Journal of Chromatography, 375 (1986) 179—183
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 2894

Note

Rapid determination of clofibric acid in human plasma by high-performance liquid chromatography

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(First received July 17th, 1985, revised manuscript received September 27th, 1985)

Clofibrate is the ethyl ester of *p*-chlorophenoxyisobutyric acid, the active metabolite of which is clofibric acid, and is used in the treatment of hyperlipoproteinaemia [1] Determination of clofibrate levels in plasma by high-performance liquid chromatography (HPLC) has been reported by Bjornsson et al [2] and Robinson et al. [3] Other authors have reported an HPLC method or a radioisotopic method measuring the glucuronide conjugated in plasma and urine [4, 5]

We propose a reversed-phase HPLC method with UV detection after an easy extraction, which can be used readily to study clofibric acid pharmacokinetics, this metabolite being alone responsible for the therapeutic activity [1]

EXPERIMENTAL

Chemicals and drugs

The following reagents were used glacial acetic acid UCB (Leuven, Belgium),

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methanol, diethyl ether and sodium hydroxide (Normapur Prolabo, Paris, France), *p*-chlorophenoxyacetic acid (internal standard), clofibric and fenofibric acid, graciously provided by Fournier Laboratory (Dijon, France).

Standard solutions of clofibric acid and internal standard were prepared by dissolution in the mobile phase at concentrations of 1000 and 100 μg/ml.

Apparatus and technique

The chromatographic system consisted of a Chromatem 38 solvent delivery pump (Touzart et Matignon, Paris, France) and a Rheodyne injector fitted with a 50-μl loop. The column was a μBondapak C_{18} (30 cm × 4 6 mm I.D., particle size 10 μm) column (Waters Assoc., Millford, MA, U.S A) The detector was a variable-wavelength Schoeffel spectrophotometer. The detection wavelength was 230 nm (0.2 a.u.f.s). All chromatograms were recorded on a 10-mV recorder (CSA, Paris, France).

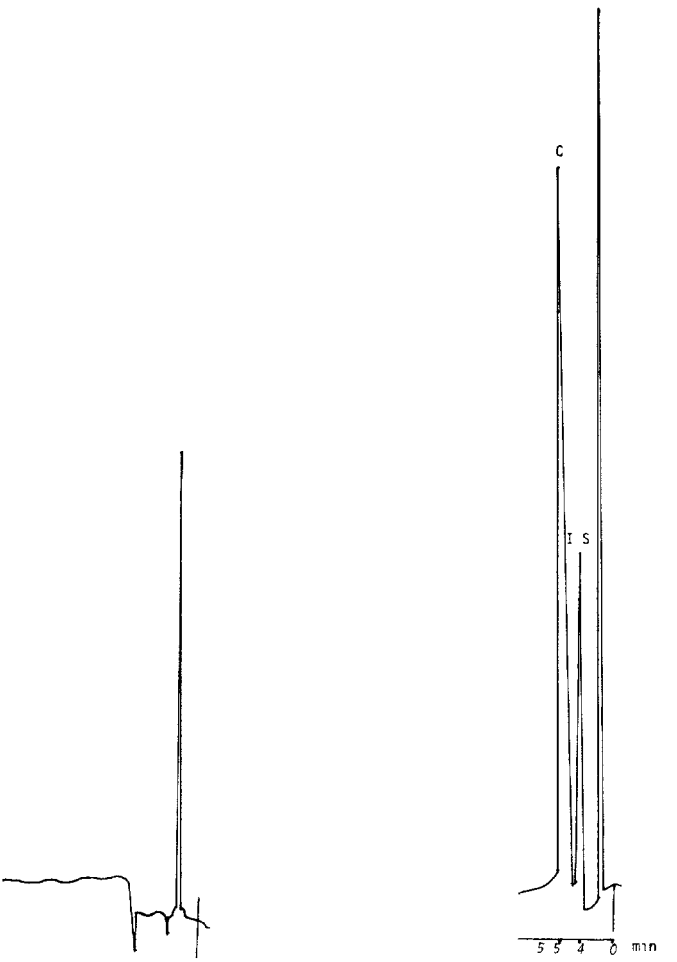

Fig 1 Chromatogram of an extract of blank plasma

Fig 2 Typical chromatogram of plasma extract from a treated subject (containing 18 μg/ml clofibric acid) Peaks C = clofibric acid, I S = internal standard

Mobile phase The mobile phase was methanol—water (45·55) with 1% glacial acetic acid. The flow-rate was 1.30 ml/min. This mobile phase was thoroughly degassed by ultrasound.

Extraction To 500 μl of human plasma were added 30 μl of a solution of 100 μg/ml internal standard, 100 μl of 0 017 *M* glacial acetic acid and 7 ml of diethyl ether The mixture was shaken for 20 s using a vortex mixer (Bioblock, France). The solution was then centrifuged for 5 min at 2000 *g* and 4° C and the supernatant was discarded The lower organic phase was transferred to a clean glass tube and then evaporated to dryness using a vortex evaporator. The residue was dissolved in 200 μl of the mobile phase a 50-μl aliquot was injected into the chromatograph with a Hamilton syringe (Fig. 1).

Calibration The standard curve was obtained by adding clofibric acid to drug-free plasma, to achieve concentrations of 10, 20, 40, 80, 100 and 150 μl/ml (Fig. 2). Standard plasma samples were extracted under the experimental conditions as described above. The peak-height ratios of clofibric acid to internal standard were plotted versus clofibric acid concentration.

RESULTS

Assay technique

The calibration curve was linear from 4 to 150 μg/ml. Its equation was $y = 0\ 55x + 0.05$ ($r = 0.998$), where y is the peak-height ratio of clofibric acid, x is the concentration of clofibric acid (in μg/ml) and r is the correlation coefficient.

Precision

The reproducibility of the method was checked for four plasma concentrations (10, 40, 80 and 150 μg/ml) and the coefficients of variation were respectively 5.1, 6 2, 4 2 and 6.1%.

Recovery

Recovery of clofibric acid was assessed by comparing the peak height after injection of a pure solution of clofibric acid to that obtained after injection of extracted plasma containing the same quantity of clofibric acid The mean recovery of the extraction procedure for sample concentrations of 10, 80 and 100 μg/ml was 83 3 ± 5.1%.

Sensitivity

The threshold of sensitivity of this technique (defined as a double high signal in comparison with background noise) was 2 μg/ml of plasma

APPLICATION AND DISCUSSION

The developed method using HPLC—UV is reproducible, sensitive enough for the determination of clofibric acid in human plasma, easy to perform and cheap This assay has been applied to pharmacokinetic studies of clofibrate with twelve healthy subjects, the corresponding mean curve of plasma concentration is presented in Fig. 3.

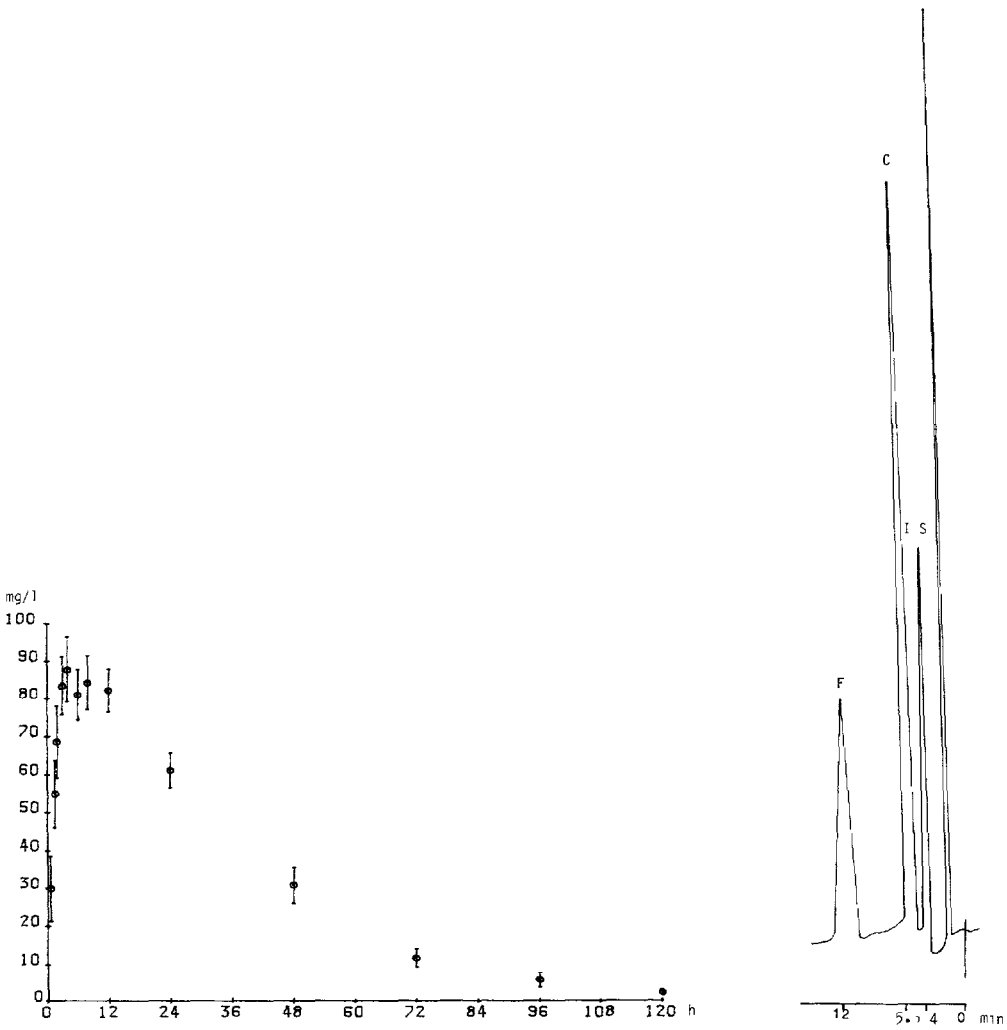

Fig 3 Mean plasma concentrations of clofibric acid in twelve healthy subjects after a single oral dose of 1 g of clofibrate

Fig 4 Typical chromatogram obtained from a plasma extract containing 30 μg of internal standard, 20 μg of clofibric acid and 40 μg of fenofibric acid Peaks C = clofibric acid, F = fenofibric acid, I S = internal standard

We could also evaluate simultaneously fenofibric acid, which is another hypolipidaemic drug, using the same internal standard (Fig 4) The fenofibric acid retention time was twice as long as for clofibric acid Nevertheless, to obtain a better separation, we tried a mobile phase consisting of methanol—water—0 017 *M* glacial acetic acid (60 40 1), but in this case, resolution between clofibric acid and the internal standard was unsatisfactory Using the conditions described for clofibric acid, the assay is linear between 5 and 40 μg/ml (y = 0 44x + 0 257, r = 0 985) The coefficients of variation for 5 and 40 μg/ml were 2 8 and 9 7%, respectively

CONCLUSION

In this paper we have described a simple and rapid technique for the determination of clofibric acid in plasma Sensitivity and reproducibility are sufficient to allow us to elucidate the pharmacokinetics of this drug in healthy subjects

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