GLC Determination of Ibuprofen [(±)-2-(p-Isobutylphenyl)propionic Acid] in Plasma

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Abstract □ To evaluate the pharmacokinetics and drug availability from various dosage formulations, a method for the determination of ibuprofen [(±)-2-(p-isobutylphenyl)propionic acid] in human plasma was required. A simple, rapid, sensitive, and specific procedure, based on a benzene extraction of the acidified specimen and subsequent GLC analysis of the methyl esters of the extract residue, was developed. The method is sensitive to 0.5 μg ibuprofen/ml plasma. Statistical analyses indicate an average recovery of 94.8 ± 6.6% *(SD),* which is adequate to differentiate assay error from normal biological variation. Mass spectrometric analysis, in conjunction with GLC, confirmed the specificity of the method for intact drug. The procedure was successfully applied to drug absorption studies in humans.

Keyphrases □ Ibuprofen—GLC analysis in human plasma □ (±)-2-(p-Isobutylphenyl)propionic acid (ibuprofen)—GLC analysis in human plasma □ GLC—analysis, ibuprofen in human plasma

Based on a variety of tests in animals, ibuprofen[1] [(±)-2-(p-isobutylphenyl)propionic acid, I] was observed (1, 2) to be one of the more potent orally active anti-inflammatory, antipyretic, and analgesic agents of a large number of substituted 2-phenylalkanoic acids. The extensive toxicological and biochemical studies (3, 4) in animals and man (5) were comprehensively reviewed (6).

To study the absorption, metabolism, and excretion of these agents in animals and man, Adams and Cliffe (7) developed a method based on paper chromatographic separation and reaction of the drug with bromcresol purple. The lower level of assay detection sensitivity was estimated to be 5–10 μg drug/ml serum, and assay precision was ±15%. Unfortunately, the assay required 48 hr for completion. Since I was selected for extensive biological evaluation (8), a simple, rapid, sensitive, and specific GLC method of analysis for this compound in human plasma was developed. Subsequent to these investigations, Nash *et al.* (9) described a GLC method for the measurement of a structurally related compound, fenoprofen [*dl*-2-(3-phenoxyphenyl)propionic acid], in human plasma. After extraction from the acidified plasma, the compound was converted to the silyl ester and measured *via* GLC utilizing a flame-ionization detector. The lower level of assay detection sensitivity for measurement of fenoprofen in human plasma was 0.25 μg/ml, and assay precision was approximately ±10%.

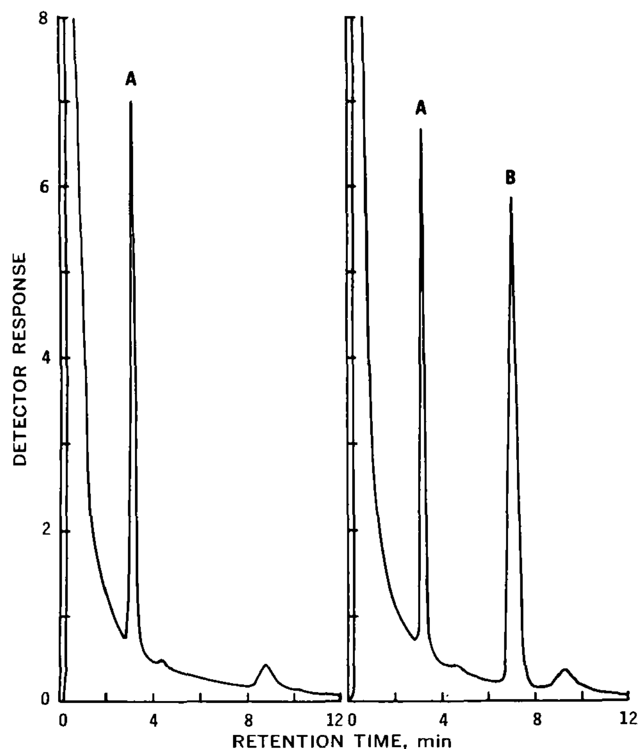
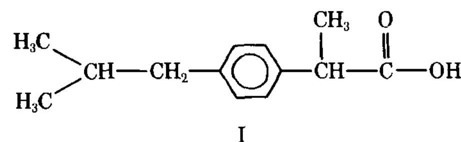


Figure 1—*Gas–liquid chromatograms of human plasma extracts. Left: normal plasma specimen. Right: plasma specimen from subject at 1 hr after single-dose oral administration of 200 mg ibuprofen. Key: A, naphthalene internal standard; and B, ibuprofen methyl ester.*

EXPERIMENTAL

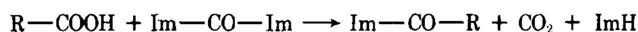
Reagents and Materials—The I used in this study was synthesized[2], and naphthalene, triethylamine, and hydrocarbon-stabilized chloroform were used as supplied[3]. The stock solution of 1,1′-carbonyldiimidazole[4] (II) (65 mg/ml) and naphthalene (100 μg/ml) in hydrocarbon-stabilized chloroform was prepared fresh daily. Stock solutions of I in hydrocarbon-stabilized chloroform (100 μg/ml), triethylamine (III) in methanol (10% v/v), aqueous hydrochloric acid (1 *N*), and aqueous sodium hydroxide (1 *N*, saturated with hydrocarbon-stabilized chloroform) were stored in glass containers. All other solvents were analytical reagent grade. Diethylene glycol succinate[5] on 80–100-mesh Diatoport-S (6% w/w) was used as supplied.

Instrumentation—A two-speed reciprocating shaker[6] was used for shaking the samples in the horizontal position. A mixer[7] was used to aid in preparing the methyl esters. Chromatographic measurements were made with a gas chromatograph[8] equipped

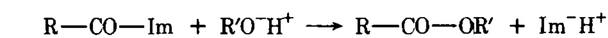


[1] Motrin, The Upjohn Co., Kalamazoo, Mich.

[2] Within the Research Division of Boots Pure Drug Co. Ltd., Nottingham, England.
[3] Matheson, Coleman and Bell, Milwaukee, Wis.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] LAC-728, Hewlett-Packard Co., Avondale, Pa.
[6] Eberbach and Sons, Ann Arbor, Mich.
[7] Vortex model K-500, Scientific Industries, Inc., Queen's Village, N.Y.
[8] F&M model 400, Hewlett-Packard Co., Avondale, Pa.

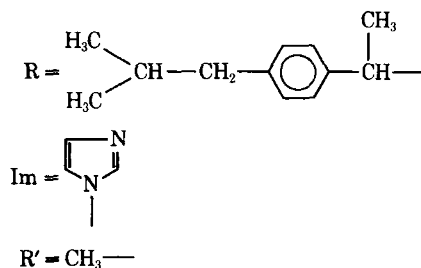


$$R—COOH + Im—CO—Im \longrightarrow Im—CO—R + CO_2 + ImH$$



$$R—CO—Im + R'O^-H^+ \longrightarrow R—CO—OR' + Im^-H^+$$

where:



Scheme I—Reaction scheme for esterification of carboxylic acids

with a hydrogen flame-ionization detector and a −0.2–1.0-mv recorder[9]. All cylinders of gases used for chromatography (*i.e.*, helium, hydrogen, and oxygen) were fitted with filters containing molecular sieve 4A.

Chromatographic Conditions—All chromatography was conducted on U-shaped or coiled stainless steel columns [1.82 m (6 ft) × 0.63 cm (0.25-in.) o.d.] of 6% (w/w) diethylene glycol succinate on 80–100-mesh Diatoport-S. All newly prepared columns were preconditioned at 200° for: *(a)* 1 hr without carrier gas flow, and *(b)* 16 hr with a carrier gas flow of 10 ml/min. During analysis, the column, injection port, and detector block were maintained isothermally at 150, 190, and 210°, respectively. Helium, hydrogen, and oxygen flow rates were 60, 40, and 400 ml/min, respectively. Under these conditions, naphthalene and the I methyl ester have retention times of 3.4 and 6.8 min, respectively (Fig. 1).

Assay Procedure—*Preparation of Standards*—Pipet aliquots of the I chloroform stock solution equivalent to 2, 5, 10, 20, 40, and 60 μg into glass-stoppered centrifuge tubes. Evaporate to dryness with a gentle stream of nitrogen gas. Add 1 ml of control plasma to each centrifuge tube and mix well with the mixer. Prepare an appropriate blank. Extract all standards in the same manner as described for the plasma specimens.

Preparation of Samples—Place 1 ml of plasma in a glass-stoppered centrifuge tube. Add 0.25 ml 1 *N* aqueous hydrochloric acid and 5 ml benzene and shake in the horizontal position for 10 min. Centrifuge for 10 min at 2000 rpm. Transfer a 4-ml aliquot of the benzene layer to a fresh glass-stoppered centrifuge tube and evaporate to dryness with a gentle stream of nitrogen gas. Wash down the walls of the centrifuge tube with 0.5 ml chloroform and evaporate to dryness with nitrogen. Add 0.1 ml of II reagent to each benzene extract residue. Rotate each tube to permit the reagent to contact the lower 2.54 cm (1 in.) of the centrifuge tube wall, allowing the reagent to react for at least 1 min. Add 0.1 ml 10% (v/v) III in methanol and mix thoroughly using the mixer. Allow the reagent to react for 5 min. Add 1 ml 1 *N* aqueous sodium hydroxide and shake vigorously by hand. Centrifuge for 10 min at 2000 rpm. Inject a 1-μl aliquot of the chloroform layer for analysis into the chromatograph.

Calculations—The peak heights for naphthalene and the I methyl ester are measured. Peak height ratios are obtained by dividing the peak height of the I methyl ester by the peak height of naphthalene. Calibration curves from known concentrations of I in plasma are prepared by plotting peak height ratios *versus* free acid concentration, expressed as micrograms per milliliter of plasma. Values for unknown concentrations of I in plasma specimens, obtained in the same manner, are then read directly from the graph or calculated from the slope of the standard curve.

Drug Administration to Man—Informed written consent was obtained from each of 20 normal human male volunteers prior to participation in this study. All subjects were between the ages of 22 and 50 years; they ranged in body weight from 63.6 to 81.8 kg and in height from 1.66 to 1.88 m. All subjects were fasted for 16 hr prior to drug administration. Each received a 200-mg dose of I, as a solution in polysorbate 80, contained in soft elastic gelatin capsules. Food was withheld for an additional 4 hr. Blood specimens (10 ml) were withdrawn in heparinized syringes at predetermined time intervals from 0 to 24 hr after drug administration. The plasma was harvested and stored at −18°.

[9] Electronik 15, Honeywell, Inc., Philadelphia, Pa.

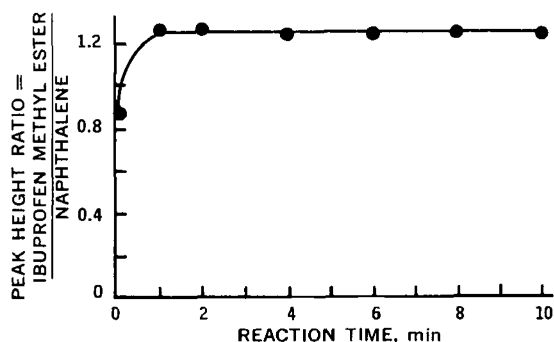


Figure 2—*Effect of reaction time (1,1′-carbonyldiimidazole in chloroform) on formation of ibuprofen methyl ester (reaction time for triethylamine in methanol = 10 min).*

RESULTS AND DISCUSSION

Esterification of Ibuprofen—Studies by Ko and Royer[10] indicated that II was a useful reagent for the facile esterification of free fatty acids found in serum extract residues. The general reaction scheme for the esterification (10) of carboxylic acids is presented in Scheme I. A series of samples, containing known amounts of I, was prepared to determine the optimal reaction times for: *(a)* imidazolide formation and *(b)* methyl ester formation from the imidazolide. The results indicated that imidazolide formation was completed within 1 min (Fig. 2) and that methyl ester formation was completed almost instantaneously (Fig. 3). Reaction times of 1 and 5 min, respectively, were selected for convenience. Synthesis of standard material indicated that the methyl ester of I was a liquid at room temperature. Attempts to isolate crystalline material were unsuccessful. GLC (using a solid sample injector) indicated that the material submitted for elemental analysis was greater than 98% pure.

Anal.—Calc. for $C_{14}H_{20}O_2$: C, 76.33; H, 9.15. Found: C, 76.16; H, 9.08.

IR and mass spectrometric analysis, before and after GLC, supported the proposed structure and confirmed that the I methyl ester chromatographed as the intact molecule.

Selection of Internal Standard—Pilot studies, using 2-(*p*-isobutylphenyl)acetic acid as an internal standard, showed that the methyl ester had a retention time of about 8.8 min as compared to 6.8 min for I methyl ester. As shown in Fig. 1, benzene extracts of plasma specimens from normal human subjects contained an interfering material of approximately the same retention time as the methyl ester 2-(*p*-isobutylphenyl)acetic acid. Naphthalene was subsequently selected to replace 2-(*p*-isobutyl

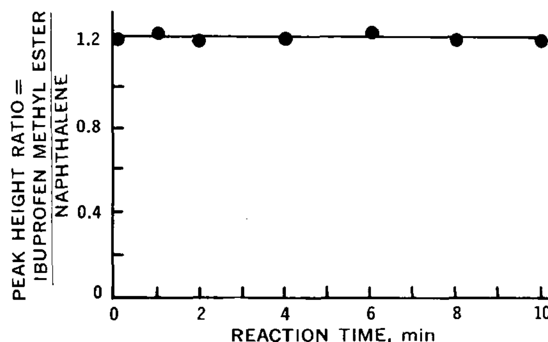


Figure 3—*Effect of reaction time (triethylamine in methanol) on formation of ibuprofen methyl ester (reaction time of 1,1′-carbonyldiimidazole in chloroform = 10 min).*

[10] H. Ko and M. E. Royer, The Upjohn Co., Kalamazoo, Mich., personal communication.

Table I—Recovery of Ibuprofen from Human Plasma

Added, μg/ml	Found, μg/ml	Recovery, %
2.0	1.82	91.0
5.0	4.42	88.4
10.0	9.45	94.5
20.0	17.60	88.0
40.0	42.76	106.9
60.0	57.96	96.6
80.0	78.64	98.3
	Mean ± *SD* =	94.8 ± 6.6

phenyl)acetic acid as the internal standard. Although naphthalene eluted from the chromatograph in a region where there was no interference, it only served as a measure of sample injection reproducibility.

Solution Stability of 1,1′-Carbonyldiimidazole—A chloroform solution of II was allowed to stand at room temperature for a total of 5 days. Forty-eight hours after the preparation of the solution, it began to turn yellow. Esterification reactions conducted at predetermined time intervals indicated that the reagent, as observed under these experimental conditions, was stable for at least 24–48 hr. Ancillary studies using NMR techniques suggested that the diimidazole was slowly degrading to imidazole in the chloroform solution.

Assay Sensitivity and Specificity—At a sensitivity of 1.28×10^{-9} amp/mv, 1.2 μg of I as its methyl ester produced a full-scale response. However, under the described assay conditions, the lower limit of detection sensitivity for I in extracts of human plasma is 0.5 μg/ml of the original sample aliquot. This value is based on a sample signal equivalent to 2% of full-scale response. Under these assay conditions, a linear relationship between detector response and concentration is obtained for I over the 0–80-μg/ml range. Quantification from a standard curve was adequate. Analysis of plasma specimens from drug-treated human subjects, using GLC in conjunction with mass spectrometry, showed that the material responding to the assay is identical to known I methyl ester.

Recovery Experiments—Known amounts of I in chloroform were evaporated to dryness in centrifuge tubes, and water or plasma was added. The samples were thoroughly mixed and extracted with benzene. All extract residues were esterified and analyzed chromatographically. The results (Table I) indicated that recovery of I from plasma was essentially quantitative (94.8 ± 6.6%) as compared to simple aqueous samples.

Plasma Levels of Ibuprofen in Man—Results from the measurement of plasma I concentrations in 20 normal human subjects, after single-dose oral drug administration, demonstrated the utility of the analytical methodology (Fig. 4). A peak mean (±*SD*) level of I (21.8 ± 4.2 μg/ml) was observed at 1 hr after drug administration, indicating rapid drug absorption from the polysorbate 80 solution. Beyond 12 hr, plasma drug concentrations were below the level of assay detection sensitivity (*i.e.*, less than 0.5 μg/ml), indicating rapid drug disappearance from peripheral circulation. The plasma drug disappearance half-life, as estimated graphically from the average plasma drug concentrations, was 1.93 hr. The combined results from these investigations showed that the GLC method could be used for: *(a)* evaluating

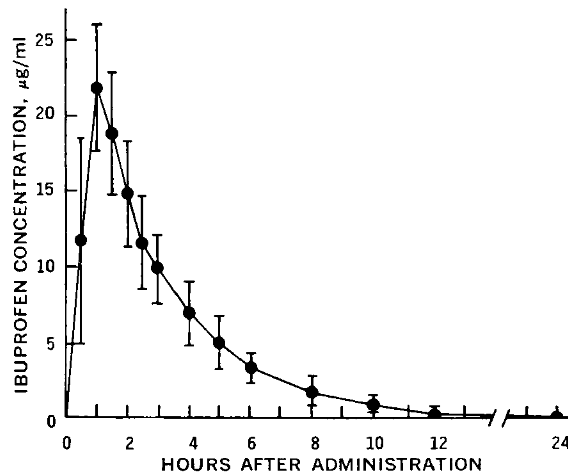


Figure 4—*Average* (±SD) *plasma concentrations of ibuprofen* versus *time in humans* (n = *20*) *after single-dose oral administration of 200 mg drug as a solution in polysorbate 80.*

pharmacokinetics, *(b)* evaluating drug availability from various dosage formulations, and *(c)* selecting an optimum dosage regimen for I administration to man.

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