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Note

A high-speed liquid chromatographic analysis of indomethacin in plasma

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Spectrofluorimetry has been the usual method for the determination of indomethacin in biological fluids[1,2]. However, concomitantly administered aspirin and frusemide both interfere with the assay and have to be removed by additional extractions[1], or chromatography[2]. Recently a gas–liquid chromatographic method has been reported[3] based on the derivatisation of indomethacin. This report describes a high-speed liquid chromatographic method which obviates the necessity for separating aspirin and frusemide from indomethacin or its derivatisation prior to assay.

EXPERIMENTAL

A Waters Ass. Model ALC/GPC 501 liquid chromatograph, with a single-wavelength detector operating at 254 nm, was used. The stainless-steel column (60.93 cm × 0.21 cm I.D.) was dry-packed with Bondapak C_{18}/Corasil (Waters Ass., 37–50 μm particle range), and the mobile phase, consisting of an acetonitrile–0.1 *M* acetic acid (40:60) mixture, was pumped through at a rate of 1 ml/min.

Plasma (1 ml) was placed in a stoppered test-tube, along with citrate buffer pH 5 (1 ml), water (1 ml) and 1 ml of an aqueous solution of flufenamic acid (concentration approximately 20 μg/ml) as internal standard. The mixture was extracted with diethyl ether (5 ml) by mechanically shaking for 15 min. The ether phase (4 ml) was transferred to a tapered tube and evaporated to dryness on a water bath at 30°, under a stream of nitrogen. The extract was reconstituted with mobile phase (100 μl) and aliquots (10 μl) were injected on to the column. A series of plasma samples containing known amounts of indomethacin was used to calibrate the instrument.

RESULTS AND DISCUSSION

The chromatograms obtained for plasma extracts are shown in Figs. 1a–d. Since the flufenamic acid and indomethacin peaks were well resolved (with retention times of 3.3 and 5.7 min, respectively) and symmetrical, peak heights were used as measures of concentration. A plot of the ratio of indomethacin to flufenamic acid peaks against indomethacin concentration proved linear (Fig. 2). The recovery of indomethacin was at least 85% and under the conditions used both aspirin and frusemide were eluted in the void volume without interfering with the quantitative

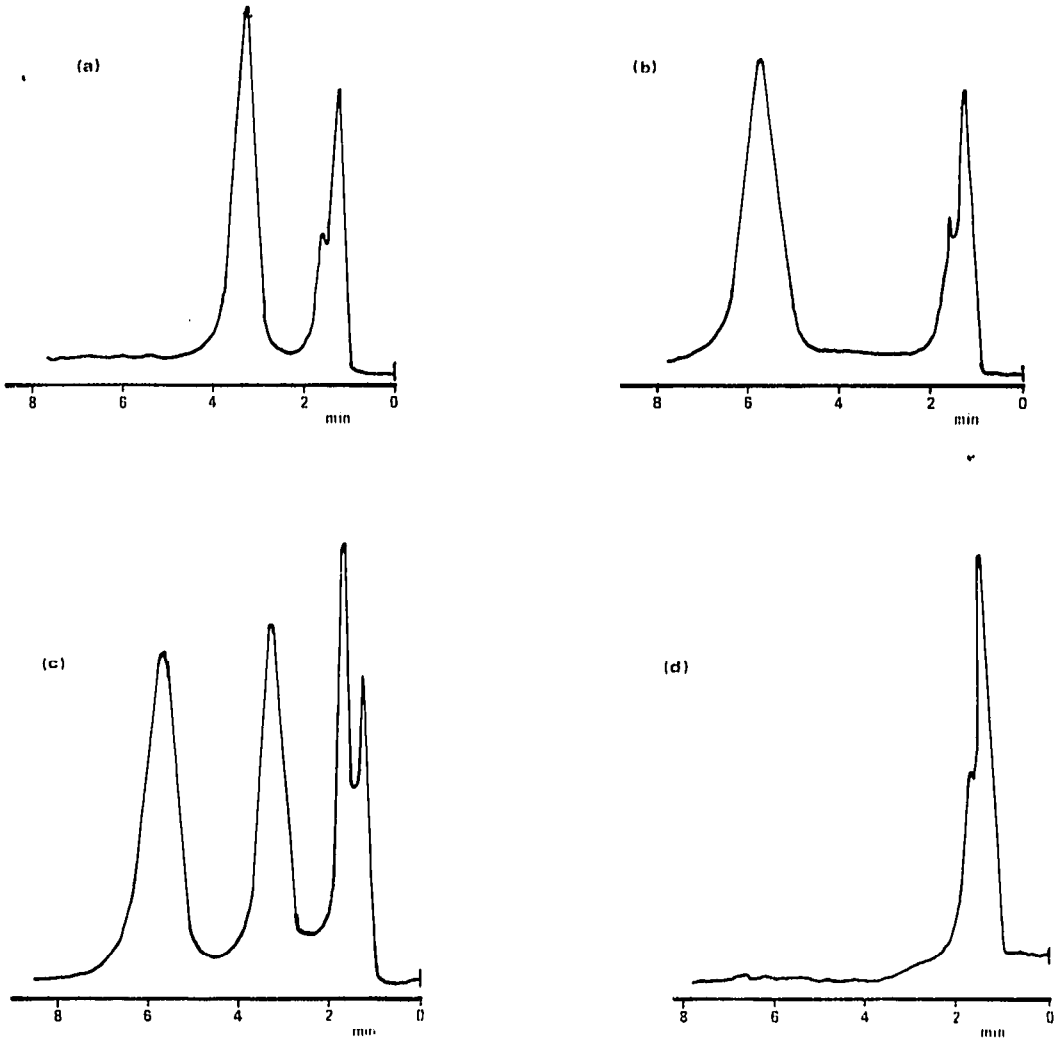

Fig. 1. Chromatograms of plasma samples. a, Indomethacin extract; b, flufenamic acid extract; c, extract of indomethacin with flufenamic acid as internal standard; d, control extract.

determination of indomethacin. No other interfering substances were extracted from plasma.

The relatively poor absorbance of flufenamic acid at 254 nm made it necessary to use a concentration approximately six times greater than that of indomethacin. Following the procedure described, the method was found to be reproducible and quantitatively sensitive to plasma concentrations as low as 0.1 μg/ml.

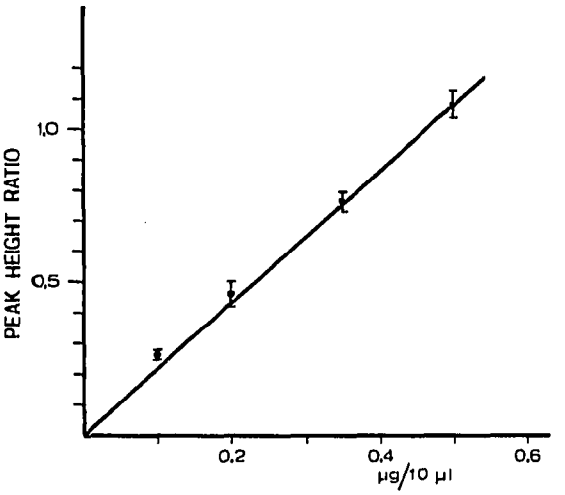

Fig. 2. Calibration curve for the determination of indomethacin in plasma; each point is the mean of three indomethacin/flufenamic acid peak ratios, with its standard deviation.

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