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Note

High-performance liquid chromatographic determination of indomethacin in human plasma and urine

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Indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid, is a non-steroidal anti-inflammatory drug widely used in the treatment of arthritis Recently, indomethacin has also been found effective in the treatment of neonates with patent ductus arteriosus and in patients with acute cystoid macular oedema following cataract surgery [1,2] For these various therapeutic uses indomethacin has been formulated into different dosage forms, including formulations designed for long duration of activity Therefore, interest in the pharmacokinetics and bioavailability of the drug from the various dosage forms has increased and necessitated the development of specific assay methods for biological fluids

Many of the reported methods require tedious sample preparation [3–5], extraction techniques [6–11] or a fluorimeter for detection [12–14] Injection of deproteinized plasma samples into a high-performance liquid chromatographic (HPLC) system has been utilized for the determination of indomethacin in rabbits (concentration range 1–100 μg ml^{-1}) [15] Recently a measurement has been suggested for plasma indomethacin and its prodrug apyramide, an ester of indomethacin and of acetaminophen, by using a simple procedure (solvent-demixing method) [16]

This paper reports a similar simple and rapid method with minimum sample preparation, that is sufficiently sensitive for the determination of indomethacin in both human plasma and urine, after oral administration of a single dose (concentration range 0 1–10 μg ml^{-1} for plasma and 0 1–100 μg ml^{-1} for urine)

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EXPERIMENTAL

Apparatus and chemicals

A Model G-802 high-performance liquid chromatograph (Gilson, Villiers-le-Bel, France) equipped with a variable-wavelength detector (set at 258 nm) and a Gilson NI chart recorder was used. Separation was performed at room temperature on a stainless-steel column (25 cm × 4.5 mm I.D.) packed with Spherisorb 5 μm (C_{18} reversed phase) (Perkin-Elmer, Norwalk, CT, U.S.A.). Analytical samples were introduced on to the column using a 20-μl loop valve (Rheodyne, Cotati, CA, U.S.A.).

The mobile phase was acetonitrile–0.1 *M* sodium acetate (35:65, v/v), the pH of which was adjusted to 6.9 by the dropwise addition of glacial acetic acid. The flow-rate was 2.5 ml min^{-1} and the inlet pressure ca. 20.68 MPa (3000 p.s.i.).

Indomethacin and phenacetin were obtained from Geofarma (Milan, Italy) and Merck (Darmstadt, F.R.G.), respectively. Solvents for HPLC were purchased from Carlo Erba (Milan, Italy). All the reagents were of analytical-reagent grade and were used without further purification.

Internal standard solution

A 10-mg amount of phenacetin was dissolved in 10 ml of methanol. Further dilutions were made with methanol to obtain a 0.5 μg ml^{-1} standard solution.

Sample preparation

Plasma. Samples were prepared by the addition of 0.5 ml of internal standard solution and 1.0 ml of methanol to 0.5 ml of heparinized plasma. The samples were mixed and centrifuged at 2105 *g* for 10 min and a 20-μl aliquot of the supernatant was injected onto the HPLC column.

Urine. Samples of 1.0 ml were analysed after the hydrolysis of the glucuronides of indomethacin by the addition of 4 *M* hydrochloric acid (100 μl). After vortex-mixing, the acid-treated samples were left to stand for 10 min at room temperature and 1.0 ml of internal standard solution was added. No precipitate was formed on the addition of hydrochloric acid and methanolic internal standard solution. After mixing, 20 μl were injected onto the column. The peak-height ratio method was used to calculate the concentration of indomethacin by reference to the internal standard from previously prepared calibration graphs. Drug-free plasma and urine spiked with indomethacin over the range 0.1–10 and 0.1–100 μg ml^{-1}, respectively, were employed for the preparation of the calibration graphs.

RESULTS AND DISCUSSION

The retention times of phenacetin (internal standard) and indomethacin were 3.4 and 4.0 min, respectively. In the chromatograms of plasma from a

volunteer following the oral administration of 50 mg of indomethacin (Fig. 1A) and in the corresponding chromatograms of urine (Fig 1B), no interfering peaks due to endogenous constituents were observed Particularly with urine no interfering peaks were observed either with or without the acid treatment Acid treatment was applied in order to hydrolyse the ester-linked glucuronides and the ether-linked glucuronide conjugates of O-demethylated indomethacin Also, acid hydrolysis is to be preferred to alkaline hydrolysis, as it avoids possible analytical problems associated with the lability (N-deacylation) of the indomethacin molecules on alkali treatment [2,12]

A series of calibration graphs of the indomethacin to internal standard peak-height ratios was prepared over the concentration ranges 0 1–10 and 0.1–100 $\mu g\ ml^{-1}$ for plasma and urine, respectively. All calibration graphs were linear and almost passed through the origin The regression equations were $x = 1\ 795(y - 0\ 007)$ and $x = 1\ 557(y + 0\ 106)$ for plasma and urine, respectively, where x is the indomethacin concentration ($\mu g\ ml^{-1}$) and y is the indomethacin-to-phenacetin peak-height ratio The correlation coefficients (r) were 0 999 or better for at least eight points

The reproducibility and precision of the method were examined by repeated

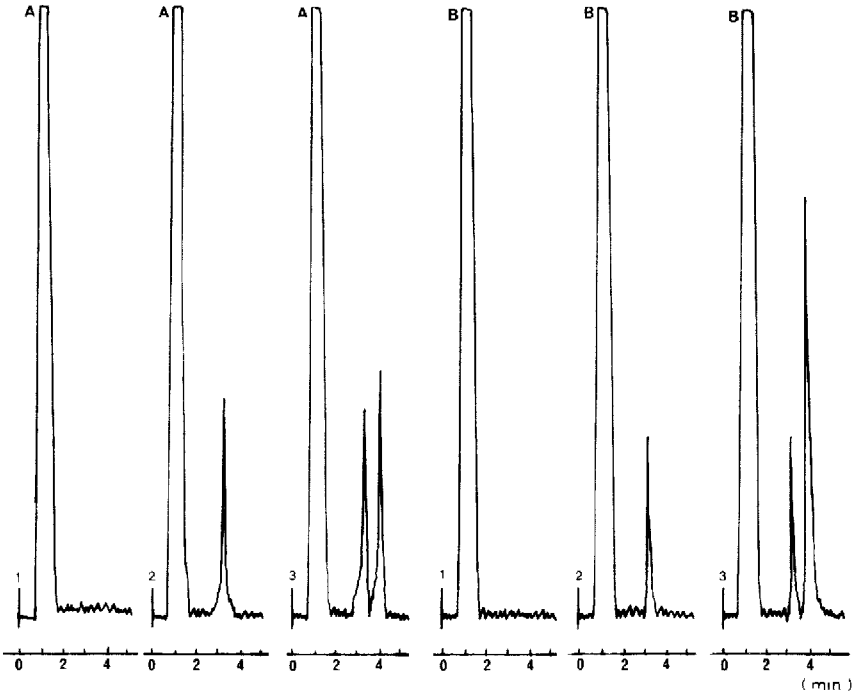

Fig 1 Chromatograms of (A) plasma and (B) urine samples 1, Blank, 2, with internal standard (0 5 $\mu g\ ml^{-1}$), 3 from a volunteer 2 h after oral administration of 50 mg of indomethacin (2 4 and 3 7 $\mu g\ ml^{-1}$)

TABLE I

REPRODUCIBILITY OF INDOMETHACIN DETERMINATION IN PLASMA AND URINE SAMPLES ($n = 10$)

Sample	Nominal concentration ($\mu g\ ml^{-1}$)	Determined concentration (mean ± S D) ($\mu g\ ml^{-1}$)	Relative standard deviation (%)
Plasma	2 00	2 00 ± 0 08	4 00
	4 00	3 99 ± 0 15	3 75
Urine	2 00	2 00 ± 0 02	0 98
	4 00	4 01 ± 0 02	0 52

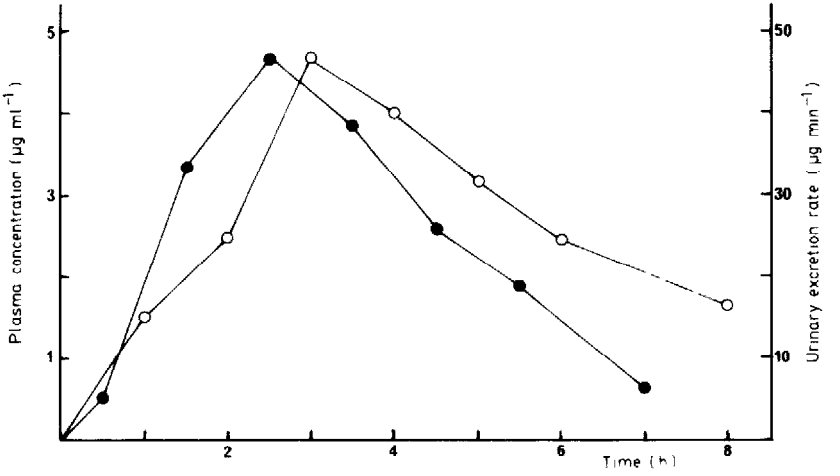

Fig 2 Plasma indomethacin concentration versus time curve (○) and urinary excretion rate versus time curve (●) following oral ingestion of 50 mg of indomethacin (as a hard capsule) by a normal volunteer

analyses of ten plasma and urine samples spiked with indomethacin at concentrations of 2 and 4 $\mu g\ ml^{-1}$ and the results are given in Table I The relative standard deviations in Table I and the correlation coefficients (r) for the regression equations (0 999 or better) indicate that the method is sufficiently precise for the determination of indomethacin in plasma and urine for biopharmaceutical and therapeutic studies The typical assay time was less than 5 min, therefore, about fifty plasma or urine samples can be examined within a working day For the determination as little as 0 1 ml of plasma can be used, with a proportional reduction in the volume of the internal standard solution and the methanol added to the samples

The method is currently being used for determinations of indomethacin in both plasma and urine for a pharmacokinetic and bioequivalence investigation

of controlled-release formulations Representative results for plasma concentration and urinary excretion rate versus time for a normal volunteer after oral administration of 50 mg of indomethacin are shown in Fig 2

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