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Note

Improved procedure for determination of indomethacin in plasma by capillary gas chromatography after solid-phase extraction

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Indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid, is an anti-inflammatory drug that has been widely used for the treatment of arthritis. In recent years, new drug forms of indomethacin, such as external application and suppositories, have been developed and a simple and sensitive method for the determination of indomethacin in body fluids is required for precise pharmacokinetic studies. Gas chromatography with electron-capture detection is very sensitive and useful for this purpose [1–9], whereas high-performance liquid chromatographic methods lack sensitivity [10–15]. However, previous gas chromatographic methods are not suitable for routine analysis, because they include a time-consuming solvent extraction step [1–9], use explosive esterification reagents such as diazoalkanes [1–6] and often provide only incomplete separation from components in body fluids as they use packed column conditions [1–9]. We have improved the pretreatment step and established a simple and sensitive procedure in which indomethacin is determined by capillary gas chromatography after solid-phase extraction and ethylation with 1-ethyl-3-*p*-tolyltriazene.

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EXPERIMENTAL

Reagents and chemicals

Indomethacin standard was obtained from Sumitomo Chemical (Osaka, Japan). 1-Ethyl-3-*p*-tolyltriazene and 1-*n*-propyl-3-*p*-tolyltriazene were purchased from Tokyo Kasei (Tokyo, Japan). A disposable extraction column, Bond-Elut C_{18}, was purchased from Analytichem International (Harbor City, CA, U.S.A.). Hydrochloric acid, methanol and diethyl ether from Wako (Osaka, Japan) were of analytical-reagent grade.

Indomethacin propyl ester was used as an internal standard with reference to the method reported by Yata et al. [5] and was synthesized from indomethacin using 1-*n*-propyl-3-*p*-tolyltriazene as a propylating reagent. This internal standard was purified twice by thin-layer chromatography and it was confirmed to show a single peak on the gas chromatogram.

Apparatus and conditions

A Model GC-9APE gas chromatograph (Shimadzu, Kyoto, Japan) with a ^{63}Ni electron-capture detector was used. A fused-silica capillary column, ULBON HR-52 (25 m×0.32 mm I.D., 0.25 μm film thickness), was obtained from Shinwa Kakou (Kyoto, Japan). The injector and detector temperatures were 330°C and the column oven temperature programme was 110°C for 1 min, increased to 300°C at 8°C/min and held at 300°C for 5 min. A Shimadzu SPL-G9 splitless injection system was used with a splitless time of 1 min after injection. The carrier gas was helium and the make-up gas was nitrogen at a flow-rate of 40 ml/min.

Procedure

To 1 ml of plasma in a test-tube was added 1 ml of 0.1 *M* hydrochloric acid and the tube was shaken vigorously. The pH of the sample solution was maintained at 4–5. This sample solution was applied to a Bond-Elut C_{18} column that had been conditioned with methanol (6 ml, twice) and distilled water (6 ml, twice). Subsequently, the column was washed with distilled water (6 ml, twice) and 15% methanol (4 ml, once) and then the sample was eluted with 4 ml of 80% methanol. These procedures of washing and elution from the column were carried out slowly under reduced pressure.

The eluate was evaporated to dryness under nitrogen at 40°C. To the residue was added 1-ethyl-3-*p*-tolyltriazene in diethyl ether (0.1 mg/ml) and the mixture was allowed to stand at room temperature for at least 6 h, then evaporated to dryness under nitrogen and the residue dissolved in 0.5 ml of toluene including internal standard. A 2-μl volume of this sample solution was injected into the gas chromatograph.

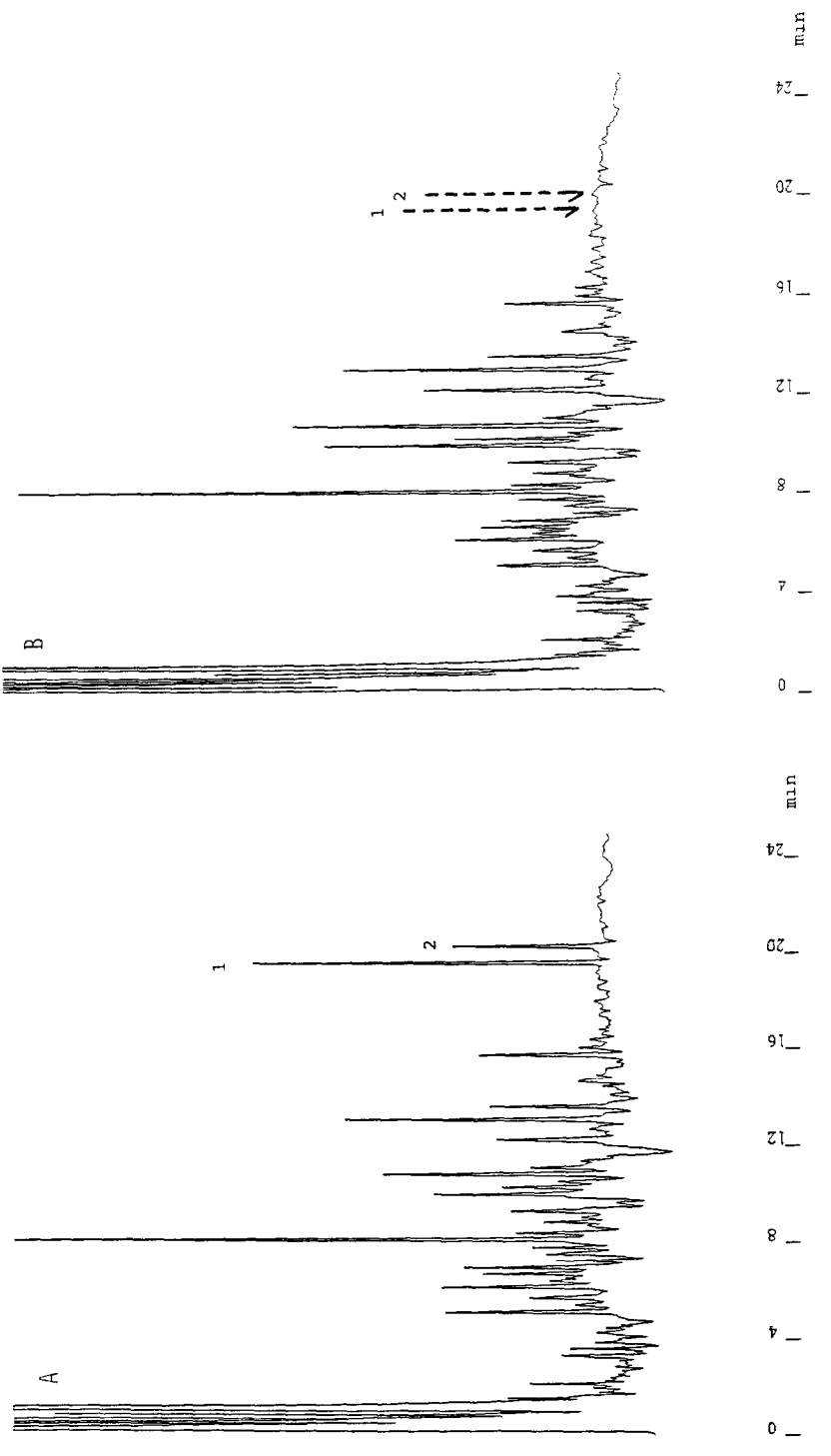

Fig. 1. (A) Chromatogram from plasma spiked with indomethacin. Peaks: 1 = indomethacin ethyl ester; 2 = indomethacin propyl ester (internal standard). Peak 1 corresponds to about 40 ng of indomethacin in 1 ml of plasma. (B). Chromatogram from blank plasma. Retention times of indomethacin (1) and internal standard (2) are indicated by dotted lines. For conditions, see text.

RESULTS AND DISCUSSION

For the extraction of indomethacin from biological fluids before gas chromatographic analysis, liquid–liquid extraction methods were used in all previous studies [1–9], but they are not suitable for routine analysis because the phase separation is time consuming and often results in emulsion formation. We investigated the solid-phase extraction of indomethacin from plasma using a disposable pretreatment column. In order to obtain the carboxylic group of indomethacin in the free form, 1 ml of 0.1 *M* hydrochloric acid was added to the plasma sample and the pH of the sample solution was adjusted to 4–5. Under these conditions, the absolute recovery of indomethacin from the column was about 87% (mean value, $n=9$). The accuracy and reproducibility of this method are shown in Table I.

Several reagents for the esterification of indomethacin have been reported, such as diazomethane [1,2], diazoethane [3–5], diazopropane [6], pentafluorobenzyl bromide [7] and pentafluoro-1-propanol [8]. However, diazoalkanes and fluorine-containing reagents are very poisonous. The reagent proposed here, 1-ethyl-3-*p*-tolyltriazene, is comparatively low in toxicity and stable in diethyl ether solution for a long period. The esterification of indomethacin using this reagent proceeded completely in 4–6 h at room temperature. Derivatized indomethacin ethyl ester was stable in diethyl ether solution for at least 24 h.

Typical chromatograms obtained from control plasma spiked with indomethacin and blank plasma are shown in Fig. 1. Peaks of coexisting substances in plasma and reagent do not interfere with quantification because of the excellent separation with the capillary column. Good selectivity and accuracy in the determination of indomethacin were achieved.

A linear calibration graph passing through the origin was obtained in the range 2–50 ng/ml indomethacin in plasma and the correlation coefficient was 0.9995. The detection limit of indomethacin was 2 ng/ml in plasma. The concentration of indomethacin in human plasma after external application is less

TABLE I

REPRODUCIBILITY AND ACCURACY OF THE PROPOSED METHOD FOR DETERMINATION OF INDOMETHACIN

Indomethacin added (ng/ml)	Indomethacin found (mean, $n=3$) (ng/ml)	Relative standard deviation (%)
2.1	2.23	6.8
10.0	9.90	6.1
50 0	50.00	2.7

than 20 ng/ml, hence this method is very useful for monitoring lower plasma levels of indomethacin.

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