Journal of Chromatography, 434 (1988) 259-264
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4462

Note

High-performance liquid chromatographic method for the determination of miconazole in vaginal fluid

KRZYSZTOF SELINGER*, DIAMANDO MATHEOU and HOWARD M. HILL

Bio-Research Laboratories Ltd., 87 Senneville Road, Senneville, Quebec H9X 3R3 (Canada)

(First received June 13th, 1988; revised manuscript received August 26th, 1988)

Miconazole nitrate is an imidazole antifungal agent and antibacterial drug. It has been in widespread use for the topical treatment of candidal infections for ca. fifteen years. As a well established and useful drug it has generated significant analytical interest. Several methods have been described for its determination in plasma or serum [1,2], saliva [3] and vaginal fluids [4]. These assays employed the usual arsenal of analytical methods, i.e., gas chromatography (GC) [2,5–7], high-performance liquid chromatography (HPLC) [2,3] and microbiological procedures [4,8,9].

While all of these methods proved to be useful for certain studies, some of them showed deficiencies usually associated with specific procedures, e.g., laboriousness of sample preparation in GC and lack of specificity in microbiological assays as metabolites could show antifungal activity.

The goal of this study was to develop a simple HPLC method suitable to quantitate miconazole in vaginal fluids.

EXPERIMENTAL

Apparatus

Chromatography was performed on a system consisting of a Model 6000 solvent delivery module, Model 481 UV detector, and WISP 710B autosampler, all from Waters Assoc. (Milford, MA, U.S.A.). The column was 5 cm × 0.46 cm I.D., in-house packed with Spherisorb C_8, 5 μm particle size, obtained from Phase Separations (Norwalk, CT, U.S.A.). Data were collected on a Hewlett-Packard 3357 laboratory automation system.

0378-4347/88/$03.50 © 1988 Elsevier Science Publishers B.V.

Materials

Miconazole nitrate was obtained from Ortho Pharmaceuticals (Canada) (Don Mills, Canada) and hydrocortisone 21-caprylate (the internal standard) and octanesulfonic acid from Sigma (St. Louis, MO, U.S.A.). All the other reagents and solvents were of HPLC grade and used without further purification.

Chromatographic conditions

Mobile phase was prepared by adding 600 ml of acetonitrile to a 1-l volumetric flask and 10 ml of 1 *M* sodium phosphate adjusted to pH 2.5 with 85% phosphoric acid. The flask was then brought to volume with water. After stirring, the mobile phase was filtered through 0.45-μm Nylon filter. The flow-rate was 1.5 ml/min, which resulted in a back-pressure of ca. 1.4 MPa. The column was kept at room temperature. The detector was set at 205 nm, 0.02 a.u.f.s. The volume of injection was 10 μl. Retention times were 2.2 and 6.5 min for the internal standard and miconazole, respectively. The total run time was 11 min.

Standard and quality control (QC) preparation

The standards and QCs were prepared by way of spiking 0.01 *M* phosphate buffer pH 2.5, the same as used for mobile phase but diluted 100-fold, with standard solutions of miconazole nitrate. QC samples were prepared from separately weighed stock solutions. The calibration curve ranged from 0.2 to 200.0 μg/ml. The standards and QCs were aliquoted (200 μl per tube) immediately after spiking and stored at -15°C until needed.

Sample collection

Full details of the procedure will be given in a separate publication [10]. Briefly, six healthy female volunteers received once daily, for three days, intravaginal 400-mg doses of miconazole nitrate. Vaginal secretions were sampled 12 h prior to initial drug administrations and at the following times after the final dose: 12, 24, 36, 48 and 72 h. The samples were collected from three sampling sites: the introitus (A), the external cervical os (B) and the posterior fornix (C). After collection, each microsponge was placed in the barrel of a 5-ml syringe. The combined weight of each sponge and syringe was recorded before and after the sponge was used. The tip of the syringe was sealed, 3.0 ml of 0.01 *M* sodium phosphate buffer pH 2.5 were added to the syringe, the plunger was placed in the barrel and the liquid was vortex-mixed for 30 s. After vortex-mixing, contents of the syringe were expelled into a polypropylene tube and stored at -15°C until analyzed.

Extraction procedure

To an aliquot (200 μl) of standard, QC or clinical sample, 0.01 *M* sodium octanesulfonic acid (200 μl) was added, as well as 100 μl of hydrocortisone 21-caprylate (100.0 μg/ml) as internal standard. Water (100 μl) was added to the blanks. After vortex-mixing, samples were extracted with 5 ml of chloroform by shaking on a roto-rack for 10 min at high speed and then centrifuged at 700 *g* for a further 10 min. The upper layer was aspirated off and the lower organic phase transferred into a clean tube where the solvent was evaporated under a gentle

stream of dry nitrogen at 35 °C. The residue was dissolved in 300 μl of mobile phase with the exception of the two most concentrated standards and QCs, in which case 1.0 ml of mobile phase was used.

RESULTS AND DISCUSSION

Precision and accuracy

A set of ten calibration standards, a zero and a blank were analyzed with every batch of clinical samples. Such a large number of calibrations was deemed to be necessary to confirm linearity of the calibration curve, since variability of results were expected to cover many orders of magnitude. The between-run precision and accuracy were assessed by the repeated analysis of QC samples containing different concentrations of miconazole. The results are shown in Table I. Linear response of miconazole and internal standard peak-height ratio was observed over the concentration range 0.20–200.0 μg/ml. A linear regression analysis using a least-squares fit was performed with the reciprocal of the drug concentration as weight. The correlation coefficients, an indication of linearity, were equal to or better than 0.9963 over five curves.

Specificity

No interfering peaks were detected at retention times of the drug or internal standard while analyzing eighteen samples collected 12 h before the drug was administered. This is not surprising, because the vaginal fluids were highly diluted in phosphate buffer; this in turn justifies use of a pure buffer as the medium for standards and QCs.

Extraction

Our empirical data suggested that miconazole is more stable in acidic solutions. For this reason we developed an ion-pair extraction procedure which allows the drug to be kept permanently in an acidic solution. At pH 2.5 miconazole is completely dissociated, an excess of octanesulfonic acid is added and the resulting ion pair extracted with chloroform. The internal standard, hydrocortisone 21-cap-

TABLE I

BETWEEN-RUN PRECISION AND ACCURACY OF THE METHOD FOR THE DETERMINATION OF MICONAZOLE IN VAGINAL FLUIDS ($n=5$)

Concentration added (μg/ml)	Concentration found (mean ± S.D.) (μg/ml)	C.V. (%)	Percentage of nominal concentration
0.20	0.180 ± 0.0158	8.8	90.0
0.50	0.460 ± 0.0430	9.4	92.0
2.00	2.056 ± 0.0808	3.9	102.8
10.0	10.36 ± 0.387	3.7	103.6
100	99.34 ± 4.891	4.9	99.3
200	190.0 ± 6.04	3.2	95.0

rylate, does not behave in the same way as the drug since it is a neutral compound and does not form an ion pair with octanesulfonic acid; nevertheless it is well extracted.

Recovery

Two extraction processes and the corresponding recoveries should be considered. The first one is the extraction of the drug absorbed on the microsponge. In order to calculate this recovery, a known volume of miconazole standard solution was added onto a microsponge and then the latter extracted as with all the other clinical samples and compared with the calibration curve. Recovery, using this process, was 85.2% [$n=8$, coefficient of variation (C.V.) = 2.4%].

The second extraction is that of the drug and the internal standard from buffer pH 2.5 into chloroform. Recovery was calculated by comparing extracted QCs with an additionally prepared calibration curve, which represented 100% recovery, correcting for the losses in volume of the organic solvent due to sample transfer. The recovery of miconazole in the second extraction is 93.8% at 0.60 μg/ml ($n=4$, C.V. = 9.8%) and 103.4% at 140 μg/ml ($n=8$, C.V. = 5.5%), while the internal standard had a recovery rate of 63.5% ($n=8$, C.V. = 5.6%).

Chromatography

Fig. 1 shows a blank sample from a subject, 12 h before the drug administration. This is followed by a sample obtained from the same subject 36 h after the last dose. Finally there is a chromatogram representing a standard 10.0 μg/ml miconazole.

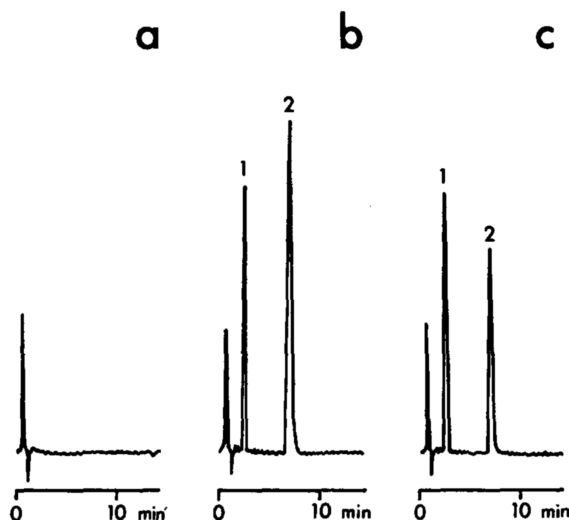

Fig. 1. Representative chromatograms of miconazole extracted from vaginal fluids. (a) Blank sample from a subject, 12 h before drug administration. (b) Sample from a subject, 36 h after the last dose, 15.9 μg/ml. (c) Standard, 10 μg/ml. Conditions: wavelength, 205 nm, 0.02 a.u.f.s.; flow-rate, 1.5 ml/min. Peaks: 1 = hydrocortisone 21-caprylate (internal standard) (retention time 2.2 mm); 2 = miconazole (retention time 6.5 min).

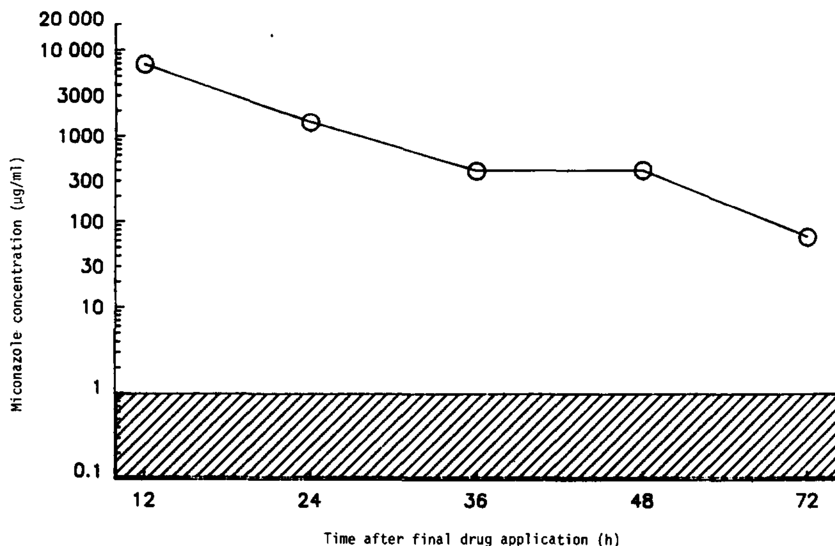

Fig. 2. Average miconazole concentrations in vaginal fluid at all sites across subjects. The shaded area represents the minimum inhibitory concentration of *Candida albicans*.

The chromatography conditions were appropriate for running big batches of samples, since back-pressure on the the column was minimal, the volume of injection small (10.0 μl) and the run time short. All these factors contributed to the consistency and robustness of the assay.

Clinical data

The concentration of miconazole in the acidic extract that resulted from extraction of microsponge with absorbed vaginal fluid ranged from 0 to 820.40 μg/ml with the highest values from initial samples and decreasing over the following four sampling times. (A typical value was between 1.0 and 100 μg/ml.) In those cases where concentration of the drug in the sample exceeded the highest standard (200 μg/ml), analysis was repeated using 50.0 μl of the sample instead of 200 μl. Actual concentration of miconazole in vaginal fluid was calculated using recovery of the drug from the microsponge and the weight of the vaginal fluid; the latter ranged from 0.0053 to 0.455 g (with typical value of ca. 0.05–0.1 g). The mean miconazole concentrations in vaginal fluid (average of areas A, B and C, all six subjects) are shown in Fig. 2. No metabolites of miconazole were seen as the drug was applied topically.

CONCLUSIONS

A simple, rapid HPLC method was developed for analysis of miconazole in vaginal fluids. The method allows one to monitor the drug concentration, 72 h after the last dose of 400 mg of miconazole nitrate.

ACKNOWLEDGEMENT

Thanks are due to Ortho Pharmaceuticals (Canada) Ltd. for their financial support.

REFERENCES

1 L.A. Sternson, T.F. Patton and T.B. King, J. Chromatogr., 227 (1982) 223.
2 T.K. Daneshmend, J. Antimicrob. Chemother., 18 (1986) 507.
3 A. Turner and D.W. Warnoch, J. Chromatogr., 227 (1982) 229.
4 F.C. Odds and F. Macdonald, Br. J. Vener. Dis., 57 (1981) 400.
5 S.M. Wallace, V.P. Shah, S. Riegelman and W.L. Epstein, Anal. Lett., B11 (1970) 461.
6 P.J. Lewi, J. Boelaert, R. Daneels, R. Demeyere, H. Van Landuyt, J.J.P. Heykants, J. Symoens and J. Wynants, Eur. J. Clin. Pharmacol., 10 (1976) 49.
7 J. Heylsants, M. Michaels, A Knaeps and J. Brugeuous, Arzneim.-Forsch., 24 (1974) 1649.
8 H.B. Levine, D.A. Stevens, J.M. Cobb and A.E. Gebhardt, J. Infect. Dis., 132 (1975) 407.
9 A. Espinel-Ingroff, S. Shadomy and J.F. Fisher, Antimicrob. Agents Chemother., 11 (1977) 365.
10 M. Watson, J.-M Houle, J. Zilber, H.M. Hill and C. Haussmann, Pharm. Res., submitted for publication.