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Note

Determination of miconazole in human saliva using high-performance liquid chromatography

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Miconazole nitrate [1-(2,4-dichloro-β-((2,4-dichlorobenzyl)oxy)phenethyl)-imidazole nitrate] is a broad-spectrum antifungal drug. It is established as a useful drug for the topical treatment of superficial fungal infections, and it has been used in the treatment of certain systemic fungal infections [1]. Several methods have been described for its determination in serum. These methods have been based on microbiological [2,3] or gas chromatographic [4] procedures and are not suitable for the rapid investigation of large numbers of samples. A sensitive and specific high-performance liquid chromatographic (HPLC) method has been described for the related imidazole antifungal drug econazole [5] using miconazole as the internal standard. This method did not, however, give good results with our chromatograph.

The method described in this paper was devised to permit the investigation of the persistence of miconazole in the mouth after dosing with an oral gel formulation of the drug. Chromatography was performed on de-proteinated saliva samples using a C_8 reversed-phase radial compression column and UV detection.

The method is simple, rapid and sensitive and will measure concentrations of miconazole down to 0.5 mg/l.

MATERIALS AND METHODS

Apparatus

The chromatograph consisted of a Waters Model M6000A pump, a Model U6K injector, a Bondapak C_{18} Corasil guard column, an RCM 100 radial compression module containing a Radial-Pak C8 analytical column (5 mm I.D.), and a Model M450 variable-wavelength detector operating at 230 nm (Waters Assoc., Hartford, Great Britain). A Model M730 data module plotter/integrator (Waters Assoc.) was used to record the chromatograms.

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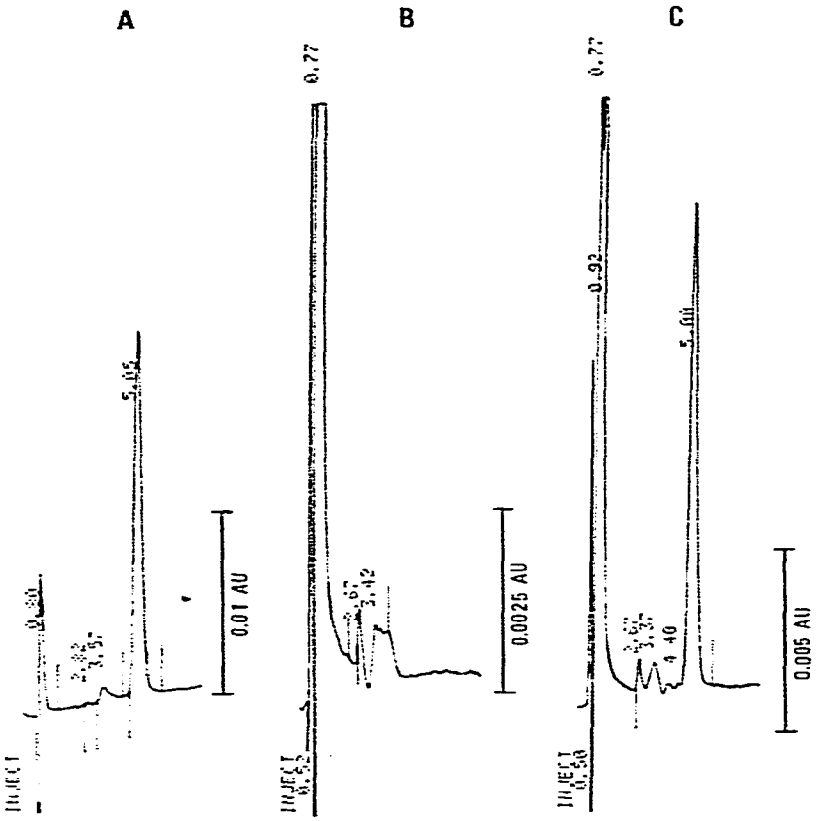

Fig. 1. (A) Chromatogram of miconazole (retention time 5.05 min) standard in water. (B) Chromatogram of predose saliva from subject No. 4. (C) Chromatogram of saliva from subject No. 4, 15 min after dosing with 10 ml miconazole oral gel.

TABLE I

CONCENTRATIONS OF MICONAZOLE IN SALIVA OF HUMAN SUBJECTS AFTER SINGLE DOSES OF 5 OR 10 ml MICONAZOLE ORAL GEL

Time after dosing (min)	Miconazole concentration (mg/l)							
	Subject 1		Subject 2		Subject 3		Subject 4	
	5-ml dose	10-ml dose	5-ml dose	10-ml dose	5-ml dose	10-ml dose	5-ml dose	10-ml dose
0	0	0	0	0	0	0	0	0
15	9.2	45.8	3.5	5.3	12.5	45.8	40.0	51.6
30	1.8	4.7	5.0	1.6	2.3	10.0	5.3	13.9
45	0.7	3.1	2.8	0.9	2.0	6.7	7.6	8.1
60	0.4	2.8	<0.5	0.9	0.8	4.8	1.2	0.8
90	0.6	2.5	<0.5	0.7	1.9	2.3	1.2	5.3
120	<0.5	1.4	<0.5	0.5	1.0	2.6	<0.5	1.2
150	0.7	1.6	<0.5	<0.5	1.5	2.9	1.8	10.9
180	<0.5	1.3	<0.5	0.6	<0.5	1.4	0.6	0.7

Reagents and solvents

Miconazole nitrate was obtained from Janssen Pharmaceutica (Beerse, Belgium). A stock solution was prepared in distilled water (acidified to pH 3 with concentrated phosphoric acid) at a concentration of 1000 mg/l. All chemicals and reagents except *n*-nonylamine were analytical grade.

Chromatographic eluent

The mobile phase was composed of 77% methanol in 0.01 *M* EDTA with 0.005 *M* *n*-nonylamine and was used at a flow-rate of 1.5 ml/min. The eluent was filtered and degassed under reduced pressure before use.

Sample preparation

Saliva samples (150 μl) were mixed with an equal volume of acetonitrile, allowed to stand for 5 min and then centrifuged at 1000 *g* for 2 min. The supernatant was collected and 20 μl injected into the liquid chromatograph. Most saliva samples were analysed on the same day they were prepared. However, no deterioration was found in deproteinated samples stored at −20°C for up to seven days.

Quantitation

Standards were prepared by spiking normal human saliva with small volumes of a concentrated stock solution of miconazole. A standard curve was produced by plotting peak area counts against miconazole concentration.

Saliva samples

The method of analysis was applied to samples obtained from volunteers who were participating in studies of the persistence of miconazole in the mouth. Samples were collected at intervals for 3 h after a 5- or 10-ml dose of miconazole oral gel. A predose sample was also collected from each volunteer on each occasion.

RESULTS AND DISCUSSION

The mean recovery of miconazole from saliva samples was determined to be 101% (S.D. 6.93, n=5), over the range of 10—100 mg/l. This was calculated by comparing peak area counts of standards prepared in saliva to those of standards prepared in acidified water. The calibration curve was linear over the concentration range 0.5—100 mg/l (y=0.1476+0.000017x, correlation coefficient, r=0.9831) with the intercept close to zero.

Fig. 1 shows some representative chromatograms. None of the control or predose samples of saliva showed interfering peaks at the retention time of miconazole.

The results of the assays on the saliva samples from some of the volunteers are shown in Table I. There are considerable variations in the peak concentrations of miconazole detected 15 min after dosing and in the increase in peak concentration obtained in response to doubling the dose. Most subjects had reached the limit of detection (0.5 mg/l) of miconazole at 3 h after dosing.

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