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Note

Quantification of ciclopirox by high-performance liquid chromatography after pre-column derivatization

An example of efficient clean-up using silica-bonded cyano phases

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6-Cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone, 2-aminoethanol salt (ciclopirox olamine, HOE 296, Batrafen®) (A, Fig. 1) is a broad-spectrum antimycotic drug [1]. Application is either dermal or vaginal. After absorption of ciclopirox olamine its free acid (ciclopirox) is rapidly conjugated with glucuronic acid. The concentration of unconjugated ciclopirox in serum is about 1—3% of the total concentration. Other metabolites were only observed in urine in negligible quantities [1].

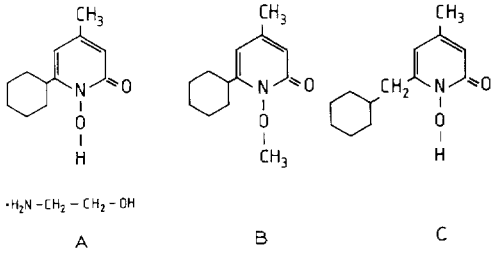

Fig. 1. Structural formulae of (A) ciclopirox olamine, (B) ciclopirox methyl derivative, and (C) internal standard [6-cyclohexylmethyl-1-hydroxy-4-methyl-2-(1H)-pyridone].

To determine the rate of systemic absorption, sensitive assay methods were required for the drug in plasma. Therefore, we tried to measure ciclopirox using high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) both on normal and reversed phases.

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It was not possible to quantify it in trace amounts in situ on TLC plates. Neither the free acid nor its salt with ethanolamine, zinc(II) and iron(II, III) migrate as uniform spots. Severe tailing was always observed. Similar phenomena were observed with HPLC. Small quantities of 1-hydroxy-2(1H)-pyridone were irreversibly retained on the column, and the injection of large amounts of 1-hydroxy-2(1H)-pyridone resulted in several tailing peaks.

This paper describes a derivatization reaction for the drug to form a derivative showing regular chromatographic behaviour and a method for the quantification of ciclopirox olamine in human plasma by means of this derivatization reaction.

Formation of derivatives

The problems mentioned above presumably arise from the complexing properties of the compound. Obviously, the chelating function of the N-hydroxypyridone group (Fig. 2) interacts strongly with silica-gel-based stationary phases. This functional group has thus to be blocked prior to chromatography which is accomplished by methylating the weak acidic N-hydroxyl group ($pK_a \approx 7$) of the 1-hydroxy-2(1H)-pyridones with dimethyl sulphate. The resulting 1-methoxypyridones (Fig. 2) show a normal chromatographic behaviour even on silica.

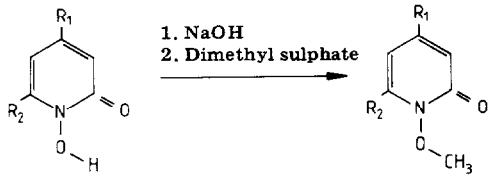

Fig. 2. 1-Hydroxy-2-(1H)-pyridone: derivatization of the chelating function to a 1-methoxy-2-(1H)-pyridone.

The optimal condition for derivatization of 1-hydroxypyridones found is described in the experimental section. Removal of excess dimethyl sulphate is indispensable for obtaining reliable analytical results. This can be achieved by reaction with triethylamine, the result of which is a non-extractable reaction product not interfering with the assay.

EXPERIMENTAL

Materials

The following solvents and reagents were used without special purification: dimethyl sulphate, *n*-hexane AR, methanol AR, toluene AR, triethylamine (Riedel-de Haën, Seelze-Hannover, F.R.G.), acetonitrile HPLC-grade S (Rathburn, Walkerburn, U.K.), 1/15 *M* phosphate buffer solution (pH 5), β-glucuronidase solution from *Escherichia coli* (Boehringer, Mannheim, F.R.G.; Cat. No. 127680). The bonded-phase cartridges containing 100 mg of cyanopropyl packing (Bond-Elut^{TM} CN cartridges, Cat. No. 6131101) were obtained from ICT-Handelsgesellschaft (Frankfurt, F.R.G.).

Compounds A, B and C (the internal standard) (for structures see Fig. 1) were synthesized by Dr. Lohaus (Hoechst, Frankfurt, F.R.G.).

Chromatographic conditions

The chromatograph consisted of a Waters M-45 solvent pump with a Rheodyne 7100 injection port (100-μl sample loop). We used a 125 mm × 4.6 mm I.D. column (Bischoff, Leonberg, F.R.G.) packed with Nucleosil 5 C_{18}, particle size 5 μm (Macherey, Nagel & Co., Düren, F.R.G.). The mobile phase was acetonitrile—water (40:60). The chromatogram was monitored by a Biotronic BT 3030 spectrophotometer at 300 nm. The chromatograph was operated at ambient temperature with a flow-rate of 2.0 ml/min. Under these conditions the retention time was approximately 5 min for the ciclopirox derivative and 8.5 min for the derivative of the internal standard. Quantification was based on the peak height ratio of ciclopirox derivative/internal standard derivative.

Sample preparation

Plasma or serum (1 ml) was incubated with 1 ml of 1/15 *M* phosphate buffer (pH 5) and 10 μl of β-glucuronidase solution at 37°C for 24 h; 40 μl of the internal standard solution (10 μg/ml in water) were then added. For derivatization, 0.5 ml of 2 *M* sodium hydroxide solution and 200 μl of dimethyl sulphate were added, the mixture was vortexed for a short time and kept at 37°C for 15 min. Subsequently, 200 μl of triethylamine were added and the test tube was vortexed again. The mixture was extracted for 20 min with 5 ml of *n*-hexane using a Rotary mixer. The phases were separated by centrifugation, and 4 ml of the organic phase were applied to a 1-ml Bond-Elut CN cartridge which had previously been conditioned twice with 1 ml of acetonitrile.

The columns were rinsed with 1 ml of toluene and aspirated to dryness under reduced pressure for 3 min. Following this, 300 μl of mobile phase (acetonitrile—water, 40:60) were applied onto the column and eluted in a conical glass tube by centrifugation for 3 min; 100 μl of this eluate were injected.

RESULTS

Ciclopirox olamine was admixed to blank plasma in six concentrations between 20 and 1000 ng/ml. Each sample was split into eight portions forming eight identical series of samples. Each series was then analysed on consecutive days so that a total of eight independent analytical results was obtained for each concentration.

Linearity, accuracy and precision

Linearity, accuracy and precision of the method were assessed over a concentration range of 20—1000 ng/ml of serum (Table I).

Extraction and reaction yield

The reaction yield of the methylation and the extraction yield of the methyl derivative together are 75% over all steps of the sample preparation.

Sensitivity

The limit of quantification of ciclopirox olamine in plasma, defined as three

TABLE I

ACCURACY AND PRECISION OF THE ASSAY OF CICLOPIROX OLAMINE IN SPIKED HUMAN PLASMA SAMPLES (n = 8)

Amount added (ng/ml)	Amount measured (mean ± S.D., ng/ml)	Coefficient of variation (%)
20	18.5 ± 1.9	10.3
50	47.9 ± 2.6	5.5
100	97.0 ± 4.0	4.1
200	195 ± 5	2.6
500	483 ± 17	3.5
1000	999 ± 21	2.1

times the standard deviation at the lowest amount measured, is 6 ng/ml. Most of the patients investigated showed no detectable blank values; in a few cases (cf. Fig. 4), blank values up to 15 ng/ml were observed.

Plasma concentration profile in man

Single doses of 5 g of Vaginal Cream P containing 1% ciclopirox olamine were administered by disposable vaginal applicator to patients suffering from vaginal candidiasis. Plasma concentrations of ciclopirox were monitored using the assay method described. In topical administration, absorption of the drug should be minimal. A typical concentration profile (in ciclopirox olamine equivalents) is shown in Fig. 3. (We thank Dr. P.U. Witte (Hoechst AG) for permission to publish these data.)

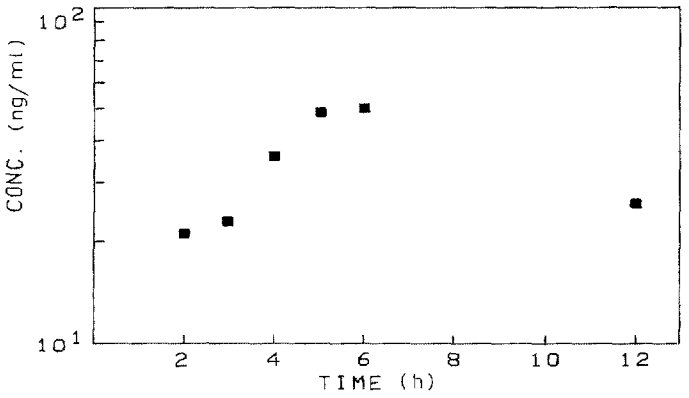

Fig. 3. Profile of plasma concentration (ng/ml) in a patient following vaginal administration of 50 mg of ciclopirox olamine.

DISCUSSION

Alkylation of ciclopirox was shown to produce a single non-tailing peak. To obtain a reproducible extraction yield, the method of alkylating ciclopirox in plasma first and then extracting the methyl derivative is given preference to the inverse procedure in which the free ciclopirox acid is first extracted and then

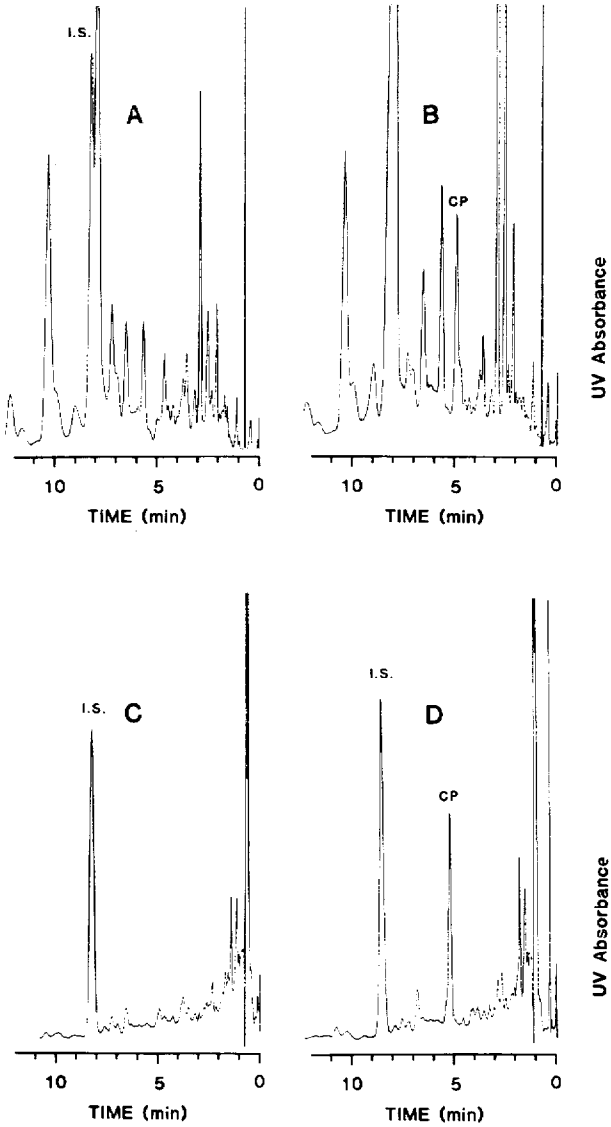

Fig. 4. Chromatograms of plasma samples without clean-up (A, B) and after clean-up (C, D) from patients without (A, C) and with (B, D) ciclopirox olamine treatment. Samples B and D each contain 184 ng/ml. Peaks: I.S. = internal standard derivative. CP = ciclopirox olamine derivative.

alkylated. However, as the methylation in plasma transforms many compounds into hexane-soluble methyl derivatives, it is necessary to introduce a further clean-up after extraction. We have found that this is done very efficiently by using cartridges filled with polar material such as cyanopropyl-modified silica (CN phases). In Fig. 4, the chromatograms of patient sera with a high proportion of endogenous interfering substances are shown before and after clean-up with the CN cartridges.

By this means, a selectivity is obtained which combines two separation principles, namely the interaction with polar groups on CN phases and Van der

Waals interactions on the RP phases. A further advantage of the method described is that there is no need to evaporate the organic phases used for extraction. The ciclopirox methyl derivative is eluted from the polar clean-up cartridge with a volume of the mobile phase small enough to allow direct injection onto the analytical column.

REFERENCE

1 H.-N. Kellner, Ch. Arnold, O.E. Christ, H.G. Eckert, J. Herok, I. Hornke and W. Rupp, Arzneim.-Forsch., 31 (1981) 1337—1353, and references mentioned therein.