancer treatment and in prevention of rejecting in organ transplant recipients, to diseases that produce defects in the host's defence mechanisms and to the abusive use of broad spectrum antibiotics which eliminate or decrease the bacterial population which normally compete with fungi [1]. Therefore, the development of antifungal drugs has been important in the last years, in order to find agents which arrive at the infection focus, which are efficient and whose toxicity is very small. Among the agents used most, the imidazolic group is characterized by having a wide range of action. They work inhibiting the biosynthesis of ergosterole, main esterole of the fungi wall [2]. Different analytical techniques have been used for the determination of azolic antifungals, among them: TLC [3], GC [4], HPLC [5–7], UV–Vis spectrophotometry [8], NMR [9], potentiometry [9], amperometry [10] and polarography [11]. Capillary electrophoresis (CE) presents important advantages in comparison with these techniques, such as high-efficiency separations, short analysis times, small sample volumes and small amounts of reagents required, high versatility and complete automatization of the whole proceeding [12]. With regard to CE, we have only found one paper in which Zhang et al. [13] studied the electrophoretic behaviour of some hydrophobic or positively chargeable substances, including ketoconazole and itraconazole. They used ECZ, adding organic substances and decreasing the pH to values down the p*K* of the substances studied. They also considered the addition of cyclodextrins and surfactants (micellar electrokinetic capillary chromatography, MECC).

*Corresponding author. Fax: +43-945-130-756.
E-mail address: qaparvaj@vc.ehu.es (J.F. Arranz)

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PII: S0021-9673(99)01228-5

However, they carried out neither any quantitative studies nor its application to real samples. In this paper, a method for the simultaneous separation and determination of the three imidazolic antifungal drugs ketoconazole, clotrimazole and econazole has been carried out by means of CZE. The parameters which affect the electrophoretic separation of these drugs: buffer composition and concentration, pH, applied voltage, temperature and different ways for the sample introduction have been optimized. Quantitative aspects have also been studied and a method to determine the antifungals in pharmaceutical formulations has been proposed.

2. Experimental

2.1. Apparatus

A Beckman P/ACE 5510 CE system provided with a diode-array detection system and Beckman P/ACE software station 1.0 was used (Beckman Instruments, Fullerton, CA, USA). CZE was carried out in an uncoated fused-silica capillary tubing (40 cm effective length × 75 μm I.D.). The apparatus was thermostated and a temperature of 25°C was selected to carry out subsequent experiments.

2.2. Reagents and pharmaceuticals

Acetone, acetic acid glacial, (Tris), methanol, NaOH and KOH pellets were of analytical-grade and used as received; deionized water was used throughout. Econazole nitrate (M_r 444.70), clotrimazole (M_r 344.84) and ketoconazole (M_r 531.44) all of 99.9% purity were supplied by Vencaser (Bilbao, Spain). Ketoconazole was determined in pessaries Fungarest 400 (Janssen-Cilag), clotrimazole in vaginal tablets Gine-Canestén 100 (Bayer) and econazole nitrate in pessaries Etramón Ginecológico 50 (Janssen-Cilag). Stock solutions of each antifungal and the standard mixture of the three antifungals were prepared in methanol–water (40:60, v/v). All solutions were filtered by passing through a 0.45-μm nylon membrane filter (Tracer, Tecnocroma, Barcelona, Spain).

2.3. Procedures

Before the CE analysis, the capillary tubing was conditioned by flushing with deionized water for 2 min, then 0.1 *M* NaOH for 5 min, then with deionized water again for 2 min, and finally with running buffer for 10 min. Further, the capillary was equilibrated for 3 min with 20 kV, in order to avoid strange baseline behavior. Between runs, the following sequence was used: 0.5 min with deionized water, 1 min with 0.1 *M* NaOH, 0.5 min with deionized water and 6 min with running buffer. The running buffer was acetic acid–Tris buffer (75 m*M*) in methanol–water (20:80, v/v) (pH 5.18), with a voltage of 25 kV, average current of 50.2 μA and temperature of 25°C. Samples were injected by hydrodynamic injection for 5 s at 0.5 p.s.i. (1 p.s.i. = 6894.76 Pa). Electropherograms were recorded at 196 nm. The separation was carried out from the positive to the negative electrode. The experiments in the same set were performed at random, that is to say, the analyses of the same concentration were not performed consecutively. In order to extract the drug from the pharmaceutical forms, five pessaries or tablets were selected at random, crushed in an agate mortar and treated with methanol for 20 min in an ultrasonic bath. The excipient was separated by filtration. The solutions were made up to volume in a volumetric flask using methanol. An aliquot of this solution was diluted with methanol–water (40:60, v/v) and the analysis was performed following the analytical conditions described above.

3. Results and discussion

Several inorganic and organic buffers were tested in the search of an electrolyte solution that was optimum for the CZE separation of the three imidazolic drugs, and a 75 m*M* acetic acid–acetate buffer was chosen. Electroosmotic flow and electrophoretic mobility of imidazoles are affected by pH; therefore, the choice of pH is substantial to separate these antifungals, and the best overall separation for mixture components was obtained at pH 5.18. Due to the low solubility of these antifungals in water, the peak areas rose when the methanol concentration in the run buffer increased, changing its viscosity and

the migration times [14], and the best resolution was obtained for a methanol concentration of 20% (v/v). The voltage was increased between 15 and 30 kV, obtaining shorter analysis times at the highest applied voltages, while the peaks appeared narrower. A value of 25 kV was selected because high currents were obtained at voltages above this value. The smallest relative loss of absorbance for the three imidazolic compounds was obtained at a wavelength of 196 nm, and an optimum injection time of 5 s for the hydrodynamic injection of the sample was chosen, because the increase of peak areas was linear and worse peak resolutions were obtained at highest time values. A typical electropherogram, obtained under these optimum conditions, appears in Fig. 1.

The calibration lines were calculated using PROGRESS software [15], which performs a robust regression [16]. A linear range was observed for concentrations between 0.8 and $12.0 \cdot 10^{-6}$ *M* (Table 1) with determination coefficients (r^2) bigger than 0.996, for the three studied antifungal drugs. The detection limits (Table 1), obtained using the DETARCHI software [17], for ketoconazole, clotrimazole and econazole were: 0.63, 0.46 and $0.27 \cdot 10^{-6}$ *M* respectively. The variation coefficients were: 2.25, 2.88 and 1.86% for econazole, clotrimazole and ketoconazole respectively, at a concentration level of. $6.0 \cdot 10^{-6}$ *M* ($n=6$). The accuracy was 99.89, 100.05 and 100.06% for econazole, clotrimazole and ketoconazole respectively, at a concentration level of $6.0 \cdot 10^{-6}$ *M*.

A comparative study to extract the antifungal drugs from their respective pharmaceutical forms (Fungarest, Gine-Canestén 100 and Etramón 50) following the procedures described above was performed, using methanol and acetone as solvents. Recovery percentages are better when methanol is used: 98.50, 99.96 and 99.58%, for econazole, clotrimazole and ketoconazole; with variation coefficients of 2.04, 1.63 and 1.26, respectively. No interferences with excipients were observed. Although the limits of detection obtained by HPLC are lower than those obtained by the proposed method, CE has got some important advantages as short migration times and small reagent amounts required to perform the analysis.

Table 1
Parameters of the calibration line obtained for each antifungal using the optimum conditions[a]

Parameters	Econazole	Clotrimazole	Ketoconazole
L.R. (μ*M*)	0.8–12.0	0.8–12.0	0.8–12.0
b_1	817.83	826.90	1045.08
b_0	−78.76	413.13	397.50
r^2	0.9992	0.9983	0.9965
$s(b_1)$	7.12	9.77	19.48
$s(b_0)$	38.52	67.27	123.91
$s_{y/x}$	86.11	149.24	237.20
D.L. (μ*M*)	0.272	0.462	0.626
$\alpha=0.05$			
$\beta=0.05$			
n	16	16	16

[a] L.R.: linearity range, b_1: slope, b_0: intercept, r^2: determination coefficient, $s(b_1)$: standard deviation of slope, $s(b_0)$: standard deviation of intercept, $s_{y/x}$: error standard deviation, D.L., detection limit, α, false positive probability, β: false negative probability, *n*: experimental points number.

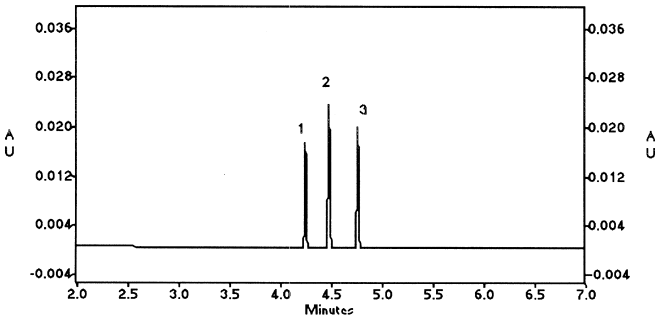

Fig. 1. Electropherogram obtained by CZE using the optimized method: 1=econazole, 2=clotrimazole and 3=ketoconazole. AU: absorbance units; $C=5.0 \cdot 10^{-6}$ *M* of antifungals.

Acknowledgements

The authors thank the University of Basque Country for financial support (UPV 171.123-EA101/97). We are also grateful to Angel González for linguistic revision of the text.

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