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## Note

# High-performance liquid chromatographic determination of ibuprofen in rat and human plasma

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Ibuprofen is a clinically important non-steroidal anti-inflammatory analgesic and antipyretic drug widely used in the treatment of several forms of arthritis and for mild to moderate pain. A number of methods for the analysis of ibuprofen in biological fluids have been published; included are paper chromatography [1], gas chromatography [2–8] and gas chromatography–mass spectroscopy [9–11]. These methods are very time-consuming and/or possess inadequate sensitivity for pharmacokinetic determinations.

High-performance liquid chromatographic (HPLC) techniques [12–20] have been reported for the determination of ibuprofen and appear to offer the most useful methodologies. Most of these methods require 0.5–1.0 ml plasma and still lack adequate sensitivity. Characterization of the pharmacokinetics of ibuprofen in rats requires a highly sensitive drug assay utilizing small sample volumes. Therefore, a simple, accurate and sensitive HPLC method has been developed which requires only 100 µl of rat or human plasma and which is suitable for the determination of large numbers of blood samples in pharmacokinetic studies.

## EXPERIMENTAL

### *Materials*

The equipment for HPLC consisted of a Waters Model M45 solvent delivery system, a Model 710B Waters Intelligent Sample Processor and a Model 481

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variable-wavelength ultraviolet absorbance detector (Waters Assoc., Milford, MA, U.S.A.). Data analysis was performed by a Model 3390A reporting integrator (Hewlett Packard, Avondale, PA, U.S.A.) measuring peak areas. A 25 cm × 0.46 cm I.D. stainless-steel column packed with 5 μm $C_{18}$ particles (Alltech Assoc., Deerfield, IL, U.S.A.) protected by a 4 cm × 0.46 cm guard column hand-packed with 30–40 μm reversed-phase material was used. HPLC-grade acetonitrile and extraction solvents were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.). Analytical standards were obtained from Sigma (St. Louis, MO, U.S.A.).

*Preparation of standards*

Standard solutions of ibuprofen were 100, 10 and 1.0 μg/ml in 0.01 *M* phosphate buffer at pH 6.0, containing 1.0, 0.1 and 0.01% of acetonitrile, respectively. Internal standard solutions of ketoprofen and mefenamic acid were 10 μg/ml in 0.01 *M* phosphate buffer at pH 6.0, containing 0.1% of acetonitrile for ketoprofen and 0.1% of 1.0 *M* sodium hydroxide for mefenamic acid. Standard solution was added to rat plasma and to human plasma to provide concentrations of 50–50 000 and 250–50 000 ng/ml ibuprofen, respectively.

*Extraction procedure*

Samples of plasma (100 μl) were placed in 125 mm × 16 mm glass centrifuge tubes followed by the addition of internal standard (30 μl of ketoprofen solution for rat samples or 75 μl of mefenamic acid solution for human samples). Proteins were precipitated by the addition of 0.05 ml of 4.0 *M* perchloric acid. Tubes were vortex-mixed, followed by the addition of 0.5 *M* phosphate buffer at pH 2.0 to obtain a final volume of 2 ml. Tubes were again vortex-mixed, followed by the addition of a 5-ml mixture of isooctane–isopropanol (90:10, v/v). The tubes were capped, vortex-mixed and shaken for 15 min at high speed and then centrifuged for 10 min at 2000 *g*. The upper organic phase was transferred by a Pasteur pipette to a disposable 15-ml culture tube and evaporated to dryness at ambient temperature under a stream of nitrogen gas.

*Chromatography*

The extraction residue was reconstituted with 300 μl of mobile phase, vortex-mixed for 15 s and transferred to a disposable 300-μl polypropylene injection tube. Injection volumes ranged from 15 to 200 μl. The mobile phase for analysis of rat plasma samples consisted of 0.04 *M* phosphate buffer at pH 7.0–acetonitrile (73:27, v/v). For the quantitation of human plasma samples, a slight modification of the mobile phase to 0.04 *M* phosphate buffer at pH 8.0–acetonitrile (69:31, v/v) was necessary. Ibuprofen and internal standards were eluted at a flow-rate of 1.1 ml/min and monitored at 223 nm. The detector was set at 0.01 and 0.05 a.u.f.s. for rat and human plasma samples, respectively. Chromatography was performed at ambient temperature.

*Quantitation*

Standard curves were prepared by plotting the peak-area ratio (drug/internal standard) versus concentration of the drug. Slopes were determined using a linear regression analysis weighted $1/y$. Use of this weighting factor generated a normal distribution of weighted residuals around the standard curve over the entire range of drug concentrations.

*Extraction recoveries*

The assay recovery was assessed at 200 and 20 000 ng/ml for rat plasma and at 750 and 20 000 ng/ml for human plasma. The peak areas from six extracted plasma samples (0.1 ml) and from six direct injections of the same amount of drug in mobile phase were compared. The assay recovery of each compound was computed using the following equation:

$$\text{recovery} = \frac{\text{peak area, extracted drug}}{\text{mean peak area, direct injection}} \times 100\%$$

RESULTS

Fig. 1 depicts chromatograms corresponding to the extracts of (A) 0.1 ml of blank rat plasma and (B) a sample taken 6 h after an intravenous bolus administration of 25 mg/kg ibuprofen. The calculated concentration in this sample is 4.7 μg/ml. Fig. 2 shows typical chromatograms of (A) blank human plasma and (B) a sample taken 8 h following administration of a 400-mg ibuprofen

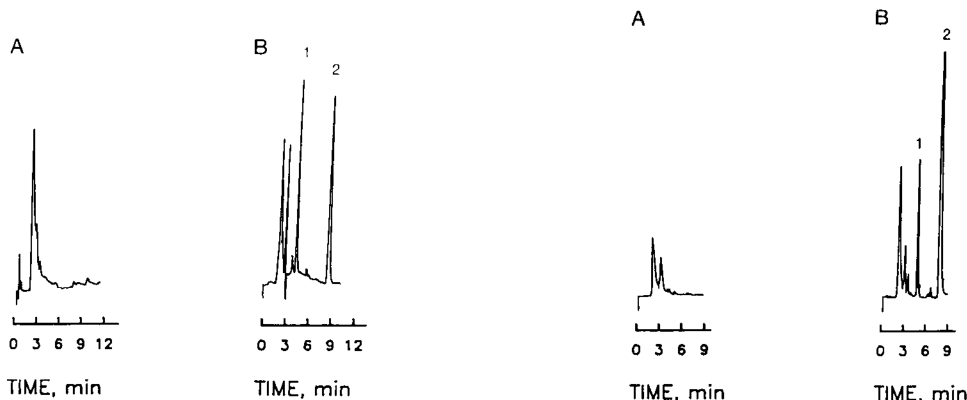


Fig. 1. Typical chromatograms for internal standard, ketoprofen (1), and ibuprofen (2) in rat plasma. (A) Blank plasma; (B) sample taken 6 h after an intravenous dose of 25 mg/kg.

Fig. 2. Typical chromatograms for ibuprofen (1) and internal standard, mefenamic acid (2), in human plasma. (A) Blank plasma; (B) sample taken 8 h after administration of a 400-mg tablet.

TABLE I

ASSAY PRECISION

| Sample | Concentration (ng/ml) | Coefficient of variation (%) | |
|---|---|---|---|
| | | Intra-day | Inter-day |
| Rat plasma | 200 | 6.76 | 5.08 |
| | 20 000 | 1.45 | 0.89 |
| Human plasma | 750 | 7.00 | 5.03 |
| | 20 000 | 1.48 | 2.84 |

tablet (Motrin, Upjohn) to a normal adult male volunteer and represents a concentration of 3.3 μg/ml. Each compound eluted with a sharp peak and distinct separation at baseline. Drug-free plasma samples were consistently free of endogenous contaminants at the retention times corresponding to ketoprofen and ibuprofen (4.7 and 9.5 min) in rat plasma and to ibuprofen and mefenamic acid (4.9 and 8.2 min) in human plasma. Extraction recovery of ibuprofen was independent of concentration with a mean recovery of 98%. The recovery of internal standards from plasma was 91 and 97% for ketoprofen and mefenamic acid, respectively. Coefficients of variation for extraction recovery were less than 7%. The limit of quantitation for the assay in rat plasma was 50 ng/ml with a coefficient of variation of 2.35%. The limit of quantitation for the human plasma assay was 250 ng/ml with a coefficient of variation of 6.20%. Calibration plots of peak-area ratio versus drug concentration were linear over the range 50–50 000 and 250–50 000 ng/ml for rat and human plasma, respectively.

The intra- and inter-day precision of the method was determined by analysis of six plasma samples containing high (20 000 ng/ml) and low (200 ng/ml for rat plasma and 750 ng/ml for human plasma) concentrations. To determine inter-day variability, the quality control samples were divided into 0.1-ml aliquots and frozen. Over a period of six assay days, high and low samples were thawed and assayed. The results are presented in Table I. The low coefficients of variation indicate good stability of frozen plasma samples and reproducibility of the assay over this period of time. Accuracy, calculated by comparing the results from the precision study to the known values, measured less than 2% in each case.

## DISCUSSION

The HPLC method outlined is efficient, precise and sensitive. The low limits of quantitation, 50 ng/ml for rat plasma and 250 ng/ml for human plasma, are achieved in 0.1-ml samples by use of the optimal wavelength of 223 nm. As

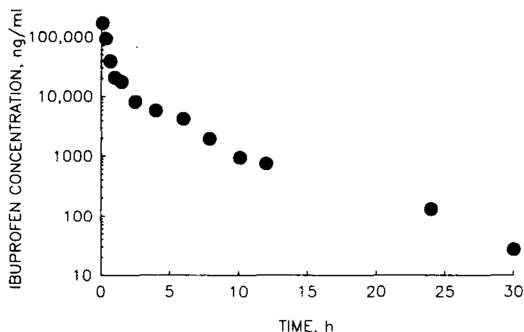


Fig. 3. Plasma concentration-time profile in a rat given a 25 mg/kg intravenous dose of ibuprofen.

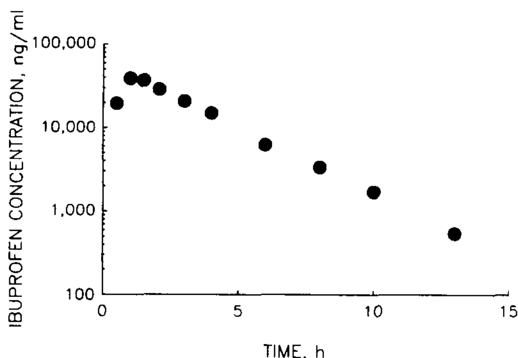


Fig. 4. Plasma concentration-time profile following administration of a 400-mg ibuprofen tablet to a normal adult male volunteer.

Figs. 1 and 2 illustrate, there are no detectable interfering peaks, thus resulting in the lower limits of sensitivity.

The extraction procedure for ibuprofen from rat and human plasma was identical. However, the determination of ibuprofen in rat and human plasma required a slight variation in the mobile phase and internal standard. These differences were necessary to attain complete resolution of drug and internal standard from endogenous interferences. Both mobile phases eluted ibuprofen and internal standard with retention times of less than 10 min.

The determination of ibuprofen by this method has been applied to a series of animal studies using rats and may be used to analyze human plasma samples from clinical studies. Typical plasma concentration versus time profiles obtained with this assay are shown in Fig. 3 for rat plasma and in Fig. 4 for human plasma.

The HPLC procedure reported has proven to be simple, reliable and sufficiently sensitive for the complete pharmacokinetic characterization of ibupro-

fen in rat plasma utilizing a small sample volume. With slight modifications, the method is also applicable for clinical use in pharmacokinetic studies.

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