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Note

Improved method for the determination of procetofenic acid in human plasma by gas—liquid chromatography

CLAUS LULEY* and HAGEN KEGEL

Lipidmetabolic Outpatient Department, Medical Department and Policlinic, Klinikum Charlottenburg, Free University of Berlin, Soorstr. 83, D-1000 Berlin 19 (G.F.R.)

and

CORNELIUS JAKOBS

Department of Pediatrics, Kaiserin-Auguste-Viktoria-Haus, Free University of Berlin, Heubnerweg 6, D-1000 Berlin 19 (G.F.R.)

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Procetofenic acid {2[4'-(*p*-chlorobenzoyl)phenoxyl]-2-methylpropionic acid} is the active metabolite of Procetofen (Lipanthyl) (LF 178; isopropyl ester of procetofenic acid). In order to be able to determine the level of this compound routinely in serum samples, we simplified the gas—liquid chromatographic (GLC) method described by Desager [1]. This improvement was achieved by the use of Meth-elute (0.2 *M* trimethylanilinium hydroxide in methanol) for the derivatization and of Bezafibrate (Cedur) (2-{4-[2(4-chlorbenzamido)-ethyl] phenoxyl}-2-methylpropionic acid) as an internal standard.

EXPERIMENTAL

Reagents

All chemicals were of analytical-reagent grade. Meth-elute (0.2 *M*) was obtained from Pierce Eurochemie (Rotterdam, The Netherlands), procetofenic acid from Fournier (Lyon, France) and Bezafibrate from Boehringer (Mannheim, G.F.R.).

Preparation of samples

To 1 ml of serum in a 12-ml stoppered glass tube were added 5 ml of diethyl ether, 100 μl of internal standard solution (Bezafibrate, 200 μg/ml) 200 μl of

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water and 100 μl of concentrated hydrochloric acid. After continuous mechanical stirring for 30 sec and centrifugation for 10 min at 3000 *g*, the upper layer was transferred to another tube. After evaporation to dryness at 50°C under nitrogen the residue was dissolved in 250 μl of 0.02 *M* Meth-elute and a 0.2-μl aliquot was injected into the gas chromatograph.

Gas chromatography

A Hewlett-Packard 5830A gas chromatograph equipped with a nickel-63 electron-capture detector was used. The column was a 1.6 m × 2 mm I.D. glass tube packed with 8% OV-101 on Gas-Chrom Q (80—100 mesh) (Boehringer) and was conditioned at 320°C for 24 h under nitrogen at a flow-rate of 20 ml/min. The oven temperature was 300°C, injection port temperature 320°C and detector temperature 320°C. The carrier gas was argon—methane (95:5) at a flow-rate of 50 ml/min.

RESULTS

A calibration graph of the peak-height ratio of procetofenic acid to internal standard versus amount of procetofenic acid was linear in the range from 2 to 200 μg/ml and passed through the origin. The accuracy and reproducibility of the method were evaluated by ten repeated determinations on three serum samples that contained 2, 20 and 200 μg/ml of procetofenic acid, respectively. The coefficients of variation were 4.7, 2.7 and 4.9%, i.e., below the 5% level that is generally accepted for quantitative analyses of drugs. The detection limit was 1 μg/ml.

A typical gas chromatogram is shown in Fig. 1. The retention time for proce-

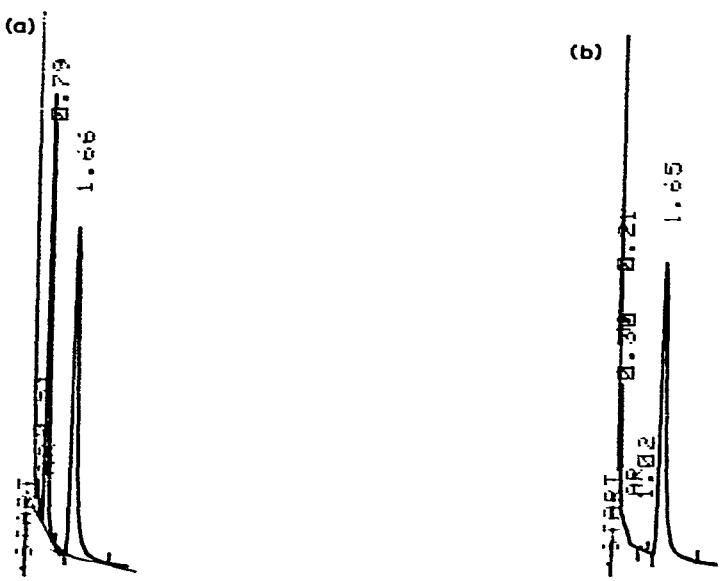

Fig. 1. Gas—liquid chromatograms of serum extracts: (a) procetofenic acid, retention time (RT) = 0.79 min, with Bezafibrate (RT = 1.66 min) as internal standard; (b) Bezafibrate alone (RT = 1.65 min).

tofenic acid (methyl ester) was 0.79 min and that of the internal standard was 1.66 min.

The specificity of the method was checked using a coupled GLC—mass spectrometric system. The mass spectrum was identical with that described by Elsom et al. [2].

DISCUSSION

In order to be able to establish a relationship between serum levels of procetofenic acid and its effect on the one hand and to control patients' compliance on the other, it was necessary to establish a method for specific and relatively rapid determinations. The method of Desager [1] could be simplified in two ways: diazomethane, which was used for the methylation, was replaced with Meth-elute, which need not be specially prepared and is less hazardous, and instead of Procetofen we used Bezafibrate as an internal standard. In comparison with Bezafibrate, Procetofen has two disadvantages: it cannot be excluded that some unmetabolized Procetofen may remain in the patients' serum [3], which would invalidate the evaluation, and there is a peak [1] which, being independent of the drug intake, has the same retention time as Procetofen and could therefore influence the evaluation. Under the conditions described here Procetofen will appear separately at a retention time of 1.11 min.

The proposed modification allows the specific and exact determination of procetofenic acid with good linearity in the range of serum levels that occur during continuous drug intake (2—20 μg/ml). Using this simplified method, one technician can handle about 120 serum samples per day if an automatic sampler is available.

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