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Note

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

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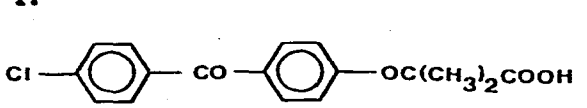
Procetofenic acid (I, Fig. 1) is the active metabolite of the new drug procetofene, or fenofibrate, which is its corresponding isopropyl ester derivative. This acid is structurally related to the well-known hypolipidaemic agent, clofibrate, in which the *p*-chlorine atom has been replaced by a *p*-chlorobenzoyl group in order to improve its activity and tolerance.

I.



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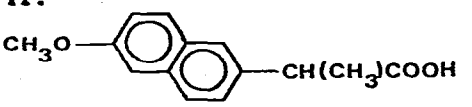

Fig. 1. Chemical structure of procetofenic acid (I) and naproxen (II).

The metabolism and pharmacokinetics of fenofibrate have been recently reported [1, 2]. The analytical methods used for the active metabolite determination in plasma include gas chromatography [3] and gas chromatography—mass spectrometry [4]. The compound was determined as its methyl ester and the parent drug, procetofene, was used as the internal standard. However, under the reported conditions [3] a small interfering peak appears in plasma samples.

The method described in this paper makes use of high-performance liquid chromatography (HPLC), and is faster, more sensitive and more specific than the gas chromatographic method. It is therefore suitable for the routine analysis of large numbers of biological samples.

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EXPERIMENTAL

Reagents and materials

Diethyl ether, methanol and potassium dihydrogen phosphate were of reagent grade (E. Merck, Darmstadt, G.F.R.) and used as received. Procetofenic acid and the internal standard, naproxen (II, Fig. 1), were of analytical purity.

Plasma samples were obtained from male Wistar rats of approx. 300 g, orally medicated with doses of 50 mg of procetofenic acid per kg of body weight. Sets of eight rats were used for each determination.

Extraction procedure

A 3-ml aliquot of plasma was pipetted into a glass-stoppered centrifuge tube, acidified with 1.5 ml of 3 *M* hydrochloric acid and mechanically shaken (Vortex mixer) for 15 sec. After standing for 5 min, 1 ml of the internal standard solution of naproxen (150 μg/ml in methanol) and 15 ml of diethyl ether were added to the acidified plasma. The mixture was shaken again for 30 sec and centrifuged at 1500 *g* for 5 min. The upper layer was transferred to another tube and the extraction was repeated once more. The ether extract were carried to dryness under vacuum at 40°C in a rotary evaporator. The resulting residue was dissolved in 1 ml of methanol and 10 μl were injected into the chromatograph.

Apparatus and chromatography

An M 600 pump and a U6K injector (Waters Assoc., Milford, MA, U.S.A.) were coupled to a flexible-walled cartridge packed with 5-μm reversed-phase material (100 × 5 mm, μBondapak C_{18}) fitted in a radial compression module (RCM-100)*. A Waters M440 UV detector, with a fixed wavelength of 254 nm, and a DM730 data processing module were used to measure the concentrations of compounds I and II (Fig. 1).

The mobile phase consisted of a mixture of 70% (v/v) methanol and 30% water containing 0.4% (w/v) phosphate—phosphoric buffer to maintain the pH at 4. A constant flow-rate of 2 ml/min was used with a pressure of 103 bar. Under these conditions, the retention times of naproxen and procetofenic acid were 2.8 and 5.2 min, respectively.

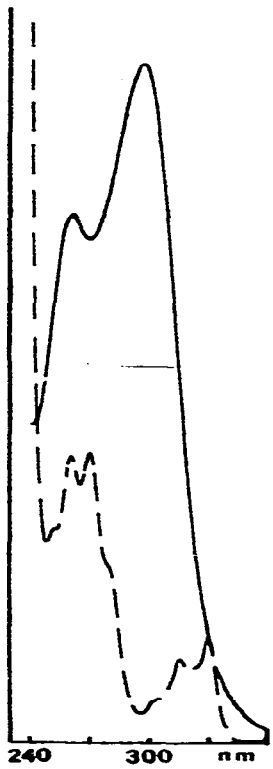
RESULTS AND DISCUSSION

The UV absorption spectra of procetofenic acid (I) and naproxen (II) show maxima at about 260 nm (Fig. 2). Therefore a 254-nm fixed-wavelength detector is suitable.

Fig. 3 shows representative chromatograms obtained and demonstrates the absence of interfering endogenous compounds. The short column length provides a satisfactory retention time, thus improving sensitivity.

A linear calibration curve of peak area ratios of procetofenic acid/naproxen vs. plasma concentrations of procetofenic acid was obtained in the range of 1—100 μg/ml. The correlation coefficient for this linear relationship was

*Trade-mark Waters Assoc.



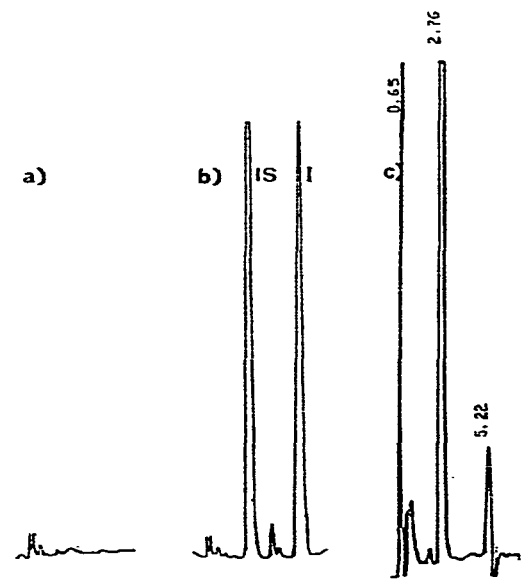


Fig. 2. Ultraviolet absorption spectra of procetofenic acid (——) and naproxen (— — —) in methanol.

Fig. 3. Typical chromatograms of: (a) control plasma; (b) plasma extract from a medicated rat (I = procetofenic acid; IS = internal standard); (c) plasma containing procetofenic acid at a level of 100 ng/ml. Retention times of procetofenic acid (5.22 min) and internal standard (2.76 min) are indicated.

consistently greater than 0.999 (n = 3) and the value of the intercept was not significantly different from zero. The corresponding equation was $y = 52.63\ x + 0.12$, where y represents the concentration of procetofenic acid in plasma in μg/ml and x the indicated peak area ratios, using 150 μg/ml of internal standard.

The reproducibility of six replicate measurements of procetofenic acid added to plasma in the concentrations 100, 50 and 10 μg/ml was found to be 1.0, 1.4 and 3.2%, respectively. The recovery was in the order of 60% in the first extraction and better than 98% with the second one.

The sensitivity of the method as described was about 50 ng/ml, which allowed the study of the pharmacokinetic parameters of this drug in rats. Table I summarizes the results of a bioavailability trial where it is clearly shown that the administration of the acid as a salt derivative does not produce any significant change in its pharmacokinetics compared to the acid itself.

However, if therapeutic levels in biological fluids need to be measured, it is possible to detect as low as 10 ng/ml. Consequently, the sensitivity of

TABLE I

PHARMACOKINETIC PARAMETERS FOR ORAL ABSORPTION OF PROCETOFENIC ACID IN RATS, IN TWO DIFFERENT FORMULATIONS

Procetofenic acid was administered (50 mg/kg) (A) as the acid and (B) as its cinnarizine salt. Values are means ± S.D. of eight animals.

Formulation	Mean peak plasma conc. (μg/ml)	Mean time to peak plasma conc. (h)	Mean area under curve (0—48 h) (μgh/ml)	Mean plasma half-life (h)
A	155 ± 18.1	2	2732	21.73 ± 1.07
B	165 ± 24.4	2	2719	18.8 ± 1.2

this method is higher than that previously described for gas chromatography [3]. In addition, it possesses several advantages for routine analysis; namely, an easier and faster handling procedure of the samples and a higher specificity. Nevertheless, the specificity must be tested when the assay needs to be applied to the plasma of patients receiving multi-drug therapy.

ACKNOWLEDGEMENT

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NOTE ADDED IN PROOF

A method for the determination of procetofenic acid in plasma by gas chromatography with electron-capture detection has been published recently [5]; the limit of detection was 1 μg/ml.

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