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# Determination of Indoprofen in Physiological Fluids by Reversed-Phase Liquid Chromatography

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Received July 25, 1977, from the **Chemical Sciences Division, Midwest Research Institute, Kansas City, MO 64110,* and ‡*Hercules, Inc., Wilmington, DE 19899.* Accepted for publication October 5, 1977.

**Abstract** □ A rapid, sensitive, reversed-phase high-performance liquid chromatographic procedure was developed for the quantitative analysis of indoprofen in plasma and urine. Minimal sample preparation is required for the analysis of unconjugated urinary or plasma drug levels. The method provided quantitative results for indoprofen levels of 0.5–50 μg/ml of plasma and 0.5–200 μg/ml of urine and had a lower detection limit of 1 ng. Total urinary indoprofen levels required enzymatic hydrolysis of the conjugated drug prior to analysis. Results are presented for the plasma and urinary excretion levels of indoprofen for a patient receiving a single oral dose.

**Keyphrases** □ Indoprofen—high-performance liquid chromatographic analysis in biological fluids □ High-performance liquid chromatography—analysis, indoprofen in biological fluids □ Analgesics—indoprofen, high-performance liquid chromatographic analysis in biological fluids

Indoprofen, *dl*-2-[4-(1-oxo-2-isoindolinyl)phenyl]propanoic acid, has analgesic activity in animals and humans (1–4). In humans, indoprofen appears unchanged in plasma (5) and enantiomeric enrichment is not significant (6). Tosolini *et al.* (7) reported that indoprofen was excreted as the glucuronide conjugate and that enzymatic hydrolysis was required prior to determining urinary excretion levels.

GLC methods for the analysis of indoprofen in plasma and urine have been reported (6–8). While each method has analytical validity and sufficient sensitivity, they all require extraction of indoprofen from the biological fluid and derivatization to provide the necessary volatility for GLC analysis. These extraction and derivatization steps are time consuming and, consequently, expensive for extensive bioavailability studies.

To overcome these difficulties in sample preparation and to maintain the necessary selectivity and sensitivity for low level analysis, a method was developed using reversed-phase high-performance liquid chromatography (HPLC). The method is applicable to both urine and plasma samples containing indoprofen and has sensitivity at the nanogram level.

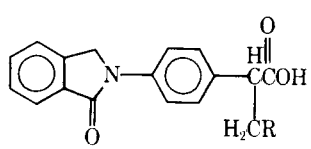
## EXPERIMENTAL

**Reagents**—Acetonitrile[1] was used as received. The water was first deionized and then distilled to remove any contaminants. For enzymatic hydrolysis of conjugated drug, aryl sulfatase[2] containing β-glucuronidase was used.

**Reference Compound and Internal Standard**—A reference standard of indoprofen[3] (I) was used to develop the analytical method. The internal standard (II) selected for quantitation was the pentanoic acid homolog of indoprofen[3].

**HPLC Conditions**—To determine the optimal wavelength for UV detection of indoprofen, reference standard was dissolved in methanol and its UV spectrum was obtained. The standard has a $\lambda_{max}$ at 282 nm with an absorptivity (ε 282) of 14,200 ($E^{1\%}_{1\,cm}$ = 505). The reported $E^{1\%}_{1\,cm}$ value for indoprofen is 500 ± 25 ($\lambda_{max}$ 283)[4]. A 280-nm filter was used.

The liquid chromatograph was constructed from components and consisted of a high pressure pump[5], a loop injector[6], and a UV detector[7]. The initial HPLC conditions used for urinary analysis consisted of a Zorbox ODS[8] (250 × 2 mm i.d.) column and an eluent of 40% acetonitrile



I: R = H
II: R = $CH_2CH_3$

[1] Nanograde, Mallinckrodt Chemical Works, St. Louis, Mo.
[2] Sigma Chemical Co., St. Louis, Mo. (31,000 units/g of solid; 1 unit will hydrolyze 1.0 μmole of nitrocatechol sulfate/hr at pH 5.0 at 37°).
[3] Adria Laboratories, Columbus, Ohio.
[4] Dr. Werner Hausmann, Adria Laboratories, Columbus, Ohio, personal communication.
[5] Model 6000A, Waters Associates, Milford, Mass.
[6] Model UK6, Waters Associates, Milford, Mass.
[7] Model 440, Waters Associates, Milford, Mass.
[8] DuPont Co., Wilmington, Del.

**Table I—Linearity and Reproducibility of Indoprofen Analysis**

| Sample | Indoprofen per Milliliter | Relative Weight Response Sample A | Sample B | Sample C |
|---|---|---|---|---|
| 1 | 25.0 ng | 1.77 | 1.65 | 1.78 |
| 2 | 50.1 ng | 1.78 | 1.76 | 1.79 |
| 3 | 100.2 ng | 1.80 | 1.79 | 1.81 |
| 4 | 200.4 ng | 1.80 | 1.75 | 1.80 |
| 5 | 501.0 ng | 1.80 | 1.81 | 1.78 |
| 6 | 1.002 μg | 1.78 | 1.77 | 1.81 |
| 7 | 2.004 μg | 1.80 | 1.80 | 1.83 |
| 8 | 5.01 μg | 1.79 | 1.81 | 1.82 |
| 9 | 10.02 μg | 1.84 | 1.82 | 1.79 |
| 10 | 25.05 μg | 1.79 | 1.80 | 1.85 |
| 11 | 50.10 μg | 1.84 | 1.83 | 1.87 |
| | Average = 1.797 | *SD* = ±0.037 | *RSD* = 2.1 | |

**Table II—Stability of Indoprofen Analysis on Succeeding Days**

| Sample | Indoprofen, ng/ml | Relative Weight Response Day 1[a] A | Day 1[a] B | Day 2[a] A | Day 2[a] B | Average |
|---|---|---|---|---|---|---|
| 1 | 25.0 | 1.77 | 1.65 | 1.67 | 1.73 | 1.71 |
| 2 | 50.1 | 1.78 | 1.76 | 1.69 | 1.82 | 1.76 |
| 3 | 100.2 | 1.80 | 1.79 | 1.77 | 1.80 | 1.79 |
| 4 | 200.4 | 1.80 | 1.75 | 1.77 | 1.79 | 1.78 |
| 5 | 501.0 | 1.80 | 1.81 | 1.81 | 1.78 | 1.80 |
| 6 | 1002 | 1.77 | 1.77 | 1.78 | — | 1.77 |

[a] Day of analysis for the same indoprofen standard solution.

in 0.175 *M* acetic acid. Later, for the plasma level determinations, a μBondapak $C_{18}$[9] (300 × 4 mm i.d.) column was used. The flow rate was maintained at 1.0 ml/min, and the temperature was ambient. Injection volumes varied between 20 and 50 μl depending on the drug concentration.

**Method for Plasma Indoprofen Levels**—A recently reported method (9) for the rapid and simple precipitation of serum proteins prior to HPLC analysis was adapted. Equal volumes of plasma and acetonitrile (containing 3.0 μg of internal standard) first were mixed thoroughly, and the sample was centrifuged at 2000 rpm to remove the precipitated protein. An aliquot of the resulting clear solution was then assayed by HPLC.

**Method for Free Indoprofen Levels in Urine**—A procedure similar to the plasma level method was employed to determine the urinary excretion level of free indoprofen. A 0.5-ml urine specimen was combined with 0.75 ml of acetonitrile (containing 22 μg of internal standard). An aliquot of this mixture was then analyzed directly by HPLC. Centrifugation to remove the precipitate was required for only a few samples.


**Figure 1**—*Chromatograms of indoprofen standards. Key: A, 10 μg of indoprofen/ml (peak 1) and 20 μg of internal standard/ml (peak 2); and B, 20 ng of indoprofen/ml (peak 1) and 200 ng of internal standard/ml (peak 2).*

[9] Waters Associates, Milford, Mass.

**Table III—Recovery of Indoprofen from Plasma**

| Sample | Indoprofen Added, μg | Internal Standard, μg | Calculated, μg | Recovery, % |
|---|---|---|---|---|
| 1 | 0.50 | 1.5 | 0.515 | 103 |
| 2 | 1.25 | 1.5 | 1.24 | 99 |
| 3 | 2.00 | 1.5 | 2.00 | 100 |
| 4 | 2.75 | 1.5 | 2.42 | 88 |
| 5 | 3.5 | 1.5 | 3.04 | 87 |
| 6 | 3.5 | 1.5 | 3.18 | 91 |
| | | | Average | 95 |
| | | | *SD* | 7 |

**Method for Total Indoprofen Levels in Urine**—To determine the total amount of indoprofen excreted in urine, the conjugated drug first must be hydrolyzed. The optimum conditions necessary for complete hydrolysis were evaluated; the conditions chosen consisted of incubating a 2-ml aliquot of urine with 2 ml of an enzyme solution at 37° for 16 hr. The enzyme solution contained 2 mg of aryl sulfatase-β-glucuronidase in 0.14 *M* sodium acetate, pH 5. After incubation, 1 ml of the hydrolyzed sample was added to 1 ml of acetonitrile containing the internal standard (22 μg/ml). An aliquot of this solution was analyzed by HPLC.

**Calculations**—The level of indoprofen (I) present in plasma and urine samples was calculated from the relative weight response (RWR) of

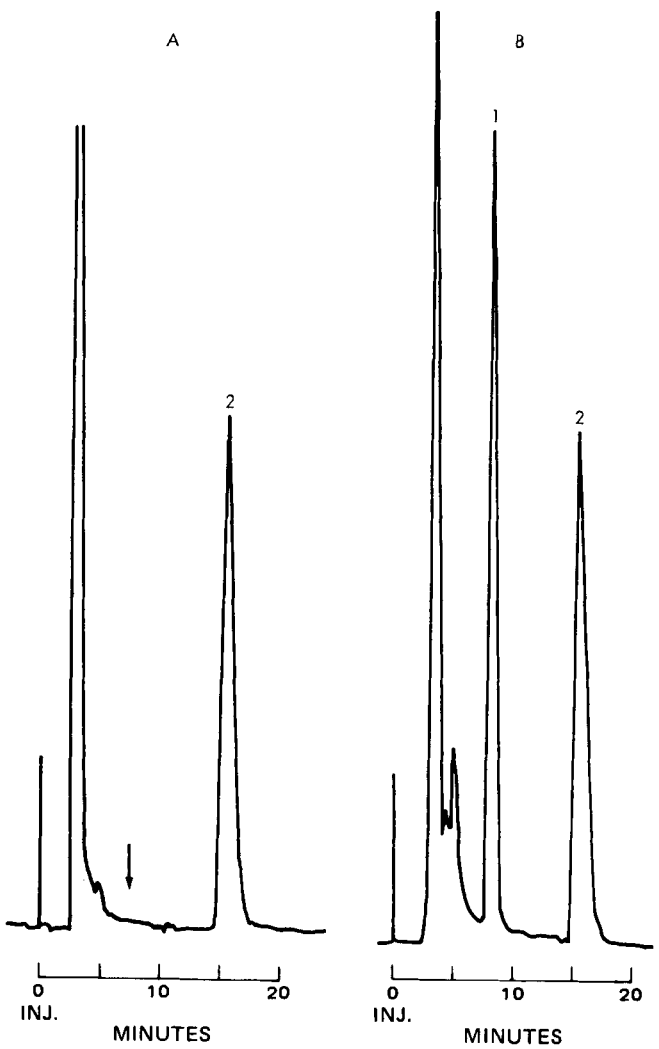



**Figure 2**—*Chromatograms of indoprofen in plasma. Key: A, blank plasma sample (arrow indicates indoprofen elution position); and B, plasma sample at 8 hr after dosing; indoprofen (peak 1) concentration = 4.2 μg/ml, internal standard (peak 2) concentration = 3.2 μg/ml.*

Table IV—Urinary Excretion of Indoprofen

| Sample | Hours | Total Volume, ml | Free I[a], mg | Average | Total I[b], mg | Average |
|---|---|---|---|---|---|---|
| 1A | 0–2 | 44 | 0.43 | 0.47 | 26 | 30 |
| 1B | | | 0.51 | | 33 | |
| 2A | 2–4 | 102 | 0.73 | 0.77 | 41 | 38 |
| 2B | | | 0.82 | | 34 | |
| 3A | 4–6 | 472 | 0.75 | 0.75 | 55 | 45 |
| 3B | | | — | | 34 | |
| 4A | 6–8 | 100 | 0.18 | 0.18 | 11 | 12 |
| 4B | | | 0.18 | | 12 | |
| 5A | 8–12 | 364 | 0.12 | 0.12 | 33 | 33 |
| 5B | | | 0.12 | | 32 | |
| 6A | 12–24 | 135 | 0.24 | 0.22 | 9 | 9 |
| 6B | | | 0.19 | | 8 | |
| Total | | | | 2.56 | | 163 |

[a] Free I: calculate level of unconjugated indoprofen in each collection period as described in text. [b] Total I: calculate level of total (free and conjugated) indoprofen in each collection period.

standard solutions. The equations used were:

$$\text{RWR I/II} = \frac{\text{PH std I}}{\text{PH II}} \times \frac{\text{wt II}}{\text{wt std I}} \quad \text{(Eq. 1)}$$

$$\mu\text{g of I/sample} = \frac{\text{PH I}}{\text{PH II}} \times \frac{\text{wt II}}{\text{RWR I/II}} \quad \text{(Eq. 2)}$$

$$\mu\text{g of I/ml} = \frac{\mu\text{g of I/sample}}{\text{ml/sample}} \quad \text{(Eq. 3)}$$

where PH is the peak height, wt is the weight of II in micrograms, μg of I/sample is the micrograms present in an aliquot of plasma or urine, and μg of I/ml is the micrograms present in a milliliter of plasma or urine.

## RESULTS

The HPLC method was evaluated to determine the linearity, reproducibility, and minimum detectable limit. Table I presents the data for the single injection of triplicate samples of standards from 25 ng/ml to 50 μg/ml. Excellent precision and linearity were obtained with an average relative weight response of 1.80 and a standard deviation and relative standard deviation of 0.037 and 2.1, respectively. The minimum detectable limit of indoprofen by HPLC was 1 ng with a 5:1 signal to noise ratio.

Succeeding day reproducibility was determined by analyzing the samples from the precision study on the next day (Table II). No differences were detected in the relative weight response of the 25–1000-ng standards analyzed on succeeding days. Figure 1 shows typical HPLC chromatograms for indoprofen and the internal standard. The recovery of indoprofen added to normal plasma was determined at concentrations of 500 ng–3.5 μg/ml of plasma (Table III). Quantitative recovery at all levels was obtained with an average recovery of 95% and a standard deviation of ±7%.

The level of indoprofen present in the plasma of a subject given a single oral dose of 200 mg was determined. Blood samples obtained at various times after dosing were centrifuged to obtain the plasma. Typical chromatograms for plasma analysis are given in Fig. 2. Figure 3 illustrates the decay of indoprofen after the single oral dose.

The urinary excretion levels of free and conjugated drug were determined for the same subject. Urine was collected in six collection periods for 24 hr after the dose, and duplicate analyses were made for both free and conjugated drug (Table IV). A total of 2.51 mg of indoprofen (1.25% of the total dose) was excreted as free drug during 24 hr, and 167 mg of

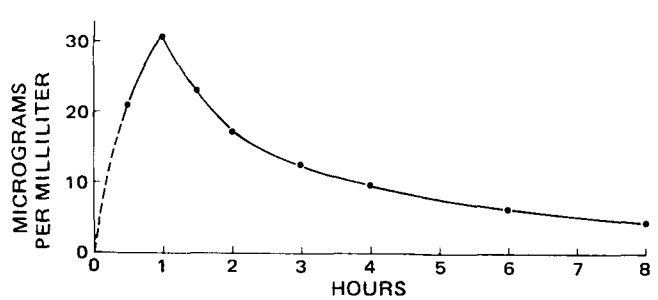



**Figure 3**—*Plasma decay for indoprofen after a single oral dose of 200 mg.*

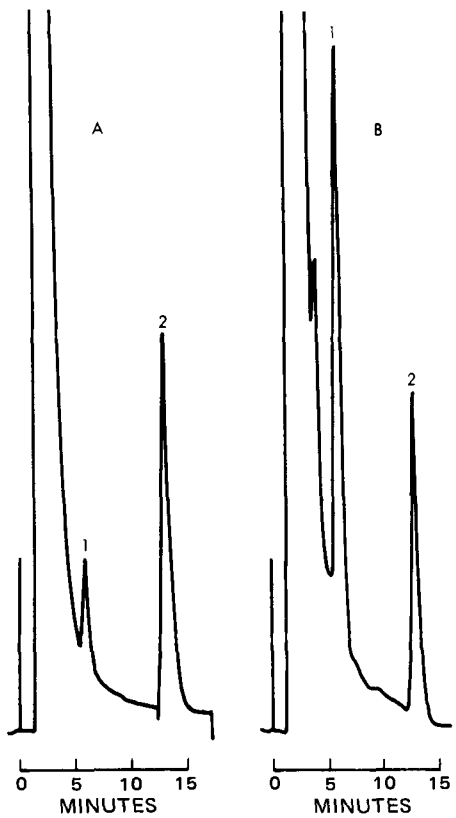



**Figure 4**—*Chromatograms of indoprofen in urine. Key: A, neat urine, 6–8-hr collection, indoprofen (peak 1) concentration = 1.8 μg/ml; and B, hydrolyzed urine, 12–24-hr collection, indoprofen concentration = 62 μg/ml.*

indoprofen (83.5% of the total dose) was excreted in 24 hr as the free and conjugated drug. Chromatograms obtained for free and total urinary indoprofen are presented in Fig. 4.

## DISCUSSION

The developed method has the required selectivity and sensitivity for low level analysis and the precision and accuracy necessary for quantitation. Plasma and urinary excretion levels (free and total) of indoprofen were determined for a subject receiving a single oral dose. The plasma decay curve agrees closely with the data of an earlier report (8).

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