High-Pressure Liquid Chromatographic Determination of Ibuprofen in Plasma

ANWER ALI, SYED KAZMI, and FOTIOS M. PLAKOGIANNIS [x]

Received April 17, 1980, from the *Division of Pharmaceutics and Industrial Sciences, Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY 11201.* Accepted for publication October 1, 1980.

Abstract ☐ A sensitive, simple, and rapid method for the quantitation of ibuprofen in plasma, using 1-(*p*-fluorobenzoyl)-5-methoxy-2-methylindole acetic acid as the internal standard, was developed. The method is based on reversed-phase high-pressure liquid chromatography with a mobile phase containing acetonitrile–0.1 *M* acetic acid (55:45 v/v). The chromatographic elution time was 8.5 min, and ibuprofen quantities as low as 0.1 μg/ml can be assayed. The suitability of the method is demonstrated.

Keyphrases ☐ Ibuprofen—analysis, high-pressure liquid chromatography, plasma ☐ Anti-inflammatory agents—ibuprofen, high-pressure liquid chromatographic analysis, plasma ☐ High-pressure liquid chromatography—analysis, ibuprofen in plasma

Ibuprofen is a nonsteroidal anti-inflammatory, antipyretic, and analgesic drug (1, 2) indicated for the relief of signs and symptoms of rheumatoid arthritis, osteoarthritis, and mild to moderate pain.

Relatively few methods are available for the measurement of blood ibuprofen levels. A paper chromatographic method involving separation and subsequent reaction with bromcresol purple was reported (3) but requires ~48 hr for completion. GLC methods, requiring extraction of the drug from plasma and tedious and time-consuming derivatization steps, were also reported (4, 5).

Recently, the use of paired-ion, reversed-phase, high-pressure liquid chromatography (HPLC) for quantitative drug analysis was demonstrated (6, 7). This paper reports a simple, rapid, and highly sensitive HPLC method for ibuprofen determination in plasma.

EXPERIMENTAL

Chemicals and Reagents—Ibuprofen[1] and 1-(*p*-fluorobenzoyl)-5-methoxy-2-methylindole acetic acid[2] were the reference and internal standards, respectively. Chromatographic grade acetonitrile[3] and acetic acid[4] were used for the mobile phase preparation. All other reagents were analytical grade or better and were used as received.

Animals—Adult, male New Zealand rabbits, 2–3 kg, were studied.

Mobile Phase—Acetonitrile–0.1 *M* acetic acid (55:45 v/v) was used.

Internal Standard Solution—Ten milligrams of the internal standard, accurately weighed, was dissolved in a 10-ml volumetric flask and diluted to volume with methanol; this solution (1 ml) was diluted to 20 ml with methanol and used as the internal standard.

Standard Solution—Accurately weighed ibuprofen (100 mg) was dissolved in, and diluted to volume with, methanol in a 100-ml volumetric flask.

Plasma Level Study—To demonstrate the applicability of the procedure to the determination of plasma ibuprofen levels from a bioavailability study, suppositories containing 150 mg of ibuprofen were administered rectally to three rabbits. Blood samples (~3 ml) were removed by cardiac puncture at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 24 hr. The samples were placed into microcentrifuge tubes and centrifuged to obtain the plasma fraction. Plasma aliquots (0.5 ml) were processed along with spiked plasma standards.

For standard curves, blood samples were removed from the heart of nonmedicated rabbits, placed in centrifuge tubes, and centrifuged to obtain the plasma fraction. Plasma aliquots (0.5 ml) were pipetted into microtest tubes and spiked with ibuprofen to yield a final drug concentration of 0.1–50 μg/ml of plasma. To each microtest tube was added 0.1 ml of the internal standard. The mixture was mixed, diluted to 2.0 ml with methanol, and filtered through an organic filter[5].

HPLC—Samples were chromatographed on a high-pressure liquid chromatograph[6] equipped with a universal liquid chromatographic in-

Table I—Recovery of Ibuprofen from Spiked Plasma [a]

Amount Added, μg/ml	Ibuprofen Found, %	*SD*, %
50	100.4	0.21
25	99.2	0.38
10	98	0.98
5	96	1.98
2	105	2.50
1	104	0.80
0.9	95.5	0.90
0.5	96	0.80
0.2	110	0.80

[a] Each value is the average of five determinations.

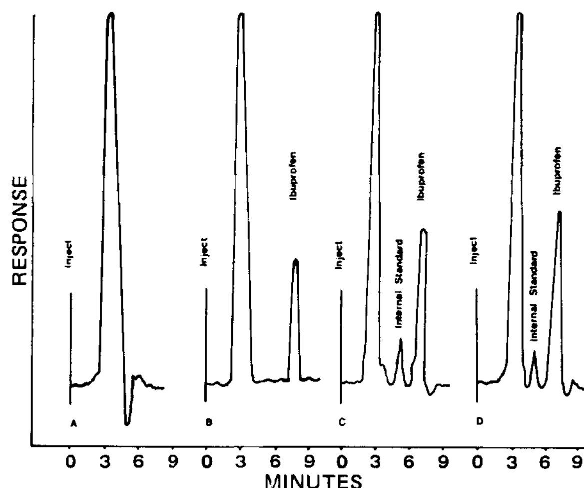

Figure 1—*High-pressure liquid chromatograms of blank plasma (A), blank plasma spiked with ibuprofen (B), blank plasma spiked with ibuprofen and the internal standard (C), and plasma obtained 60 min after ibuprofen administration and spiked with the internal standard (D).*

[1] The Upjohn Co, Kalamazoo, Mich.
[2] Merck Sharp & Dohme, West Point, Pa.
[3] Waters Associates, Milford, Mass.
[4] J. T. Baker Chemical Co., West Rochester, N.Y.

[5] Waters Associates, Milford, Mass.
[6] Model 440, Waters Associates, Milford, Mass.

0022-3549/81/0800-0944$01.00/0
© 1981, American Pharmaceutical Association

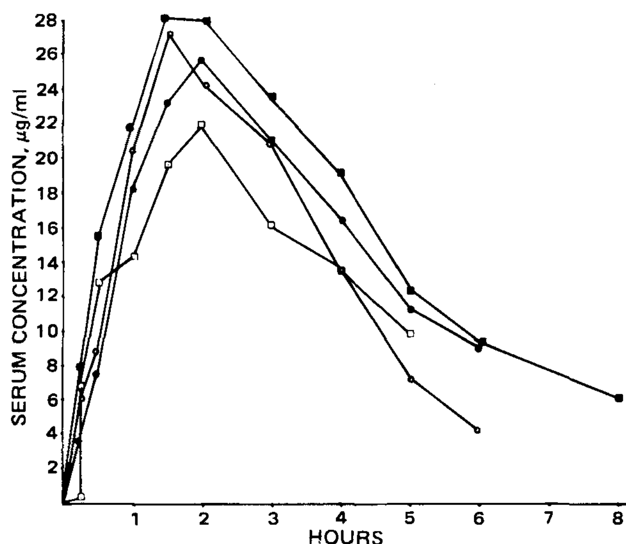

Figure 2—*Serum ibuprofen levels after administration of a 150-mg dose of a suppository of three different bases and one oral preparation. Key:* □, *theobroma oil;* ○, *polyethylene glycol 1540;* ●, *fatty acids; and* ■, *suspension.*

jector[5], a UV (254 nm) absorbance detector, and a strip-chart recorder. The deproteinated plasma samples were chromatographed at room temperature on a microparticulate (μBondapak C_{18}) reversed-phase HPLC column (4 mm × 30 cm) with an eluting mobile phase of acetonitrile—0.1 *M* acetic acid (55:45 v/v). The flow rate was adjusted to 1 ml/min with an inlet pressure of ~1500 psi. The chart speed was 0.2 cm/min. The ratio of the peak height of ibuprofen to that of the internal standard was used to calculate the ibuprofen concentration, based on a calibration curve prepared from spiked plasma samples.

Calculations—Peak height ratios were obtained by dividing the peak height of ibuprofen by the peak height of the internal standard. Calibration curves from known ibuprofen concentrations in plasma were prepared by plotting the peak height ratios *versus* the ibuprofen concentration, expressed as micrograms per milliliter of plasma. Values of unknown concentrations of ibuprofen in plasma samples were read directly from the graph.

RESULTS AND DISCUSSION

Typical chromatograms of blank rabbit plasma (A), blank rabbit plasma spiked with ibuprofen (B), blank rabbit plasma spiked with ibuprofen and internal standard (C), and plasma collected 60 min after administration of 150 mg of ibuprofen in a suppository or oral preparation and spiked with the internal standard (D) are shown in Fig. 1. Ibuprofen and the internal standard were well resolved and eluted with retention times of 8.5 and 5.5 min, respectively. No interference from metabolites was detected under these experimental conditions.

Table I illustrates recoveries of ibuprofen from plasma spiked with 0.1–50 μg/ml. The standard curve is the average of five determinations; the regression line slope was calculated to be 0.128 with a standard correlation matrix of 0.999, indicating excellent linearity.

The plasma ibuprofen time courses for four rabbits in a bioavailability study are shown in Fig. 2. The *AUC* values for the four formulations were 89.77, 74.38, 96.5, and 128.94 μg/ml/hr for 150 mg of ibuprofen in polyethylene glycol 1540, theobroma oil, esterified fatty acids (C_{10}–C_{18})[7], and an oral suspension, respectively.

REFERENCES

(1) S. S. Adams, E. E. Cliffe, B. Lessel, and J. S. Nicholson, *J. Pharm. Sci.*, **56**, 1686 (1967).

(2) S. S. Adams, K. F. McCullough, and J. S. Nicholson, *Arch. Int. Pharmacodyn. Ther.*, **178**, 115 (1969).

(3) S. S. Adams and E. E. Cliffe, *J. Pharm. Pharmacol.*, **17**, 173 (1965).

(4) G. Kaiser and J. Vaniessen, *J. Pharm. Sci.*, **63**, 219 (1974).

(5) R. F. N. Mills, S. S. Adams, E. E. Cliffe, W. Dickenson, and J. S. Nicholson, *Xenobiotica*, **3**, 589 (1973).

(6) "Paired Ion Chromatography," Bull. D61, Waters Associates, Milford, Mass., Dec. 1975.

(7) J. L. Day, L. Tterlikkis, R. Niemann, A. Mobley, and C. Spikes, *J. Pharm. Sci.*, **67**, 1027 (1978).

[7] Witepsol H-15, Kay-Fries Chemicals, Montvale, NJ 07645.

Binding of Prostaglandins E_1 (Alprostadil), E_2 (Dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (Dinoprost) to Human Serum Proteins

JOSEPH JUDIS

Received October 20, 1978, from the *College of Pharmacy, University of Toledo, Toledo, OH 43606.* Accepted for publication January 13, 1981.

Abstract □ Prostaglandins E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (dinoprost) were characterized in terms of binding to human serum, albumin, γ-globulin II, β-globulin III, α-globulin IV-1, and α-globulin IV-4. By using equilibrium dialysis and tritium-labeled ligands, the percent binding for all four ligands was found to decrease in the following order: human serum, albumin, and α-globulin IV-4. For the other three proteins, the order was not consistent with the four ligands, and <10% binding was observed. In general, prostaglandins E_1 and E_2 showed a higher percent binding for all fractions than prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$. All four ligands can be characterized as showing significant binding to human serum, albumin, and α-globulin IV-4.

Keyphrases □ Prostaglandins—binding to human serum proteins, equilibrium dialysis of radiolabeled prostaglandins □ Protein binding—prostaglandins E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (dinoprost) to human serum proteins, equilibrium dialysis of radiolabeled prostaglandins □ Binding, protein—binding of prostaglandins E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (dinoprost) to human serum proteins, equilibrium dialysis of radiolabeled prostaglandins □ Equilibrium dialysis—of radiolabeled prostaglandins E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (dinoprost) bound to human serum proteins

Since the discovery and description of the prostaglandins, a vast literature has evolved concerning their physiological and pharmacological actions (1). As circulating hormones, these compounds have different rates of disappearance from the circulation after injection. Presumably, the differences reflect differences in metabolic degradation rates (2, 3). The availability of a compound for metabolic degradation could be related to whether or not

0022-3549/81/0800-0945$01.00/0
© 1981, American Pharmaceutical Association