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Note

Sensitive and automated gas chromatographic method for the determination of miconazol in plasma samples

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Miconazol (Daktar®, Janssen, Neuss, F.R.G.) has a broad spectrum of antifugal activity and has been proved in clinical studies to be an effective drug [1,2]. The drug is administered locally or intravenously in cases of local or systemic mycotic infections. To evaluate the plasma levels of the unaltered miconazol, a gas chromatographic (GC) assay [3] has been used most frequently. This allowed a detection limit of 1 ng/ml in plasma. Recently, clinical trials have demonstrated that miconazol is a safe and effective therapy for vaginal candidiasis in gravid women [4]. In this case the administered cream or ovule should have a local effect and the absorption of the drug must be negligible to minimize the risk for mother and foetus. Therefore, a sensitive method for the determination of very low plasma levels is needed.

We report a method that is faster and more sensitive than those previously described [3,5–7]. The method allows automated injection of series of plasma samples and determination of miconazol concentrations in plasma down to 0.25 ng/ml.

EXPERIMENTAL

Chemicals

Analytical-grade miconazol, 1-[2,4-dichloro-β-(2,4-dichlorobenzyloxy)-phenethyl]imidazole, and its internal standard (I.S.), 1-[2,4-dichloro-β-(2,3,4-trichlorobenzyloxy)phenethyl]imidazole, were obtained from Janssen Pharmaceutica (Beerse, Belgium). The chemical structures of these compounds are shown in Fig. 1. Chemicals and organic solvents were of the best grade commercially available.

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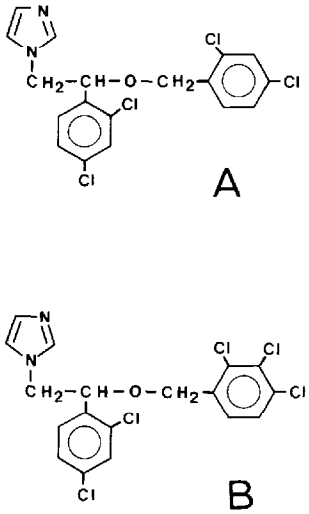

Fig. 1. Chemical structures of miconazol (A) and the internal standard (B).

Extraction procedure

The extraction was carried out as described earlier [8]. In a 15-ml glass tube 50 ng of I.S. were added to 1 ml of plasma. The tube was rapidly shaken for a few seconds. After pipetting 2 ml of 0.1 *M* sodium hydroxide into the tubes, the aqueous layer was extracted twice with 4 ml of an *n*-heptane–isoamylalcohol (98.5:1/5, v/v) mixture for 10 min and centrifuged (1900 *g*, 10 min). The water phases were discarded and the organic layers were transferred to a second centrifuge tube containing 2 ml of 0.05 *M* sulfuric acid. After the extraction the organic phase was removed and discarded. The remaining acidic phase was turned basic with 100 μl of concentrated ammonia (pH 10) and re-extracted twice with 2 ml of the heptane–isoamylalcohol mixture. The combined organic phases were dried under a stream of nitrogen at 50 °C. The residue was dissolved in 50 μl isopropanol from which sample volumes of 5 μl were taken and injected into the gas chromatograph.

Calibration and calculation procedures

Using the miconazol standard solutions, separate series of blank (control) plasma samples (1 ml) were spiked with concentrations ranging from 0.25 to 250 ng/ml. Additionally 50 ng of internal standard were added to each sample. These calibration samples were extracted as described above.

Unknown concentrations were calculated by determining the peak-area ratios of miconazol relative to the internal standard, and comparing these ratios with the standard curve obtained from the analysis of the calibration samples.

Chromatographic conditions

The instrument used was a Hewlett-Packard Model 5890A gas chromatograph equipped with a nitrogen–phosphorus detector. A fused-silica capillary column (12 m × 0.2 mm I.D.) coated with 0.1-μm OV-1 was used (C & S Chromatographie

Service, Eschweiler, F.R.G.). The injected sample was splitted in a ratio of 1:10. The make-up gas flow-rate was 30 ml/min. Injector and detector temperatures were maintained at 300 °C. The separation took place isothermally at 265 °C. The GC instrument was equipped with a Model 7673A automatic sampler which was programmed to wash the syringe properly to prevent carry-over. A Spectra-Physics Model 4270 integrator linked to a LABNET data system was used (Spectra-Physics, Darmstadt, F.R.G.).

The retention times were 3.6 min for miconazol and 5.7 min for the internal standard (relative retention time, 0.61).

RESULTS AND DISCUSSION

The overall recovery of the extraction procedure for miconazol and the internal standard from 1 ml spiked control plasma was 85%. Representative chromatograms (Fig. 2) of extracts from blank plasma, blank plasma spiked with 0.25 ng/ml miconazol and patient plasma show that the criteria of sensitivity and

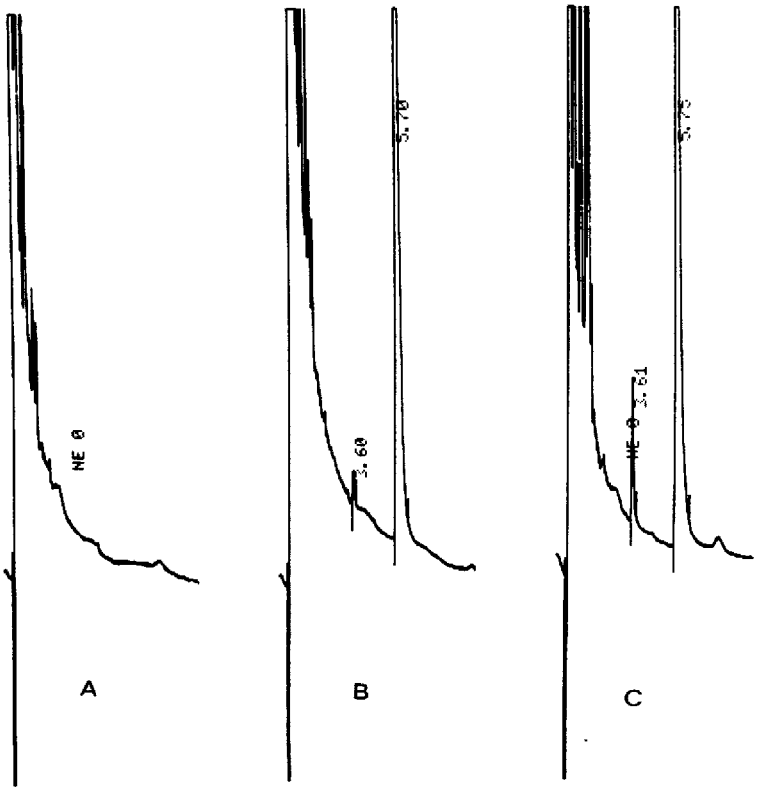

Fig. 2. Chromatograms of extracts from (A) blank (control) plasma (1 ml), (B) plasma spiked with 0.25 ng/ml miconazol and (C) plasma from a patient, 24 h after vaginal administration of 1200 mg of miconazol (plasma level: 1.2 ng/ml).

TABLE I

PRECISION AND ACCURACY FOR THE DETERMINATION OF MICONAZOL IN PLASMA

Concentration added (ng/ml)	Observed concentration (mean ± S.D.) (ng/ml)	n	Coefficient of variation (%)	Accuracy (%)
0.25	0.25 ± 0.03	7	12.0	100.0
0.50	0.49 ± 0.06	8	12.2	98.0
1.00	0.99 ± 0.10	11	10.1	99.0
2.50	2.61 ± 0.58	4	22.2	104.4
5.00	5.36 ± 0.42	8	7.8	107.2
10.00	10.00 ± 0.24	7	2.4	100.0
25.00	23.90 ± 0.78	6	3.3	95.6
50.00	51.50 ± 3.13	6	6.0	103.0
100.00	100.50 ± 4.30	7	4.2	100.5
250.00	242.10 ± 6.43	6	2.6	96.8

purity were met. The patient plasma was taken 24 h after the administration of 1200 mg miconazol ovula.

The method was validated by using spiked plasma. Good linearity and a negligible intercept were found routinely. Least-squares regression analysis yielded the regression equation $y = 1.039x - 3.667$ ($r^2 = 0.9998$). The accuracy and precision of the GC method are presented in Table I. Despite the concommitant medication of the patients with metroprolol, diazepam and fenoterol we found no interfering peaks.

The analytical method described here provides suitable sensitivity and specificity and is applicable for the determination of miconazol at very low levels in plasma. This method may also be used in other clinical pharmacokinetic trials. Additionally, the favourable injection and isothermal operating conditions allow the use of an autosampler.

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