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Note

A gas chromatographic method for the determination of Bezafibrate in serum and urine

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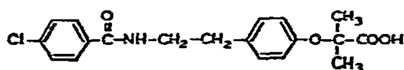
Bezafibrate, 2-{4-[2-(4-chlorobenzamido)ethyl]phenoxy}-2-methylpropionic acid, is a new lipid-lowering drug of the clofibrate type.



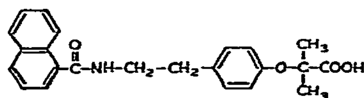
Bezafibrate

It is administered in daily doses of 450–600 mg and has been shown to decrease lipoprotein concentrations more effectively than does clofibrate[1,2]. In initial pharmacokinetic studies, unchanged Bezafibrate in serum and urine were measured by a thin-layer chromatographic (TLC) method; later, by a simple gas chromatographic (GLC) method was developed; this gave more reliable results and is described here. In principle, it involves extraction of the drug under acid conditions and conversion to the methyl ester by flash-heater methylation with trimethylanilinium hydroxide.

EXPERIMENTAL

Materials

Bezafibrate and the internal standard BM 13.217 were synthesized in our research laboratories and were of analytical grade. The methylating agent, 0.2 *M* trimethylanilinium hydroxide in methanol (Meth-Elute®) was purchased from Pierce Chemicals (Rotterdam, The Netherlands). All solvents used were of reagent quality and were not further purified.



BM 13.217

Gas chromatography

A Hewlett-Packard model 5710 A gas chromatograph equipped with a flame ionisation detector was used; the glass column (2 m × 4 mm I.D.) was packed with

8% of OV-101 on Gas-Chrom Q (80–100 mesh). The flow-rates for the carrier gas (nitrogen) and for the hydrogen were set at 60 ml/min; the air flow-rate was 240 ml/min. The injection port and detector were operated at 300°, and the oven temperature was 290°. Under these conditions Bezafibrate and the internal standard were eluted after *ca.* 4 and *ca.* 8 min, respectively.

Work-up procedure

To 1 ml of serum or urine in a 10-ml centrifuge tube are added 10 μl of conc. hydrochloric acid, 100 μl of an aqueous solution (100 mg/l) of the internal standard and 5 ml of ethyl ether. The tube is shaken for 2 min and centrifuged at 4000 *g*. Back-extraction is made by transferring the organic phase to an other centrifuge tube containing 2 ml of 2 *M* sodium hydroxide. This tube is shaken for 2 min and centrifuged, the ether layer is discarded, and the aqueous alkaline phase is washed with 5 ml of ether.

The washed aqueous phase is acidified with 50 μl of conc. hydrochloric acid and extracted with 5 ml of ether; the ether phase is transferred to an other tube and evaporated to *ca.* 1 ml under a gentle stream of pure nitrogen, and this solution is pipetted into a conical vial and evaporated to dryness. Then 10 μl of Meth-Elute are added, and 1–3 μl of the mixture are injected into the chromatograph.

Calibration graphs are prepared by assaying samples of serum and urine to which known amounts of Bezafibrate have been added. Peak-height ratios of Bezafibrate to internal standard are plotted against the known concentrations.

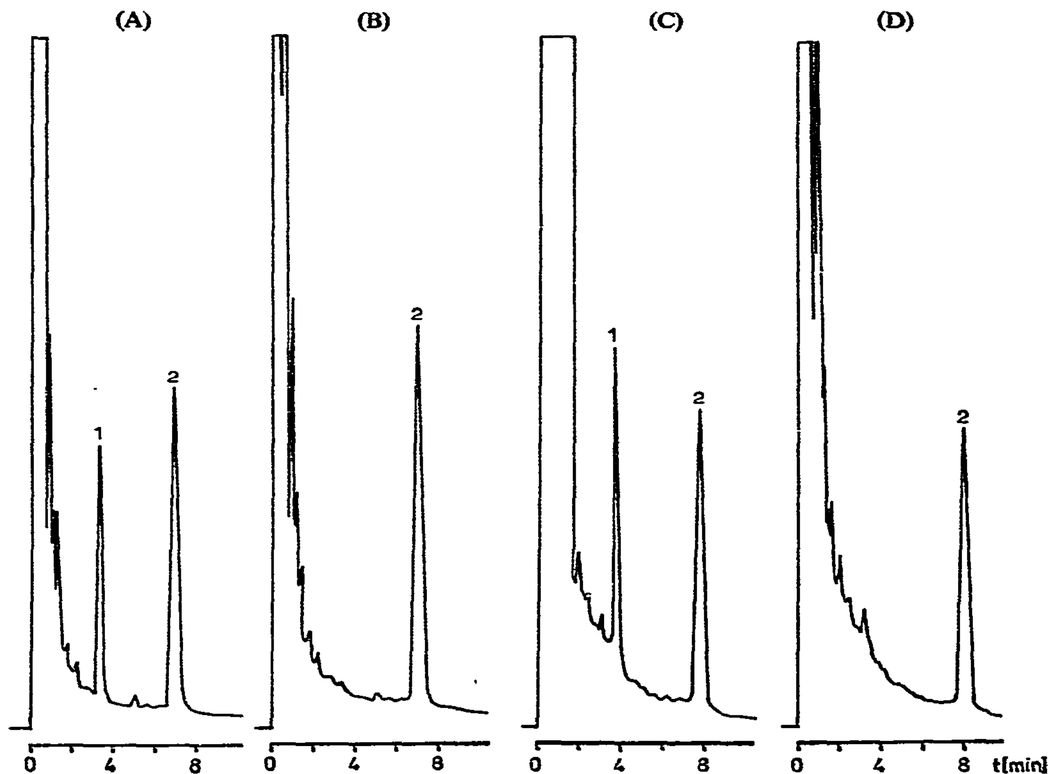


Fig. 1. Gas chromatograms for: A, Serum plus 5 mg/l of Bezafibrate; B, blank serum; C, urine plus 5 mg/l of Bezafibrate; D, blank urine. All samples contained 10 mg/l of added internal standard. Peaks: 1, Bezafibrate; 2, internal standard.

RESULTS AND DISCUSSION

As mentioned above, the normal daily dose of Bezafibrate is 450–600 mg. For such a dose, the serum and urine concentrations are expected to be in the range 1–10 mg/l, and the peak-height ratios of Bezafibrate to internal standard were linear over this range.

During toxicological studies in animals, doses of up to 70 mg/kg were administered, and serum levels reached max. values of more than 150 mg/l. Analysis of calibration samples in the range 10–200 mg/l showed that the relationship between signal and concentration was still linear. To measure serum levels as high as 200 mg/l, the concentration of the internal standard was increased 10-fold (100 μl of 1 g/l internal standard solution) and the procedure was applied without further modification.

Fig. 1 shows typical chromatograms obtained from serum and urine samples; no interfering substances were co-extracted.

Over a period of 10 months, the precision and the coeff. of variation were checked at two concentrations (5 and 2.5 mg/l) of Bezafibrate in serum samples; the mean values found (73 determinations) were 4.933 and 2.497 mg/l, respectively, and the corresponding coefficients of variation were 4.4 and 4.1%.

These samples were analyzed together with samples from patients who had received Bezafibrate and are a measure for long-time quality control. The recovery of the extraction procedure was 67 $\pm$ 3% for serum and 72 $\pm$ 3% for urine.

Fig. 2 shows a typical concentration profile for serum from a patient who had received 300 mg of Bezafibrate orally.

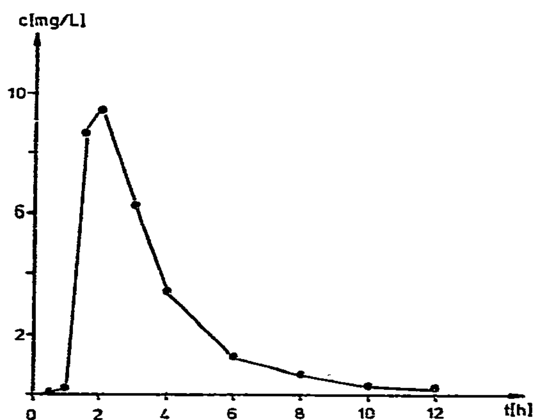


Fig. 2. Concentration profile of serum from a patient who had received an oral dose of 300 mg of Bezafibrate.

With the method described, as little as 0.2 mg/l of Bezafibrate in serum and urine can easily be assayed.

REFERENCES

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