# High-Pressure Liquid Chromatographic Determination of Ibuprofen in Plasma

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**Abstract** □ A high-pressure liquid chromatographic method is presented for the determination of ibuprofen in human plasma. Ibuprofen is extracted from plasma acidified with 1.0 *M* phosphoric acid using hexane containing *p*-phenylphenol as an internal standard. A reversed-phase octadecylsilane column was used with a liquid phase of 65% methanol and 35% 0.10 *M* acetate buffer (pH 5.0). A spectrofluorometric detector with an excitation wavelength of 253 nm and a band pass filter (230–420 nm) provided a detectable peak for 1 μg of ibuprofen/ml of plasma. The effect of the pH and molarity of the mobile phase on the capacity factor was studied.

**Keyphrases** □ Ibuprofen—high-pressure liquid chromatographic determination in human plasma □ High-pressure liquid chromatography—determination of ibuprofen in human plasma □ Anti-inflammatory drugs—clinical determination of plasma levels in humans

Ibuprofen, an arylalkonic acid, is an oral, nonsteroidal anti-inflammatory drug used in the treatment of arthritis and for the relief of mild to moderate pain (1). The wide range of plasma levels encountered during therapy with ibuprofen indicates the need for clinical determination of plasma levels, which may allow maximum therapeutic effect while minimizing adverse affects. The incidence of GI bleeding with ibuprofen is reportedly lower than with aspirin; however, severe bleeding occurs, especially with patients taking anticoagulants or with hematological disorders (2). Monitoring is critical for patients requiring concomitant administration of other antiarthritic drugs with ibuprofen (3).

Several GLC procedures for the quantitative determination of ibuprofen were reported, but the majority require either derivatization or an electron-capture detector (4–7). A high-pressure liquid chromatographic (HPLC) method using UV detection quantitated ibuprofen in dog plasma (8). Recently, an HPLC method using dual UV detectors was reported for screening solid dosage forms for drugs used in the treatment of arthritis (9). Due to the greater specificity and sensitivity of fluorescence detection, a method for the determination of ibuprofen in human plasma that combines HPLC with fluorometry is described. Conditions are discussed for the extraction, separation, and detection of ibuprofen.

## EXPERIMENTAL

**Instrumentation**—The high-pressure liquid chromatograph[1] was equipped with a fluorometric detector[2] and an octadecylsilane column[3], 250 mm long × 4 mm i.d. The degassed mobile phase was pumped through the column at 1.5 ml/min (3700–3800 psi) at ambient temperature until a stable baseline was obtained. The fluorometer was set at an excitation wavelength of 253 nm, and a band pass filter with an entrance transmission of 230–420 nm was used.

**Chemicals and Reagents**—Sodium acetate, sodium phosphate, acetic acid, phosphoric acid, hexane, ethyl acetate, ether, methylene chloride, and chloroform were reagent grade. HPLC-grade methanol[4] was used. Ibuprofen was obtained courtesy of the manufacturer[5], and *p*-phenylphenol was obtained commercially[6].

**Table I—Effect of Buffer Molarity at pH 5 on the Capacity Factor of Ibuprofen and Internal Standard**

| Buffer Molarity | Capacity Factor | |
|---|---|---|
| | Ibuprofen | Internal Standard |
| 0.01 | 2.45 | 1.95 |
| 0.02 | 2.05 | 1.60 |
| 0.03 | 2.00 | 1.65 |
| 0.04 | 2.20 | 1.70 |
| 0.06 | 2.30 | 1.70 |
| 0.08 | 2.25 | 1.55 |
| 0.10 | 2.45 | 1.70 |
| 0.12 | 3.00 | 2.12 |
| 0.15 | 2.93 | 2.06 |

**Mobile Phase**—The mobile phase was prepared by adding an appropriate amount of methanol to the aqueous phase and degassing under vacuum. The aqueous phases consisted of pH 4 and 5 acetate buffers (0.01, 0.02, 0.03, 0.04, 0.06, 0.08, 0.10, 0.12, and 0.15 *M*) and acetic acid solutions (0.01, 0.02, and 0.03 *M*). The effect of various pH levels and concentrations of the aqueous portion of the mobile phase on retention times was studied.

**Stock Solutions**—A solution containing 10 mg of ibuprofen in 25 ml of methanol was prepared fresh monthly. A solution of *p*-phenylphenol was prepared by dissolving 3 mg of *p*-phenylphenol in 100 ml of methanol; it was prepared fresh monthly. A dilution of *p*-phenylphenol was prepared from 1 ml of the original *p*-phenylphenol solution, which was brought to a volume of 100 ml with methanol. The working internal

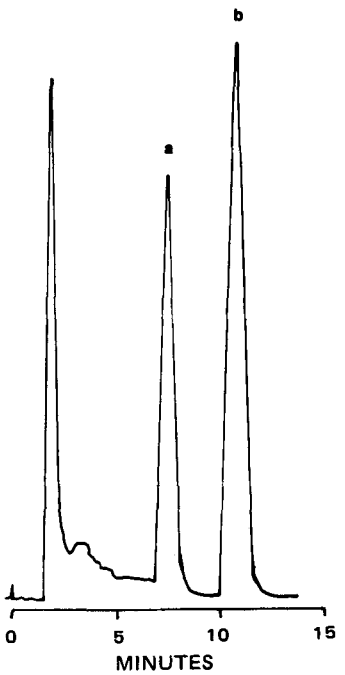


**Figure 1**—*Typical chromatogram of a plasma extract containing ibuprofen (15 μg/ml). Key: a, p-phenylphenol (internal standard); and b, ibuprofen.*

[1] Model 202 chromatograph, M600 pump, and U6K Universal injector, Waters Associates, Milford, Mass.
[2] FS970, Schoeffel, Westwood, N.J.
[3] Dupont, Wilmington, Del.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] The Upjohn Co., Kalamazoo, Mich.
[6] Eastman Organic Chemicals, Rochester, N.Y.



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**Table II—Effect of pH on Capacity Factor of Ibuprofen and Internal Standard**

| Molarity | pH | Capacity Factor | |
|---|---|---|---|
| | | Ibuprofen | Internal Standard |
| 0.01 | 5[a] | 2.45 | 1.95 |
| | 4[a] | 4.25 | 1.65 |
| | 3.37[b] | 4.70 | 2.70 |
| 0.03 | 5[a] | 2.00 | 1.65 |
| | 4[a] | 5.15 | 1.95 |
| | 3.13[b] | 4.15 | 1.40 |

[a] Sodium acetate buffer. [b] Acetic acid.

**Table III—Nonsteroidal Antiarthritics Tested for Possible Interference**

| Drug or Metabolite | Fluorescence at Assay Conditions | Retention Time, min |
|---|---|---|
| Aspirin | Yes | Solvent front |
| Fenoprofen | Yes | 5.9 |
| Indomethacin | No | — |
| Des(chlorobenzoyl) metabolite | Yes | Solvent front |
| *O*-Desmethyl metabolite | No | — |
| Naproxen | Yes | 4.0 |
| Desmethyl metabolite | Yes | — |
| Oxyphenbutazone | No | — |
| Phenylbutazone | No | — |
| Sulindac | No | — |
| Sulfide metabolite | No | — |
| Sulfone metabolite | No | — |
| Tolmetin | No | — |
| Metabolite | No | — |

standard solution was prepared by transferring 1.7 ml of the *p*-phenylphenol dilution to a 250-ml volumetric flask and diluting to volume with hexane.

**Extraction Conditions**—The extraction of ibuprofen and the internal standard (*p*-phenylphenol) from plasma acidified at several pH levels was studied using five solvents: hexane, ethyl acetate, ether, methylene chloride, and chloroform.

**Analytical Procedure**—To 1.0 ml of heparinized plasma (in 15-ml screw-capped centrifuge tubes) were added 300 μl of 1.0 *M* phosphoric acid, 5 ml of hexane containing 2.0 ng of *p*-phenylphenol/ml as the internal standard, and an aliquot of the ibuprofen solution containing 1–40 μg (2.5–100 μl of methanolic solution). The tubes were vortexed for 10 sec and centrifuged for 5 min at 900×*g*. A 4-ml volume of the organic phase was transferred to special concentration tubes[7] and evaporated to dryness at ambient temperature under a gentle nitrogen stream. The residue was dissolved in 150 μl of methanol, and 100 μl was injected[8].

A standard curve was constructed by injecting the plasma extracts

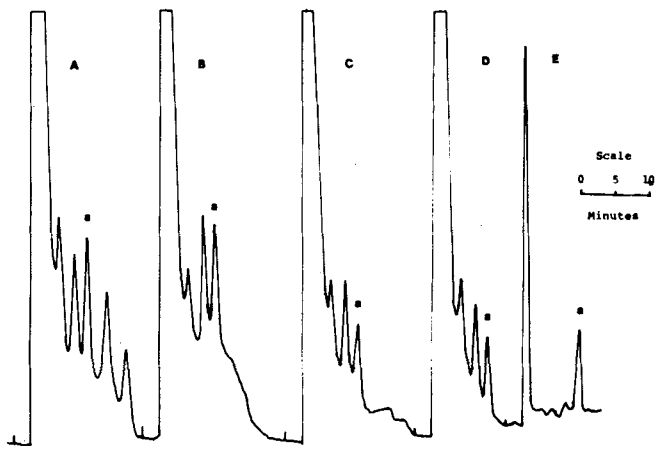


**Figure 2**—*Chromatograms of plasma extracts containing ibuprofen (5 μg/ml) (a). Key: A, methylene chloride; B, chloroform; C, ether; D, ethyl acetate; and E, hexane.*

[7] Concentratubes, Laboratory Research Co., Los Angeles, Calif.
[8] Hamilton Co., Reno, Nev.

**Table IV—Assay Accuracy (Serum Concentration in Micrograms per Milliliter)**

| Theoretical | Experimental[a] | Difference, % |
|---|---|---|
| 1.0 | 1.11 | 11.0 |
| 3.0 | 3.15 | 5.0 |
| 5.0 | 5.07 | 1.4 |
| 10.0 | 9.98 | 0.2 |
| 15.0 | 14.37 | 4.2 |
| 20.0 | 20.06 | 0.3 |
| 25.0 | 24.72 | 1.1 |
| 30.0 | 30.38 | 1.3 |
| 35.0 | 35.41 | 1.2 |
| 40.0 | 40.01 | 0.0 |

[a] Mean of five determinations.

simulating concentrations of ibuprofen from 1 to 40 μg/ml (corresponding to 0.4–16 μg of ibuprofen on the column). The chromatograms were recorded at a chart speed of 5 mm/min[9]. The peak heights were measured, and the ratios (ibuprofen to *p*-phenylphenol) were calculated and plotted *versus* ibuprofen concentrations expressed as micrograms per milliliter.

**Interferences**—The possible interferences from normal plasma constituents and other drugs that might be administered simultaneously were studied.

**Recovery**—For the recovery study, 10- and 25-μg/ml plasma standards were prepared as described under *Analytical Procedure*. After evaporation, the residue was dissolved in 150 μl of methanol containing 7.5 μg of ibuprofen. A volume of 100 μl was injected onto the column.

**Sample Preparation and Assay**—Heparinized plasma samples from patients receiving oral ibuprofen were processed in duplicate as described under *Analytical Procedure*. The amount of ibuprofen was calculated by comparison with standards prepared daily.

## RESULTS AND DISCUSSION

Increasing buffer molar concentrations first decreased and then increased the capacity factor of ibuprofen and the internal standard (Table I). The capacity factor was also affected by pH changes (Table II).

A mobile phase of 65% methanol and 35% acetate buffer (pH 5 and 0.10 *M*) gave well-resolved, sharp peaks for ibuprofen and *p*-phenylphenol with retention times of 10.9 and 7.5 min, respectively (Fig. 1). Aspirin, fenoprofen, naproxen, and other commonly used nonsteroidal antiarthritic agents (Table III) did not interfere with the analysis. The choice of the fluorometric parameters allowed a concentration of ibuprofen as low as 1 μg/ml of plasma to be quantitated.

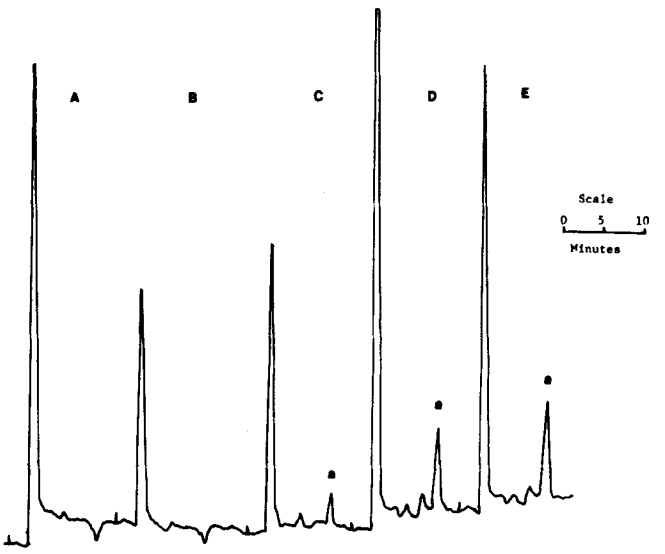


**Figure 3**—*Chromatograms of plasma extracts containing ibuprofen (5 μg/ml) (a) using hexane for extraction. Key: A, 0.1 M phosphate buffer (pH 4); B, 0.1 M phosphate buffer (pH 3); C, 0.1 M acetate buffer (pH 4); D, 2.0 M phosphoric acid; and E, 1.0 M phosphoric acid.*

[9] Model 56, Perkin-Elmer, Norwalk, Conn.

Hexane was selected for extraction of ibuprofen from plasma acidified with 1 *M* phosphoric acid. This selection was based on the highest amount of ibuprofen and/or the lowest amount of interfering fluorescent material being extracted. Ethyl acetate, ether, methylene chloride, and chloroform extracted excessive fluorescent substances from plasma (Fig. 2).

When phosphate buffers of pH 3 and 4 were used to acidify plasma, no ibuprofen was extracted. A pH 4 acetate buffer allowed extraction of only minimal amounts of ibuprofen. Phosphoric acid (1.0, 2.0, 3.0, 4.0, and 5.0 *M*) produced maximal extraction of ibuprofen; however, with 1.0 *M* phosphoric acid, minimal interfering constituents were extracted (Fig. 3). The optimum recovery of ibuprofen (74.91 ± 0.56%) from spiked plasma samples was obtained using 1.0 *M* phosphoric acid to acidify the plasma prior to extraction with hexane.

The ratio of the peak height of ibuprofen to the peak height of the internal standard was calculated. Statistical analysis indicated excellent linearity in the range of 1–40 μg of ibuprofen/ml of plasma with a correlation coefficient of 0.993, a slope of 0.0625 ± 0.0016, and an intercept of −0.11743 ± 0.0326 (Table IV).

Plasma levels of volunteers receiving one 600-mg oral dose of ibuprofen were as high as 40 μg/ml and as low as 15 μg/ml at 2 and 6 hr, respectively, after dosing.

The method reported here for the determination of ibuprofen will be a valuable tool for studying pharmacokinetic parameters and for monitoring patients, especially when they receive other nonsteroidal anti-inflammatory drugs.

## REFERENCES


(1) "Facts and Comparisons," Facts and Comparisons, Inc., St. Louis, Mo., 1980, p. 253i.

(2) *Med. Lett.*, **22**, 29 (1980).

(3) D. M. Grennan, D. G. Ferry, M. E. Ashworth, R. E. Kenney, and M. Mackinnon, *Br. J. Clin. Pharmacol.*, **8**, 497 (1979).

(4) D. J. Hoffman, *J. Pharm. Sci.*, **66**, 749 (1977).

(5) D. G. Kaiser and R. S. Martin, *ibid.*, **67**, 627 (1978).

(6) D. G. Kaiser and G. J. Vangiessen, *ibid.*, **63**, 219 (1974).

(7) K. K. Midha, J. K. Cooper, J. W. Hubbard, and I. J. McGilveray, *Can. J. Pharm. Sci.*, **12**, 29 (1977).

(8) D. Pitre and M. Grandi, *J. Chromatogr.*, **170**, 278 (1979).

(9) J. K. Baker and E. K. Fifer, *J. Pharm. Sci.*, **69**, 590 (1980).


# Chronic Dog Intestinal Loop Model for Studying Drug Absorption as Exemplified by β-Adrenoreceptor Blocking Agents, Atenolol and Propranolol


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Received December 4, 1979, from the *Pharmaceutical and Biology I Departments, Pharmaceuticals Division, ICI Limited, Alderley Park, Macclesfield, Cheshire, England.* Accepted for publication October 8, 1980.



**Abstract** □ Chronic *in situ* loops of dog small intestine (jejunum or ileum) were used to investigate the absorption of the β-adrenoreceptor blocking agents atenolol and propranolol. Absorption measurements were made in conscious dogs by monitoring drug disappearance from solution in the loop, with correction for intestinal water absorption. The jejunum had a mean resting pH of 7.3 and a slight net secretion of water into the lumen; the ileum had a resting pH of 7.9 and a strong net absorption of water. Propranolol absorption was rapid and first order in both regions, with the ileum showing faster absorption than the jejunum due to its higher resting pH. In contrast, atenolol absorption was negligible in the jejunum and only moderate in the ileum. The data were quantitatively consistent with the pH–partition mechanism for the absorption of propranolol but not for atenolol. The model was validated for atenolol by showing that, following drug administration into jejunal and ileal loops, drug disappearance rates were similar to absorption rates calculated from systemic blood levels. This technique is useful, realistic, and relatively simple for studying intestinal drug absorption without seriously perturbing normal GI conditions.



**Keyphrases** □ Atenolol—absorption studied in chronic *in situ* ileal and jejunal loops in dogs □ Propranolol—absorption studied in chronic *in situ* ileal and jejunal loops in dogs □ Absorption—atenolol and propranolol, studied in chronic *in situ* ileal and jejunal loops in dogs □ β-Adrenoreceptor blocking agents—atenolol and propranolol, absorption studied in chronic *in situ* ileal and jejunal loops in dogs


The widespread use of *in situ* intestinal loop preparations for studying drug absorption is partly a result of the difficulty of extracting absorption rate data from blood level measurements. In addition, these techniques allow drug absorption to be measured directly at the absorption site, by monitoring the disappearance of a drug introduced into the intestinal lumen, and require much less experimental effort than blood level studies. Models using rats (1, 2) and dogs (3) have been reported.

However, physiological factors that affect *in vivo* absorption rates (*e.g.*, intestinal pH, water and ion flux, and intestinal blood flow) may themselves be affected by the surgical manipulation and anesthesia involved in preparing the animal for absorption measurements in the acute situation (4–9). In some cases, these effects may render an animal model unsuitable for particular drugs. For instance, it was found (10) that the Doluisio rat *in situ* preparation (2) was a valid model for the *in vivo* absorption of one β-adrenoreceptor blocking agent (propranolol) but not for another (practolol). This result was attributed to the effects of anesthetic and surgical shock on the animal.

A technique designed to overcome some of these problems is described in this paper. It consists of an isolated intestinal loop *in situ* in the conscious dog and is a development of the classical Thiry Vella loop technique (11). The use of this model is illustrated by investigation of the absorption characteristics of two β-adrenoreceptor blocking agents, atenolol and propranolol. This comparison is relevant because of the different extent of absorption of the two drugs in humans and because of the previous observation that the Doluisio *in situ* rat technique is not a good model for this type of compound. Attempts also were made to demonstrate the validity of the technique. Preliminary observations with this model were reported previously (12), and this paper represents an expansion and continuation of this work.

## EXPERIMENTAL

**Preparation of Intestinal Loops**—Adult male beagle dogs, 13–17 kg, were trained to sit unrestrained on a table for 1 hr or more. Thiry Vella




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