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Letter to the Editor

High-performance liquid chromatographic determination of ibuprofen in human plasma and urine by direct injection

Sir,

The majority of the reported methods for the analysis of ibuprofen in plasma require tedious sample preparation and extraction techniques before high-performance liquid chromatography (HPLC) may be carried out [1—9]. Deproteinization of plasma followed by direct injection onto the HPLC system has been utilized by both Ali et al. [10] and Shah and Jung [11], the latter method requiring only 25 μl of plasma. The present report describes a similar method for the analysis of ibuprofen in both plasma and urine.

EXPERIMENTAL

Chemicals

Ibuprofen and indomethacin were supplied by Temmler-Werke (Marburg, F.R.G.), solvents for HPLC were purchased from Koch-Light (Haverhill, U.K.), all other reagents were of analytical grade or better and were used without further purification.

Chromatography

HPLC employed a Perkin-Elmer Series 10 chromatograph equipped with a Rheodyne injector, fitted with a 20-μl loop, linked to an LC-75 UV variable-wavelength detector, set at 220 nm, and a Perkin-Elmer R-100 chart recorder. Chromatography was carried out using a stainless-steel column (250 × 4.5 mm) packed with LiChrosorb 10 μm (C_{18} reversed-phase) (Perkin-Elmer) and the mobile phase was acetonitrile—0.1 *M* sodium acetate (35:65) the pH of which was adjusted to 6.2 by the dropwise addition of glacial acetic acid, flow-rate 3 ml min^{-1} at ambient temperature. The retention times of ibuprofen and indomethacin (internal standard) under these conditions were 5.2 and 3.8 min, respectively.

Sample preparation

Samples for plasma analysis were prepared by the addition of 0.5 ml of

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internal standard solution (indomethacin, 40 μg ml^{-1} in methanol) and methanol (1.0 ml) to heparinized plasma (0.5 ml). The samples were mixed and centrifuged at 300 *g* for 10 min and a 20-μl aliquot of the supernatant was injected onto the HPLC column.

Urine (1.0 ml) samples from subjects after the oral administration of ibuprofen were analysed as such and following the addition of 1 *M* sodium hydroxide (100 μl) to hydrolyse the ester glucuronide of ibuprofen. The alkali-treated samples were vortex-mixed and left to stand for 2 h at room temperature, after this time 100 μl of 4 *M* hydrochloric acid were added, followed by 1.0 ml of internal standard solution (indomethacin, 20 μg ml^{-1}). After mixing 20 μl of the whole was injected onto the column.

The quantity of ibuprofen present in both plasma and urine was estimated by comparison of the peak-height ratio of drug to internal standard with previously prepared calibration curves, using drug-free plasma and urine spiked with ibuprofen over the range 0.5—100 μg ml^{-1}.

RESULTS AND DISCUSSION

Typical chromatograms of drug-free plasma, and plasma from a volunteer following the oral ingestion of 200 mg ibuprofen are shown in Fig. 1A, corresponding chromatograms from the analysis of urine samples are shown in Fig. 1B. No interfering peaks were observed in the chromatograms owing to endogenous constituents in plasma or in urine, either before or after alkali treatment to hydrolyse the ester glucuronide of ibuprofen. The use of alkaline rather than enzymatic hydrolysis of 1-O-acyl glucuronides circumvents analytical problems associated with the intramolecular acyl migration of the carboxylic acid moiety to yield β-glucuronidase insensitive glucuronic acid esters [12, 13]. In agreement with previous studies [14] we were able to detect only trace quantities

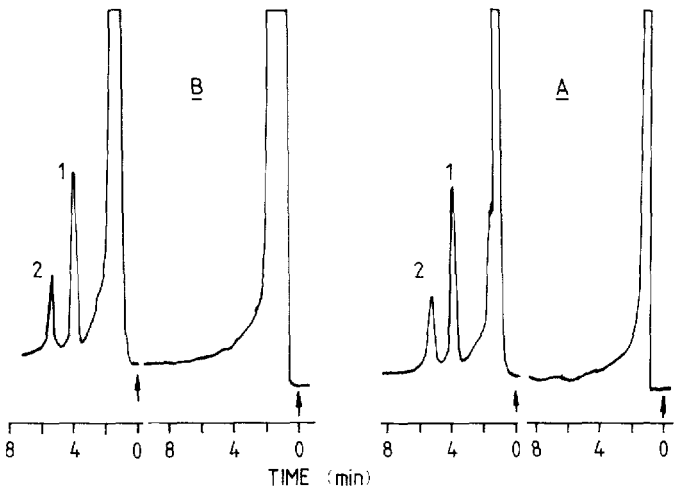

Fig. 1. Chromatograms obtained from (A) drug-free plasma and a plasma sample from a volunteer 2 h after the oral administration of 200 mg ibuprofen and (B) drug-free urine after alkali treatment and a similarly treated urine sample from a volunteer 5 h after the oral administration of ibuprofen. Peaks: 1 = indomethacin (internal standard) (3.8 min); 2 = ibuprofen (5.2 min).

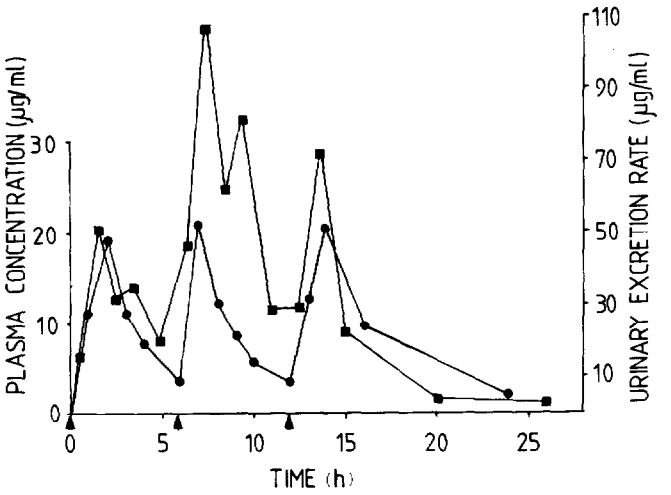

Fig. 2. Plasma ibuprofen concentration—time curve (•) and urinary excretion rate—time curve (▪) (both free and conjugated ibuprofen) following the repeated oral administration of 200-mg tablets of ibuprofen to a normal healthy volunteer (arrows indicate times of drug administration).

of non-conjugated ibuprofen in the urine of volunteers after oral drug administration.

A series of standard curves of ibuprofen to internal standard peak-height ratios were prepared over a concentration range of 0.5—100 $\mu g\ ml^{-1}$ ibuprofen in both plasma and urine. All standard curves were linear over the range examined and almost passed through the origin, the intercept values being between 8—10% of the lowest peak-height ratio examined and regression coefficients of these curves were 0.999 or better. The reproducibility of the method was examined by the repeated analysis of ten plasma samples spiked with ibuprofen at concentrations of 8.0 and 40 $\mu g\ ml^{-1}$, the results obtained (mean ± S.D.) were 8.0 ± 0.06 and 39.9 ± 0.49 $\mu g\ ml^{-1}$, respectively.

The present method allows the rapid analysis of ibuprofen in both plasma and urine, e.g. some 40 plasma samples may be examined within a working day, at a sensitivity which is suitable for the plasma levels of the drug reported in biopharmaceutical and therapeutic studies i.e. 1.0—66 $\mu g\ ml^{-1}$ [15, 16]. By proportional reduction in the volume of internal standard solution and methanol added to the plasma samples, ibuprofen concentrations may be determined in 0.1 ml. The application of the method described above to the analysis of ibuprofen in both plasma and urine following oral drug administration to a human volunteer is shown in Fig. 2.

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