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## Note

# Rapid gas chromatographic determination of underivatized phenprocoumon in plasma

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The determination of the anticoagulant drug phenprocoumon by gas–liquid chromatographic (GLC) methods has been described by several workers[1–4]. These methods either need special equipment or are time consuming owing to derivatization and additional purification steps.

This paper describes a GLC method that is simple and rapid. Its sensitivity is sufficient for the specific determination of phenprocoumon in the plasma of patients treated with this drug.

## EXPERIMENTAL

### *Reagents*

All solvents were of pro analysi grade (Merck, Darmstadt, G.F.R.) and were used without further purification. Phenprocoumon [3-(α-ethylbenzyl)-4-hydroxycoumarin], *p*-chlorophenprocoumon [3-(α-ethyl-*p*-chlorobenzyl)-4-hydroxycoumarin] and [$^3$H]phenprocoumon (specific activity 264 mCi/mmole) were gifts from Hoffmann-La Roche, Basle, Switzerland.

### *Preparation of samples*

Internal standard solution (0.1 ml of human plasma containing 6 μg of *p*-chlorophenprocoumon) and 2.0 ml of distilled water were added to plasma samples of 0.5–2.0 ml. The samples were acidified with 0.5 ml of 3 *N* hydrochloric acid and extracted into 10 ml of ethylene dichloride. After centrifugation, 8 ml of the organic phase were removed and evaporated to dryness on a rotary evaporator. The residue was dissolved in 1.0 ml of acetone and transferred into 1.5-ml custom-made evaporating vials[5]. The acetone solution was concentrated in an air bath (*ca.* 60°) with slight vibration to a volume of about 5 μl. One to two microlitres were injected on to the gas chromatograph.

### *Gas–liquid chromatography*

Analyses were performed using a (Carlo Erba Fractovap G I) gas chromatograph equipped with a flame-ionization detector and glass columns (6 ft. × 2 mm I.D.) packed with 3% (w/w) OV-17 on 100–120-mesh Chromosorb W-HP operated

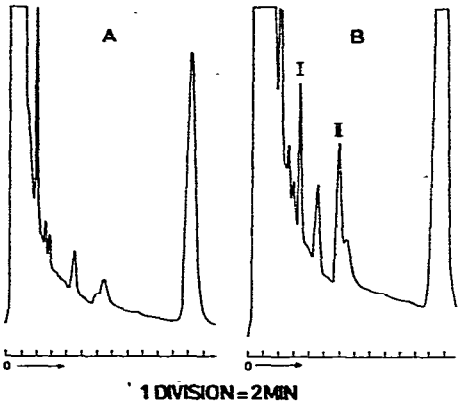


Fig. 1. Gas–liquid chromatograms of (A) blank extract of 1 ml of human plasma and (B) extract from 1 ml of human plasma containing 2 $\mu$g of phenprocoumon (I) and 6 $\mu$g of *p*-chlorophenprocoumon (II). Samples were dissolved in about 10 $\mu$l of acetone and about 1.5 $\mu$l were injected.

at an oven temperature of 265° isothermal and an injection port and detector temperature of 300° with a flow-rate of carrier gas (nitrogen) of 50 ml/min.

## RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram obtained from blank plasma (Fig. 1A) and from plasma to which phenprocoumon and *p*-chlorophenprocoumon had been added (Fig. 1B). Retention times of 6.5 and 12 min were obtained for phenprocoumon and *p*-chlorophenprocoumon, respectively. When plasma samples from more than 30 untreated subjects were analyzed, no endogenous plasma constituents were found to have the same retention time as that of phenprocoumon. Endogenous material having a slightly longer retention time than that of *p*-chloro-phenprocoumon (12.5 min) always occurred. However, this material did not interfere in the quantitative determination when the peak height was used for quantification. The pronounced signal at about 25 min also results from endogenous material in the plasma. To keep

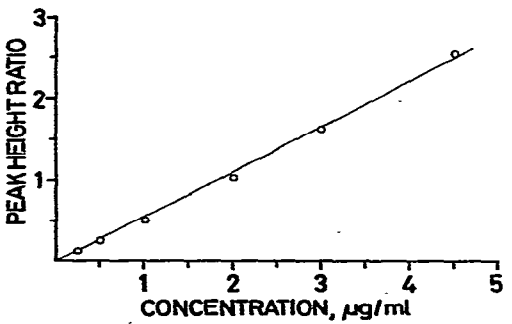


Fig. 2. Calibration graph for phenprocoumon obtained from human plasma to which different concentrations of phenprocoumon were added. Samples of 1 ml were assayed. Each point represents the mean of four determinations (standard deviations were smaller than the symbols). Peak height ratio = height of phenprocoumon peak/height of *p*-chlorophenprocoumon peak.

the method as simple as possible, no attempt was made to purify either the plasma samples or organic solvents by additional steps.

The calibration graph obtained by assaying human plasma samples to which different concentrations of phenprocoumon had been added was linear in the concentration range studied (Fig. 2).

The recovery of phenprocoumon was determined by spiking the plasma samples with [$^3$H]phenprocoumon. Aliquots of the spiked plasma samples and the final acetone solution to be injected on to the gas chromatograph were counted. Recoveries of 83 $\pm$ 10% (Mean $\pm$ standard deviation, $n$ = 10) were obtained in the 0.25 –4 $\mu$g/ml concentration range. Recoveries fo *p*-chlorophenprocoumon were determined by adding *p*-chlorophenprocoumon and phenprocoumon to the final acetone extract from blank plasma. The peak height ratio was compared with that obtained by assaying plasma samples containing 6 $\mu$g/ml of *p*-chlorophenprocoumon. In these experiments, 2 $\mu$g of phenprocoumon were added to the final acetone extract. Recoveries of 88 $\pm$ 11% ($n$ = 4) were obtained.

Concentrations of 0.2 $\mu$g/ml of phenprocoumon can be determined readily in plasma samples of 1 ml. Lower concentrations can be determined by increasing the volume of the plasma sample. When plasma samples to which 0.25 or 3.0 $\mu$g/ml of phenprocoumon had been added were analyzed, the coefficient of variation was 6% and 2%, respectively ($n$ = 4).

A large number of drugs were tested for possible interference in the method, including the coumarin derivatives warfarin, dicumarol, acenocoumarin and tromexan. These structurally related compounds were not detected by the method. Only phenylbutazone was found to interfere, its retention time being similar to that of phenprocoumon.

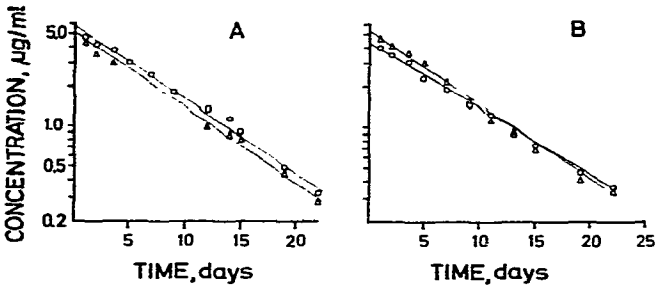


Fig. 3. Plasma concentrations of racemic phenprocoumon (A) and *S*(—)-phenprocoumon (B) as a function of time after oral administration of a single dose of 0.6 mg/kg to male subjects. Plasma samples were assayed by a fluorimetric method[6] (○) and by our GLC method (△).

Examples of the application of the method are shown in Fig. 3. Two healthy male volunteers received an oral dose of 0.6 mg/kg of either racemic phenprocoumon (Fig. 3A) or of the enantiomer *S*(—)-phenprocoumon (Fig. 3B). The elimination of the drugs was followed for about four half-lives. Plasma samples were assayed by a specific fluorimetric method[6] and by our GLC method. The values obtained by the two methods were in agreement. However, when plasma concentrations have to be monitored in patients treated with phenprocoumon and other drugs, the GLC method has a considerable advantage as most commonly used drugs do not interfere.

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