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Note

Improved high-performance liquid chromatographic assay of ibuprofen in plasma

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Ibuprofen is a widely used over-the-counter non-steroidal anti-inflammatory, antipyretic and analgesic [1—3] which is used primarily to treat rheumatoid arthritis. A variety of gas—liquid chromatographic (GLC) [4—10] and gas chromatographic—mass spectrometric (GC—MS) [11—13] methods for measuring ibuprofen in plasma or serum have been reported. Many of these assay methods involve complex extraction procedures and derivatization steps. The few GLC methods that do not use derivatization reactions, however, require at least 1 ml or more of plasma. Thus, such methods render a pharmacokinetic study of ibuprofen in small animals (e.g., mice and rats), neonates and children difficult. Many high-performance liquid chromatographic (HPLC) methods have also been reported [14—25]. Some of these methods [14, 17, 18, 20—22, 25] require 0.5—1 ml plasma and involve extraction procedures which are often time-consuming and cumbersome. Two methods [23, 24] using 50—100 μl plasma require extraction procedures. Although one method [19] uses a simple direct deproteinization method with methanol, it requires 0.5 ml of plasma. We describe herein an improved rapid, simple, sensitive and selective HPLC method for the quantitative determination of ibuprofen in plasma using 25 μl plasma or less which makes it well suited for pharmacokinetic studies in small animals as well as in the pediatric population.

EXPERIMENTAL

Apparatus

We used a Model 6000A pump (Waters Assoc., Milford, MA, U.S.A.) and a variable-wavelength ultraviolet absorption detector (Spectromonitor III,

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Laboratory Data Control, Riviera Beach, FL, U.S.A.). Samples were injected into a Model 7125 injector valve equipped with a 100-μl sample loop (Rheodyne, Cotati, CA, U.S.A.). A 30 × 4.6 mm I.D. precolumn with RP-18 packing (10 μm particle size, Brownlee Labs., Santa Clara, CA, U.S.A.) connected to a 250 × 4.5 mm I.D. octadecyl (5 μm particle size) reversed-phase column (IBM, Danbury, CT, U.S.A.) was used at ambient temperature for the assay. Detector output was quantitated using a strip-chart recorder (Model 585, Linear Instruments, Irvine, CA, U.S.A.) at a chart speed of 10 cm/h.

Reagents

Chemicals. Ibuprofen was donated by Upjohn (Kalamazoo, MI, U.S.A.) and mefenamic acid was obtained from Parke, Davis and Co. (Detroit, MI, U.S.A.). The chromatographic solvents, acetonitrile, methanol and 85% phosphoric acid (all HPLC grade), were obtained from Fisher Scientific (Itasca, IL, U.S.A.).

Mobile phase. The mobile phase, acetonitrile—water—methanol—phosphoric acid (58:37:5:0.05, v/v) was filtered through a 0.45-μm membrane and deaerated prior to use.

Procedure

To 25 μl heparinized plasma in a microcentrifuge tube were added 50 μl acetonitrile containing 40 μg/ml mefenamic acid. The mixture was vortex-mixed for 10 sec and centrifuged at 11 000 *g* for 2 min to pellet the precipitated proteins. A 20-μl aliquot of the supernatant was injected onto the column and eluted with the mobile phase at a flow-rate of 1.8 ml/min. The variable-wavelength detector was set at 196 nm with an attenuation of 0.2 a.u.f.s.

Standard curves were prepared by spiking appropriate ibuprofen stock solutions to plasma to yield final concentrations of 1, 5, 25, 50 and 100 μg/ml. They were treated as described above. Peak height ratios of ibuprofen to the internal standard, mefenamic acid, were used to construct the standard curves.

Analytical recovery

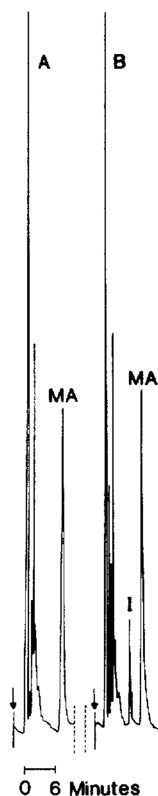
Aqueous solutions containing 5 and 50 μg/ml ibuprofen were prepared and assayed as described above. Results for these standards were compared with those for standards prepared in plasma to determine analytical recovery.

Data analysis

For all standard curves, the data on peak height ratios versus drug concentration were subjected to linear least-squares regression analysis. All results are expressed as mean ± S.D.

RESULTS AND DISCUSSION

Using the described chromatographic conditions, ibuprofen and its internal standard, mefenamic acid, yielded very sharp and well resolved peaks with no interference from endogenous compounds at 5 and 7 min, respectively. Fig. 1 illustrates representative chromatograms of plasma taken before and after intravenous administration of ibuprofen to a male rat.



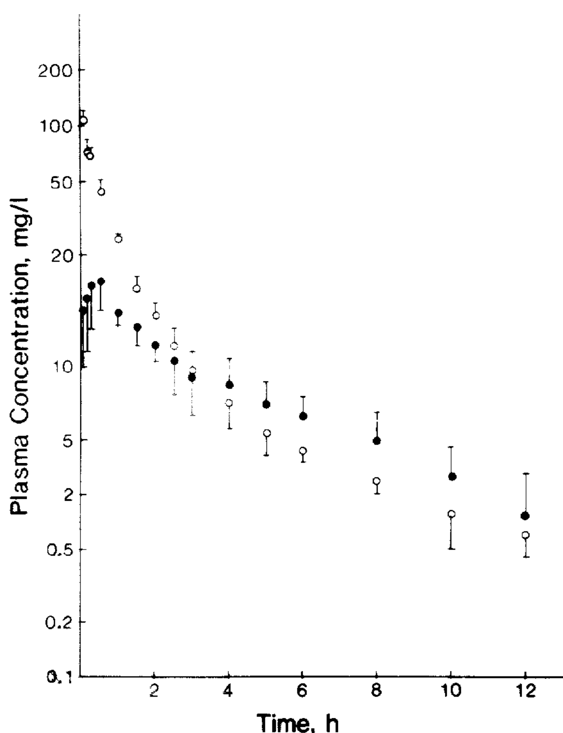


Fig. 1. Chromatogram of ibuprofen (I) in rat plasma. Range = 0.2 a.u.f.s. Arrow indicates when injection was made. (A) Blank rat plasma containing the internal standard, mefenamic acid (MA). (B) Rat plasma 2 h following intravenous injection of 20 mg/kg ibuprofen (8.9 mg/l).

Fig. 2. Plasma concentration versus time curves of ibuprofen following 20 mg/kg intravenously (○) and orally (●).

The standard curves based on peak height ratios of the drug to internal standard were all linear and highly reproducible. Eleven standard curves in plasma with ibuprofen were made at different times over a period of eight weeks. The slope of the peak height ratio of ibuprofen to internal standard versus ibuprofen concentration in plasma was 0.0355 ± 0.0032 l/mg. The coefficient of variation for the slope was 8.9% and the mean coefficient of determination (r^2) was 0.99. The corresponding intercept was 0.053 ± 0.022. The value of the intercept was not significantly different from zero in any of the eleven standard curves. The within-day coefficient of variation performed on five samples each at 5 and 50 μg/ml were 5.1 and 4.4%, respectively. Analytical recovery studies comparing spiked plasma with aqueous standards over a concentration range of 1—100 μg/ml showed an average analytical recovery of 97%. Of the 23 drugs tested (Table I), none interfered with ibuprofen or the internal standard, mefenamic acid.

Only one other HPLC method [19] is available which uses a simple, single one-step deproteinization method for determining ibuprofen in plasma. How-

TABLE I

COMPOUNDS STUDIED FOR CHROMATOGRAPHIC INTERFERENCE

Phenobarbital	Lithium	Quinidine	Chloramphenicol
Primidone	Theophylline	Disopyramide	Salicylate
Carbamazepine	Digoxin	Methotrexate	Acetaminophen
Phenytoin	Lidocaine	Caffeine	Imipramine
Ethosuximide	Procainamide	Gentamicin	Desipramine
Valproic acid	N-Acetylprocainamide	Tobramycin	

ever, since that method uses a fixed-wavelength detector set at 254 nm, it requires 0.5 ml plasma. In the method described herein, the use of a variable-wavelength detector set at 196 nm increased the sensitivity of the assay significantly so that 25 μl or less of plasma is required to determine ibuprofen accurately to a practical limit of 0.5 mg/l in plasma. The small sample size required for this method is especially important in the neonatal or pediatric population or in small animals (e.g. rats) where samples are often difficult to obtain and volume is limited.

The validity of the assay was assessed by determining the plasma concentrations of ibuprofen following intravenous and oral administration of 20 mg/kg (Fig. 2) to male Sprague—Dawley rats. The data were best fit by a biexponential equation with a terminal half-life of 200 min.

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