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Note

High-performance liquid chromatographic determination of ketoprofen and naproxen in rat plasma

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Ketoprofen and naproxen, two arylpropionic acid derivatives, are clinically important non-steroidal anti-inflammatory drugs. A number of methods for the analysis of these drugs in biological fluids have been published; included are thin-layer or paper chromatography [1–3], gas chromatography after derivatization [4–6], spectrophotometry [1, 2], radioisotope tracing [3], colorimetry or polarography [4] and combinations of these techniques. The methods are very time-consuming and/or possess inadequate sensitivity for pharmacokinetic determinations.

Several high-performance liquid chromatographic (HPLC) assays for ketoprofen and naproxen have been reported which represent an improvement in convenience [7–10]; however, these also lack suitable sensitivity. Characterization of the pharmacokinetics of ketoprofen and naproxen in rats requires a highly sensitive drug assay utilizing small sample volumes. A sensitive assay for the determination of ketoprofen in human serum has been developed by Oka et al. [11]. This procedure, however, utilizes a tedious and time-consuming two-column method and requires large sample volumes for increased sensitivity. Upton et al. [12] reported an HPLC assay for the separation of ketoprofen and naproxen in human plasma with adequate sensitivity for 1.0-ml samples. This assay has been substantially modified, resulting in a simple, accurate and sensitive HPLC method which requires only 100 μl of rat plasma and which is suitable for the determination of large numbers of blood samples in pharmacokinetic studies.

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EXPERIMENTAL

Materials

The equipment for HPLC consisted of a Waters Model M45 solvent delivery system, a Model 710B WISP autosampler and a Model 481 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 mobile phase 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 ketoprofen and naproxen 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. Standard solution was added to rat plasma to provide concentrations of 10–50 000 ng/ml and 5–50 000 ng/ml for ketoprofen and naproxen, respectively.

Extraction procedure

Samples of plasma (100 μl) were placed in 17 mm × 100 mm polypropylene centrifuge tubes and 50 μl of a 10 μg/ml solution of internal standard were added. Naproxen was used as the internal standard for the assay of ketoprofen and ketoprofen as the internal standard for naproxen assay. The pH of each sample was adjusted by addition of 0.2 ml of 1.0 *M* phosphate buffer at pH 2.0. Tubes were vortexed, followed by the addition of 5 ml diethyl ether. The tubes were capped and shaken for 15 min at low speed and then centrifuged for 5 min at about 2000 *g*. The upper organic phase was transferred by Pasteur pipette to a disposable 15-ml culture tube and evaporated to dryness at 30°C under a stream of nitrogen gas.

Chromatography

The extraction residue was reconstituted with 300 μl of mobile phase, vortexed for 15 s and transferred to a disposable 300-μl polypropylene injection tube. Injection volumes ranged from 15 to 200 μl. The mobile phase was 0.01 *M* phosphate buffer (pH 7.0)–acetonitrile (82.5:17.5). Utilizing Alltech columns, the percentage of organic solvent varied slightly from one column to the next (16.5–18.5%). Ketoprofen and naproxen were eluted at a flow-rate of 1.5 ml/min and monitored at 258 and 229 nm. The detector was set at 0.005 a.u.f.s. 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 lin-

ear 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 75 and 20 000 ng/ml for ketoprofen and at 40 and 20 000 ng/ml for naproxen. The peak areas from ten extracted plasma samples (0.1 ml) and from ten 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 rat blank plasma and (B) a sample which was taken 4 h after an intravenous bolus administration of 2.5 mg/kg ketoprofen. The calculated concentration in this sample is 1.1 μg/ml. Fig. 2 shows typical chromatograms of (A) rat blank plasma and (B) a sample which was taken 18 h after an intravenous bolus administration of 25 mg/kg naproxen and represents a concentration of 1.1 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 naproxen and ketoprofen (8.6 and 10.6 min).

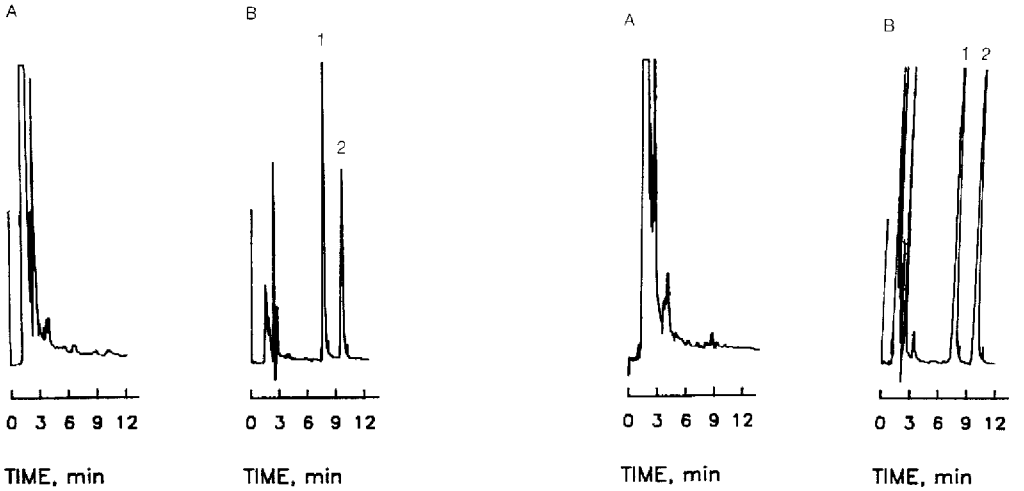

Fig. 1. Typical chromatograms for internal standard, naproxen (1), and ketoprofen (2) in rat plasma. (A) Blank plasma; (B) sample taken 4 h after an intravenous dose of 2.5 mg/kg.

Fig. 2. Typical chromatograms for naproxen (1) and internal standard, ketoprofen (2), in rat plasma. (A) Blank plasma; (b) sample taken 18 h after an intravenous dose of 25 mg/kg.

TABLE I

ASSAY PRECISION

Compound	Concentration (ng/ml)	Coefficient of variation (%)	
		Intra-day	Inter-day
Ketoprofen	75	4.47	6.44
	20 000	3.09	3.94
Naproxen	40	3.13	2.68
	20 000	1.90	2.60

At high and low concentrations, the recovery of both compounds from plasma was approximately 100% with coefficients of variation less than 8%. The sensitivity limit of the assay, determined by a signal-to-noise ratio of 3:1, was 5 ng/ml for naproxen and 10 ng/ml for ketoprofen. Calibration plots of peak-area ratio versus drug concentration were linear over the ranges 5–50 000 and 10–50 000 ng/ml for naproxen and ketoprofen, respectively.

The intra- and inter-day precision of the method was determined by analysis of ten plasma samples containing high (20 000 ng/ml) and low (75 ng/ml for ketoprofen and 40 ng/ml for naproxen) concentrations. To determine inter-day variability, the quality control samples were divided into 0.1-ml aliquots and frozen. Over a period of ten 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, was greater than 95% in each case.

Assay specificity was measured by comparing retention times of standards to those of samples. An additional assurance of specificity was provided by comparing the 258 nm/300 nm peak-area ratios of ketoprofen samples to the ratio of standards. Likewise for naproxen, ratios at 229 nm and 216 nm were compared.

DISCUSSION

The determination of ketoprofen and naproxen by this HPLC method is efficient, precise and sensitive. The optimal wavelengths (258 and 229 nm) for detection of ketoprofen and naproxen were employed which decreased sample size requirements yet improved upon previously reported methods. The limit of quantitation, 10 ng/ml for ketoprofen and 5 ng/ml for naproxen, are in a 0.1-ml sample. Greater sensitivity (1 and 0.5 ng/ml) may be achieved using 1.0 ml of plasma. As Figs. 1 and 2 illustrate there are no detectable interfering peaks, thus resulting in the lower limits of sensitivity.

The determination of ketoprofen and naproxen by this method has been applied to a series of animal studies using rats. Typical plasma concentration versus time profiles obtained with this assay can be seen in Fig. 3 for ketoprofen and in Fig. 4 for naproxen.

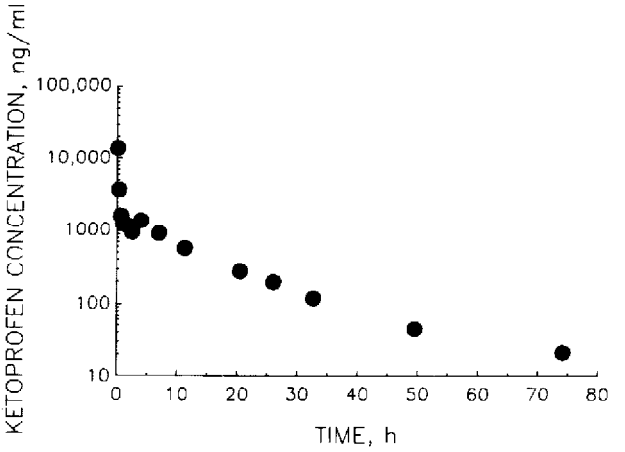

Fig. 3. Plasma concentration–time profile of a rat given a 2.5 mg/kg intravenous dose of ketoprofen.

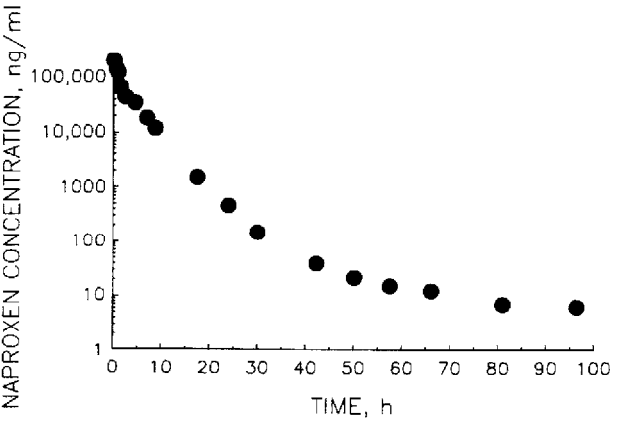

Fig. 4. Plasma concentration–time profile of a rat given a 25 mg/kg intravenous dose of naproxen.

The HPLC procedure reported here has proven to be simple, concise and reproducible. The highly sensitive nature of this assay allows the pharmacokinetic characterization of the two compounds utilizing a small sample volume.

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