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Note

High-performance liquid chromatographic determination of the antimycotic agent, econazole in plasma

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Econazole, 1-[β-(*p*-chlorobenzyloxy)-β-(2,4-dichlorophenyl)ethyl]-imidazole (Fig. 1), is a promising new broad spectrum antimycotic agent which is also active against gram-positive bacteria[1–4].

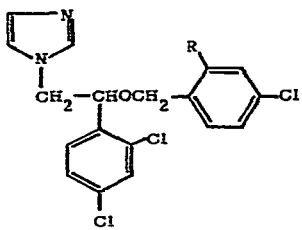

Fig. 1. Chemical structure of econazole (R = H) and the internal standard, miconazole (R = Cl).

During metabolism and pharmacokinetic studies of this drug, it was necessary to develop a rapid, sensitive and specific method for its measurement in biological fluids. High-performance liquid chromatography (HPLC) has become established as a useful technique for the analysis of drugs in biological fluids and was therefore adopted for the measurement of econazole in plasma using the structurally related imidazole miconazole[5] (Fig. 1) as the internal standard. Thus the method could equally be used for analysis of miconazole in biological fluids.

Chromatography was performed in a reversed-phase mode using a C_{18} hydrocarbon stationary phase. The method is simple, rapid and sensitive and has been applied to the measurement of econazole in the plasma of two human subjects, who received single oral doses of 500 mg of drug.

EXPERIMENTAL

Materials

Reagents were of analytical grade and inorganic reagents were prepared in freshly glass-distilled water. Diethyl ether was freshly redistilled prior to use. Standard solutions of econazole free base (1-[β-(*p*-chlorobenzyloxy)-β-(2,4-dichlorophenyl)-

ethyl]-imidazole and miconazole nitrate (the internal standard, 1-[β-(2,4-dichlorobenzyloxy)-β-(2,4-dichlorophenyl)ethyl]-imidazole nitrate) were prepared in methanol at a concentration of 0.1 mg/ml and stored at 4°. Samples of econazole free base and miconazole nitrate were supplied by Cilag-Chemie, Schaffhausen, Switzerland.

Extraction procedure

Plasma samples (0.5 ml) were transferred into conical centrifuge tubes, spiked with internal standard (10 μl, 1 μg) and made alkaline by the addition of potassium hydroxide solution (1 *M*, 100 μl). This mixture was extracted by shaking it for 1 min with diethyl ether (5 ml). After centrifugation for 10 min, the ether layer was transferred to a 10-ml conical centrifuge tube and evaporated to dryness under a stream of nitrogen at 37°. The residue was dissolved in methanol (25 μl) and an aliquot (10–25 μl) was injected into the chromatograph.

Calibration procedure

Samples of control plasma (0.5 ml) were spiked with econazole at concentrations of 0.2, 0.5, 1.0, 3.0 and 5.0 μg/ml and with internal standard at a fixed concentration of 2 μg/ml. The samples were taken through the extraction procedure described previously.

HPLC conditions

The chromatograph consisted of an M6000A pump (Waters Assoc., Cheshire, Great Britain) fitted to an LC55 variable-wavelength spectrophotometer (Perkin-Elmer, Beaconsfield, Great Britain) operated at 220 nm and at a sensitivity of 0.04 a.u.f.s. Injection was performed by syringe via a U6K universal injector (Waters Assoc.). The column was constructed of stainless steel (25 × 0.46 cm I.D.) and prepacked with Partisil 10 ODS (Whatman, Maidstone, Great Britain).

Chromatography was performed in a reversed-phase mode using a mobile phase of methanol–aqueous potassium dihydrogen phosphate (0.01 *M*, 70:30, v/v), adjusted to pH 4.5 with phosphoric acid, at a flow-rate of 2 ml/min. Fig. 2 illustrates the separation of econazole from miconazole (internal standard) which had retention times of 6 and 8 min, respectively.

Plasma samples

The method of analysis was applied to the plasma from two male volunteer subjects who were participating in pharmacokinetic studies of econazole[6].

RESULTS AND DISCUSSION

Concentrations of econazole were calculated from calibration curves constructed by plotting the peak height ratios of drug to internal standard over the concentration range 0.2 to 5.0 μg/ml econazole extracted from plasma (Fig. 3). The recovery of internal standard from plasma was 77% ± 1.7 (S.D., $n = 6$). The overall recovery of econazole from plasma (84% ± 9.2, S.D., $n = 25$) over the concentration range 0.2 to 5.0 μg/ml was calculated by comparing peak-height ratio measurements of standards to those of extracted standards corrected for recovery of internal standard (Table I).

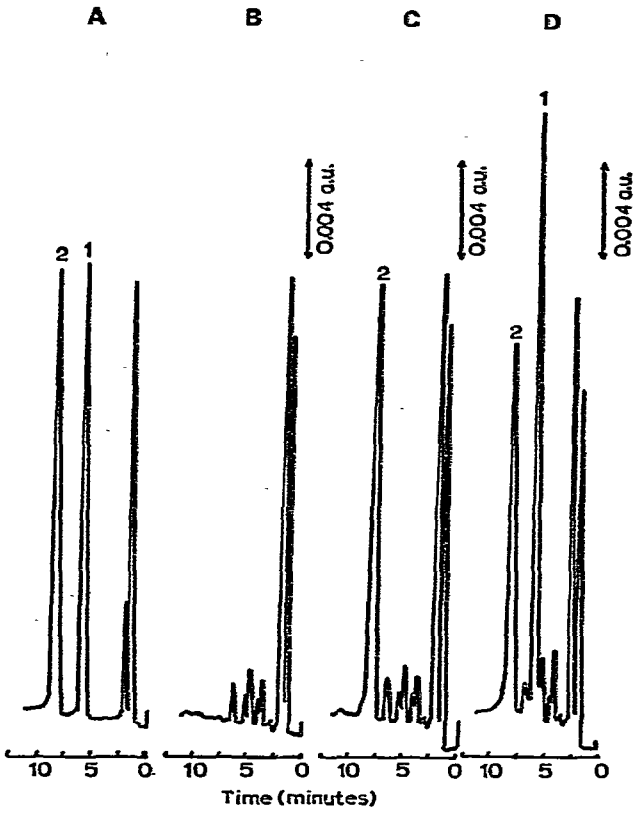

Fig. 2. A, Chromatogram of authentic standards (1) econazole and (2) miconazole (internal standard). B, Chromatogram of predose "control" plasma extract. C, Chromatogram of predose "control" plasma extract containing internal standard. D, Chromatogram of a plasma extract containing 1.6 μg/ml econazole. Column: Partisil 10 ODS (25 × 0.46 cm I.D.). Mobile phase: methanol–aqueous KH_2PO_4 (0.01 *M*, 70:30, v/v) pH 4.5. Flow-rate: 2 ml/min. Detector: UV, 220 nm.

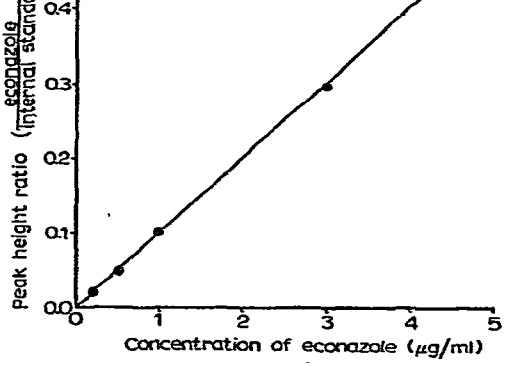

Fig. 3. Calibration curve of econazole extracted from plasma; each point represents a mean of five determinations.

TABLE I

PRECISION OF METHOD AND RECOVERIES OF ECONAZOLE FROM PLASMA

Concentration of econazole added to plasma (μg/ml)	*Recovery (%)*	*Coefficient of variation (%) (n = 5)*
0.2	99	12.5
0.5	82	3.1
1.0	83	1.9
3.0	78	4.9
5.0	81	1.8

The calibration curve was constructed from five replicate measurements at five concentrations over the range, and the plot of peak-height ratios against concentration was linear ($y = 0.0196 + 0.9988x$, correlation coefficient, $r = 0.9991$) and the value of the intercept was not significantly different from zero. The 95% confidence limits of the least-squares regression line forced through the origin were ±84.1% at 0.2 μg/ml, ±8.8% at 2.0 μg/ml and ±3.6% at 5.0 μg/ml. The precision of the method for the measurement of econazole in plasma ranged from ±12.5% at 0.2 μg/ml to ±3.1% at 0.5 μg/ml to ±1.8% at 5.0 μg/ml (Table I).

Chromatograms of predose "control" plasma samples from each subject showed a small interfering peak with the same retention time as econazole equivalent to 0.08 μg/ml (Fig. 1). This "blank" value was subtracted from measured concentrations in the postdose plasma samples. The limit of detection of econazole based on instrumental conditions (signal-to-noise ratio, 2:1) was approximately 0.04 μg/ml.

When applied to the collected samples, the method showed that peak concentrations of econazole occurred between 1.5 and 3 h after dosing (3.64 μg/ml and

TABLE II

CONCENTRATIONS OF UNCHANGED DRUG IN THE PLASMA OF HUMAN SUBJECTS AFTER SINGLE ORAL DOSES OF 500 mg ECONAZOLE

Time after dosing (h)	*Concentrations of econazole (μg/ml)*		
	Subject No. 1	*Subject No. 2*	*Mean*
0.5	0.04	0.14	0.09
1	0.78	1.18	0.98
1.5	1.98	2.72	2.35
2	3.44	2.52	2.98
3	3.64	1.60	2.62
4	2.86	0.80	1.83
6	1.20	0.30	0.75
8	0.54	0.18	0.36
10	0.30	0.08	0.19
12	0.20	0.10	0.15
16	0.12	0.04	0.08
24	0.06	<0.04	<0.04
36	<0.04	<0.04	<0.04
48	<0.04	<0.04	<0.04
72	<0.04	<0.04	<0.04
96	<0.04	<0.04	<0.04
120	<0.04	<0.04	<0.04

2.72 μg/ml in subjects 1 and 2, respectively) and were at or near the limit of detection (0.04 μg/ml) at 24 h after dosing (Table II). The descending portion of the concentration–time curves appeared to be composed of at least two linear sections, presumably associated with distribution and elimination phases respectively. The half-life of the terminal linear section was approximately 4 to 7 h (Fig. 4).

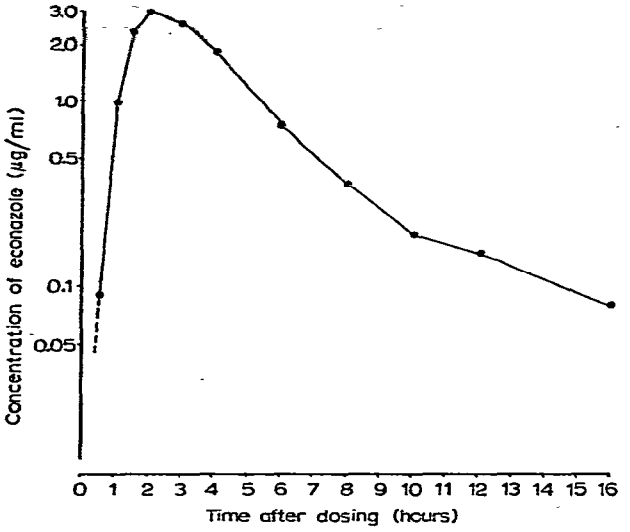

Fig. 4. Mean plasma concentrations of econazole during 16 h after an oral dose of 500 mg of drug. Semi-logarithmic scale.

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