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Short communication

High-performance liquid chromatographic analysis for the determination of miconazole in human plasma using solid-phase extraction

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Abstract

A high-performance liquid chromatographic method for the determination of miconazole in human plasma is described. A solid-phase extraction was performed on an octadecyl (C_{18}) cartridge. Miconazole was eluted with methanol, separated on a reversed-phase column and was measured by ultraviolet detection at 230 nm. The absolute extraction recovery from plasma samples was 85%. The limit of detection was established as 5 ng/ml. The coefficient of variation of the determination of plasma levels by this method over the standard curve concentration range was less than 10%, except with the concentration of 10 ng/ml. The plasma levels of miconazole in twelve healthy volunteers given a 250-mg oral dose of two tablet forms were determined by this method.

Keywords: Miconazole

1. Introduction

Miconazole [1-(2,4-dichloro-β-((2,4-chlorobenzyl)oxy)phenethyl)imidazole] is a synthetic imidazole derivative with a broad-spectrum antifungal activity. It is established as a useful drug for the treatment of various systemic mycoses, including candidiasis, coccidioidomycosis, cryptococcosis and histoplasmosis [1,2]. It is also active against Gram-positive bacteria [1]. Several HPLC methods have been described for its determination in biological fluids [3–5], but none was sensitive enough for quantitation of this drug after oral administration. These methods used a liquid–liquid extraction procedure as the sample preparation step.

In this work, we describe a simple and sensitive HPLC method to measure miconazole in human plasma which renders it applicable in bioavailability studies. The method consists of a solid-phase extraction (SPE) procedure as the sample preparation step.

2. Experimental

2.1. Chemicals

Miconazole nitrate was supplied by the Pharmacy Department of the Pharmaceutical Research Institute (Warsaw, Poland). HPLC-grade acetonitrile and methanol were purchased from J.T. Baker (Phillip-

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sburg, NJ, USA). All other reagents were of analytical grade.

2.2. Chromatographic conditions

The HPLC system consisted of a Model LC-6A pump, a Model SPD-6AV UV–Vis detector operated at 230 nm, a Model CR-6A integrator and a Model SIL-9A autosampler (Shimadzu Europa, Duisburg, Germany). The analytical column (250×4 mm I.D., Nucleosil 120-5 C_{18}; Macherey-Nagel, Düren, Germany) was preceded by a 30×4 mm I.D. Nucleosil 120-5 C_{18} guard column. The column was heated at 40°C using a Model CTO-10A oven (Shimadzu Europa). The mobile phase consisted of methanol–acetonitrile–0.01 *M* phosphate buffer, pH 7.0 (36:36:28, v/v) and was delivered at a flow-rate of 2.1 ml/min. The solution was filtered (0.45 μm nylon membrane) and sonicated prior to use.

2.3. Sample preparation

Twelve Bakerbond SPE octadecyl C_{18} columns (3 ml, 200 mg) (J.T. Baker) were placed on the Baker SPE-12G vacuum extraction manifold and were prewashed with 2 ml of methanol followed by 5 ml of water and 2 ml of 0.2 *M* borate buffer, pH 10. Then, plasma samples (1 ml) each mixed with 1 ml of 0.2 *M* borate buffer, pH 10, were loaded on the columns and drawn through by means of the vacuum manifold. The flow-rate was kept constant (<1 ml/min). The solid phases were washed again with 2 ml of water, 2 ml of methanol–water (30:70, v/v), 1 ml of acetonitrile–0.2 *M* borate buffer pH 10 (70:30, v/v), 1 ml of acetonitrile and 1 ml of *n*-hexane, at a flow-rate of 5 ml/min. Then, the SPE columns were dried under vacuum for 10 min. Miconazole was slowly eluted by 1 ml of methanol (<1 ml/min). The eluent was evaporated to dryness under an air stream at 50°C. The residue was reconstituted in 100 μl of acetonitrile and an aliquot of this solution (60 μl) was injected onto the HPLC system for analysis. The experiments were carried out in duplicate.

2.4. Drug solutions

A stock solution was prepared by dissolving miconazole nitrate in methanol (1 mg/ml calculated as free base) and was stored at 4°C.

2.5. Calibration and recovery

Calibration was performed by adding known amounts of miconazole to blank human plasma to yield concentrations over a range of 10–1000 ng/ml. These standards were than extracted according to the procedure described in Section 2.3.

The absolute recovery of miconazole was calculated by comparing the peak area obtained from extracts of spiked plasma samples and the peak area obtained from direct injection of known amounts of standard solutions of miconazole.

The precision of analysis was assessed by six replicate analyses of human plasma spiked with miconazole to give concentrations of 50, 300 and 1000 ng/ml, and then the within-day variations were calculated. Between-day variations were also calculated for the above concentrations (Table 1).

2.6. Plasma samples

The method of analysis was applied to the plasma from twelve healthy non-smoking volunteers (one male and eleven females), aged 22–40 years (mean±S.D.=29.8±6.0) who participated in pharmacokinetic studies of miconazole. Their average weight and height (mean±S.D.) were 57.5±6.7 kg and 161.8±5.8 cm, respectively. The participants were confirmed to be in good health by physical

Table 1
Within- and between-day precision of miconazole in human plasma

Concentration added (ng/ml)	Concentration determined (mean±S.D.) (ng/ml)	C.V. (%)
Within-day[a] *(n=6)*		
50	43.395± 2.618	6.03
300	318.076± 3.407	1.07
1000	1000.462±24.965	2.50
Between-day[b] *(n=6)*		
50	42.138± 3.072	7.29
300	316.692± 3.827	1.21
1000	997.265±25.095	2.52

[a] Within-day assay variance was calculated from six different assay values obtained on a single day of analysis for 1-ml aliquots of spiked plasma standards.

[b] Between-day assay variance was calculated from the assay values obtained on six different days of analysis for 1-ml aliquots of spiked plasma standards.

examination and laboratory testing. The study was performed according of the ethical guidelines of the revised Declaration of Helsinki. All subjects gave written informed consent and the study protocol was approved by the Comittee on Medical Ethics of the Warsaw Medical Academy. The subject received Mikonazol tabl. (Pharmaceutical Research Institute, Warsaw, Poland) and Daktarin tabl. (Janssen, Copenhagen, Denmark), 125 mg orally in a 250-mg dose (two tablets) in a cross-over design study with one week wash-out period. Blood samples were taken from a forearm vein into heparinized tubes immediately before (time zero) and at 0.5, 1, 1.5, 2, 4, 6 and 8 h after drug administration. The plasma was separated by centrifugation and stored at −20°C until drug analysis.

2.7. Analysis of data

Plasma concentrations and time data were analysed by the one-compartment model according to the Pharm/PCS program [6]. The maximum plasma concentrations (C_{max}) and the time to peak (T_{max}) for miconazole were directly obtained from the experimental data. The area under the concentration versus time curves (AUC_{0-t}) was calculated using the linear trapezoidal rule. The apparent terminal rate constant k_{el} was computed by log–linear regression over the last data points of the concentration versus time curve. The value of k_{el} was then used to extrapolate the AUC_{0-t} values until infinity (total AUC). For statistical analysis, the t-pair Student test was used with $p \leq 0.05$ taken as the level of significance [7].

3. Results and discussion

The chromatogram of the plasma from drug-free volunteers (Fig. 1A) did not show any interfering compound extracted from the sample. A typical chromatogram of a drug-free human plasma sample spiked with miconazole (300 ng/ml) is shown in Fig. 1B. The chromatogram of the extract of plasma sample from a volunteer receiving 250 mg of oral Daktarin tabl. per day is shown in Fig. 1C. The retention time for miconazole is 7.5 min.

The standard curve for miconazole was linear over the range 10–1000 ng/ml. The standard curve was

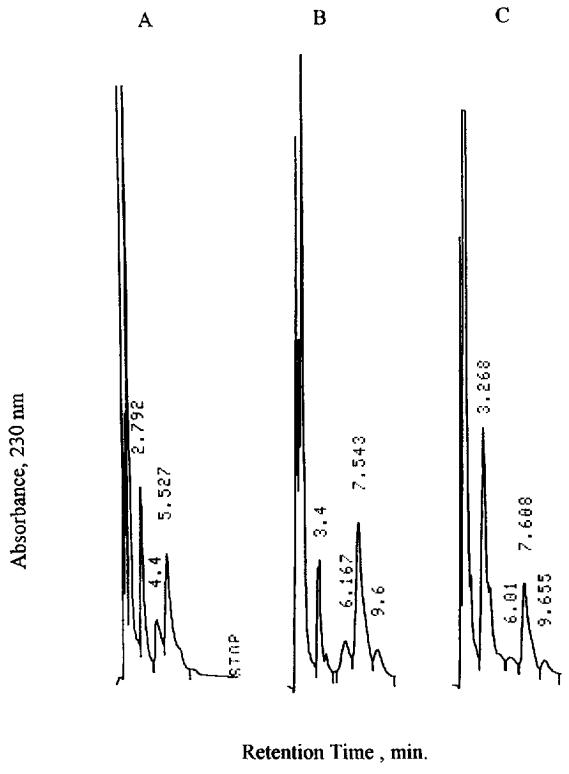

Fig. 1. Chromatograms of (A) blank human plasma, (B) spiked human plasma with 300 ng of miconazole and (C) a plasma sample collected 4 h after oral administration of 250 mg of Daktarin tabl. per day to the volunteer. The retention time for miconazole is 7.5 min. See Section 2.2 for chromatographic conditions.

fitted to a first-degree polynomial, $y=ax+b$, where y is the peak area, a and b are constants, and x is the miconazole concentration (ng/ml). Typical values for the regression parameters a (slope), b (y-intercept) and correlation coefficient were calculated to be 335.5126, 1925.7758 and 0.9995, respectively ($n=6$).

The minimum detectable concentration of miconazole was determined to be 5 ng/ml ($S/N=2$). The lowest quantifiable level was found to be 10 ng/ml and the C.V. of replicate determinations was 17.6% ($n=6$).

The within-day (intra) and between-day (inter) assay variances are given in Table 1. The within-day assay variations were determined by analyzing six 1-ml aliquots of spiked plasma samples containing 50, 300 and 1000 ng/ml of miconazole. The between-day assay variations were determined by

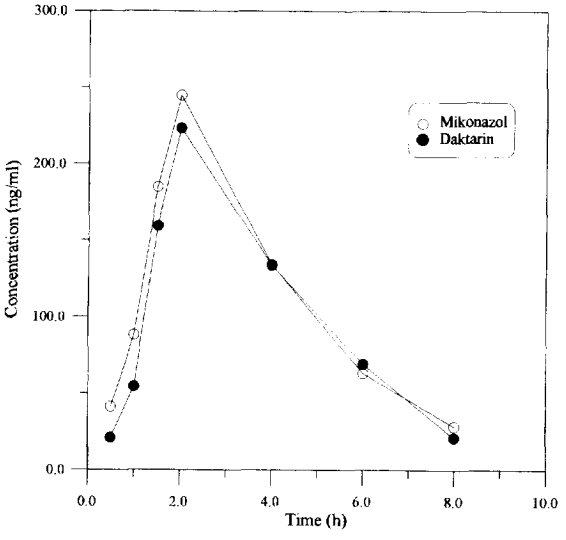

Fig. 2. Mean plasma levels of miconazole in twelve healthy volunteers following a single oral dose of 250 mg Mikonazol tabl. or Daktarin tabl.

analyzing 1-ml aliquots of spiked plasma samples containing 50, 300 and 1000 ng/ml of miconazole on six different days. In both cases, the coefficient of variation was <10% at all concentrations investigated. The absolute recovery of miconazole from the human plasma using this method was 85.82±4.83%.

The applicability of the assay procedure is illustrated in Fig. 2, which shows the average plasma concentration–time curves of miconazole after administration of Mikonazol tabl. and Daktarin tabl. to twelve volunteers. Table 2 summarizes some of the mean pharmacokinetic parameters and their standard errors. No significant differences were observed between these parameters.

Plasma level of miconazole after oral administration is very low and was estimated using a microbiological method only [1]. The problem involved in the determination of miconazole from plasma is the fact that clear separation of miconazole from plasma components is essential for optimal quantitation. Other HPLC methods used liquid–liquid extraction or acetonitrile deproteinization as the sample preparation steps [2–4]. Those methods have a sensitivity of 0.1–1 μg/ml of plasma, which is not enough to measure plasma level of miconazole after oral administration. This low sensitivity resulted from detection at 254 nm. At 254 nm, contributions to the chromatogram from plasma contaminants were minimized but the absorbance of miconazole was lower. We monitored the column eluent at 230 nm, the peak absorbance wavelength of miconazole. The column was maintained at 40°C to minimize band broadening. A one-compartment model was fitted to the observed plasma concentrations, and differed from a three-compartment open model for plasma concentrations obtained after intravenous infusion of miconazole (522 mg) over 15 min [8]. In our study, the pharmacokinetic parameters k_{el} and $t_{1/2}$ (Table 2) are similar to k_{12} and $t_{\alpha 1/2}$ calculated for a fast compartment by Lewi et al. [8] (0.390 h^{-1} and 2.080 h, respectively). Unfortunately, our method still has too little sensitivity to measure the plasma level of miconazole for longer than 8 h after oral administration and we could calculate pharmacokinetic parameters for fast compartment only.

In summary, the described method for the determination of miconazole in human plasma is sensitive and reproducible. This method would allow pharmacokinetic studies for miconazole after oral administration to be conducted. The bioequivalence of Mikonazol tabl. 125 mg was found to be not

Table 2
Pharmacokinetic parameters of miconazole in twelve healthy volunteers after a single 250-mg oral dose of Mikonazol tabl. and Daktarin tabl.

Pharmacokinetic parameter	Mikonazol (mean±S.D., $n=12$)	Daktarin (mean±S.D., $n=12$)
k_{el} (h^{-1})	0.397±0.105	0.438±0.098
k_a (h^{-1})	0.872±0.294	0.721±0.126
$t_{1/2}$ (h)	1.811±0.404	1.647±0.325
T_{max} (h)	1.739±0.196	1.807±0.274
C_{max} (ng ml^{-1})	205.985±125.567	187.233±119.180
$AUC_{0-\infty}$ (ng h ml^{-1})	967.574±606.247	837.457±457.709

significantly different from that of Daktarin tabl. 125 mg.

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