Determination of Ibuprofen in Capillary and Venous Plasma by High-Performance Liquid Chromatography with Ultraviolet Detection

K. S. ALBERT*[x], A. RAABE[‡], M. GARRY[‡], E. J. ANTAL*, and W. R. GILLESPIE*

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Abstract □ A high-performance liquid chromatographic (HPLC) method is described which determines ibuprofen in human capillary or venous plasma. Ibuprofen plus the internal standard, flurbiprofen, were extracted from acidified plasma with pentane–ether, back-extracted into base, and then extracted into the pentane–ether solution after acidification of the aqueous phase. A reverse-phase octadecylsilane column with acetonitrile–water–phosphoric acid as mobile phase and UV detection provided a quantifiable peak for 1 μg/mL of ibuprofen in 0.1 mL of plasma. Capillary and venous plasma level curves were virtually superimposable after administration of 400 mg of ibuprofen to four normal volunteers. No ibuprofen was detected in the saliva of the subjects.

Keyphrases □ Ibuprofen—HPLC, small volumes, human plasma and saliva □ Anti-inflammatory agents—ibuprofen, HPLC, capillary and venous plasma, saliva, humans

Ibuprofen, a nonsteroidal phenylpropionic acid derivative possessing potent anti-inflammatory, antipyretic, and analgesic properties (1–3), is used extensively to treat adult rheumatoid arthritis (4–6). In addition, the drug has been shown to be an effective pediatric antipyretic (7). A variety of GC (8–13) and high-performance liquid chromatographic (HPLC) (14–16) methods suitable for measuring ibuprofen in plasma or serum have been reported. With the exception of the electron-capture GC procedure (8), which detects ≥1 μg/mL in 0.1 mL of sample, all methods require >0.5 mL of plasma or serum which limits their use in juvenile or pediatric populations and in multiple-dose studies requiring small sample volumes. The electron-capture procedure (8) is tedious because it involves a TLC step prior to derivatization.

We describe a rapid, specific, and sensitive HPLC method with UV detection for measuring ≥1 μg/mL of ibuprofen in 0.1 mL of plasma or serum. Application of this method to human capillary venous plasma and to saliva is described.

EXPERIMENTAL SECTION

Instrumentation—The high-pressure liquid chromatograph[1] was equipped with a variable-wavelength UV detector[2], an octadecylsilane column[3] (15 cm × 4.6 mm), an automatic injector[4], and a recorder[5]. The flow rate was maintained at 1.5 mL/min (1250 psi) at ambient temperature. The UV detector was set at 195 nm with an attenuation of 0.2 AUFS. The mobile phase was acetonitrile–water–phosphoric acid (550:450:0.5), filtered through a membrane[6] and deaerated before use.

Chemicals and Reagents—Reagents were analytical grade or better. Acetonitrile was distilled in glass[7]. Anhydrous ether was monitored daily for peroxide formation. Stock solutions of ibuprofen[8] (1 mg/mL) and flurbiprofen[8] (1 mg/mL) were prepared in acetonitrile. Appropriate spiking solutions of ibuprofen in acetonitrile and a 10-μg/mL spiking solution of flurbiprofen internal standard in acetonitrile were prepared from the respective stock solutions. The water was deionized[9].

Analytical Method—Standards ranging from 0 to 100 μg/mL were prepared in culture tubes by combining 100 μL each of an appropriate ibuprofen spiking solution, internal standard, and drug-free matrix with 900 μL of deionized water. Samples were prepared by combining 100 μL each of acetonitrile, internal standard, and sample with 900 μL of deionized water. All tubes were vortex-mixed briefly, 0.5 mL of 1 M HCl was added to each, and the tubes were mixed again. The samples were extracted with 10 mL of pentane–ether (80:20, v/v). The upper organic layers were transferred to clean

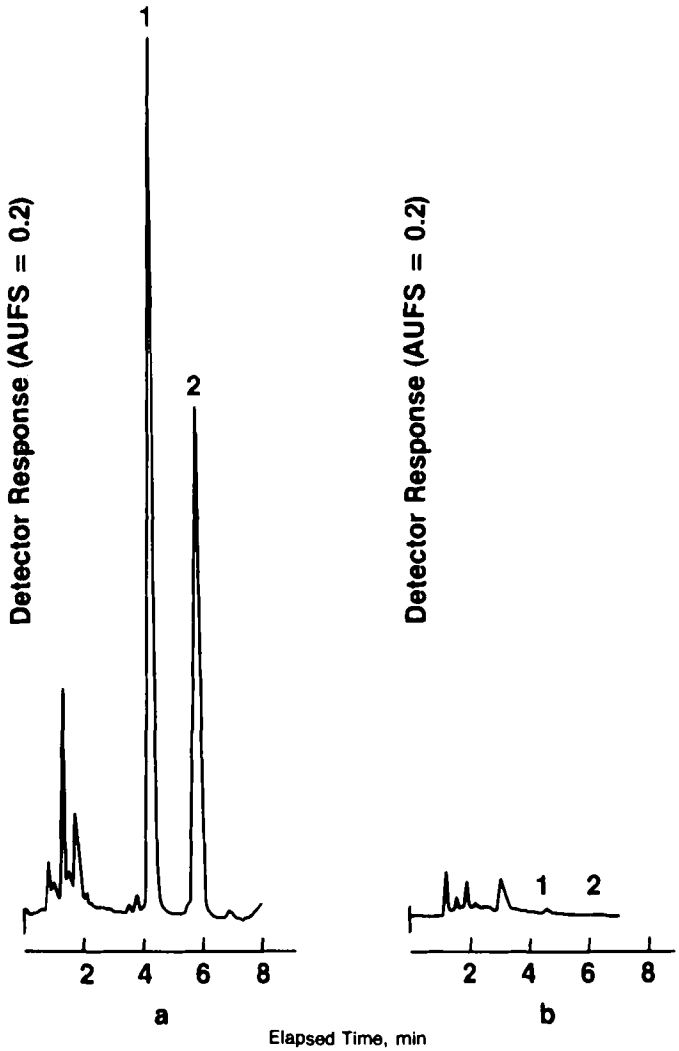

Figure 1—*Typical chromatograms of (a) normal human plasma spiked with 10 μg/mL of internal standard (1) and 6 μg/mL of ibuprofen (2) and (b) unspiked human plasma showing a region where the above peaks would be expected.*

[1] Model 112 pump; Beckman Instruments, Fullerton, Calif.
[2] Spectromonitor III variable-wavelength UV absorbance detector; Laboratory Data Control, Riviera Beach, Fla.
[3] Zorbax ODS; Dupont, Wilmington, Del.
[4] WISP 710B; Waters Associates, Milford, Mass.
[5] Linear Instruments Corp., Reno, Nev.
[6] GS Millipore membrane filtering apparatus; Millipore Corp., Bedford, Mass.
[7] Burdick and Jackson Laboratories, Muskegon, Mich.
[8] The Upjohn Co., Kalamazoo, Mich.
[9] Milli-Q deionized water; Millipore Corp.

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Table I—Sensitivity and Precision of the Assay of Ibuprofen in Plasma

Theoretical Conc., μg/mL	Mean Conc., μg/mL[a]	SD	RSD, %
1.0	1.00	0.116	11.6
3.0	3.01	0.148	4.90
6.0	5.93	0.282	4.75
10.0			
Low curve	10.0	0.191	1.91
High curve	9.39	0.536	5.71
30.0	30.5	0.978	3.20
60.0	60.5	1.43	2.36
100.0	99.5	2.82	2.83

[a] Mean of 14 determinations except for nominal concentration of 60.0 μg/mL, which was the mean of 13 determinations.

culture tubes and extracted with 1.0 mL of 1 M NaOH. After aspirating off the upper organic layer, the pH was adjusted with 2.0 mL of 1 M HCl and the samples were extracted with 10 mL of pentane–ether. The upper organic layer was transferred to clean culture tubes and evaporated to dryness[10]. The residue was dissolved in 0.4 mL of acetonitrile–water (55:45), transferred to vials, and 25 μL was injected onto the HPLC.

Calculations—Calculations were made using baseline-corrected peak height values[11]. Standards were fit to the linear equation, $y = a + bx$, where x corresponds to the concentration of ibuprofen (μg/mL) and y corresponds to the ratio of ibuprofen-to-internal standard peak heights. A split curve was used to calculate the data. The two curves consisted of standards from 1 to 10 μg/mL and from 10 to 100 μg/mL. This was done to prevent negative bias on low-end values caused by variation in the high-end standards. A correlation coefficient was calculated to judge if an adequate curve fit was obtained. Slope and intercept values were also checked to note day-to-day consistencies. Control and sample values were calculated from the appropriate standard curves.

Validation Study—A standard curve with seven data points, a blank plasma control, and two positive plasma controls were assayed in duplicate on each of 3 d. On the fourth day a saliva standard curve, a blank saliva control, and two positive saliva controls were analyzed in duplicate.

To estimate recovery, 100 μL of 10-μg/mL flurbiprofen and ibuprofen spiking solutions were either added to 0.1 mL of plasma and analyzed (matrix standard) or diluted to 0.4 mL with mobile phase and injected directly into the chromatograph (injection standard). Runs were made on four separate occasions and recoveries were determined by comparing matrix-standard peak heights with injection-standard peak heights.

Validation samples were generated by administering ibuprofen to four non-obese volunteers[12] whose average age was 39.5 years (range, 31–47 years) and whose average weight was 69.2 kg (range, 59–77 kg). Each subject fasted

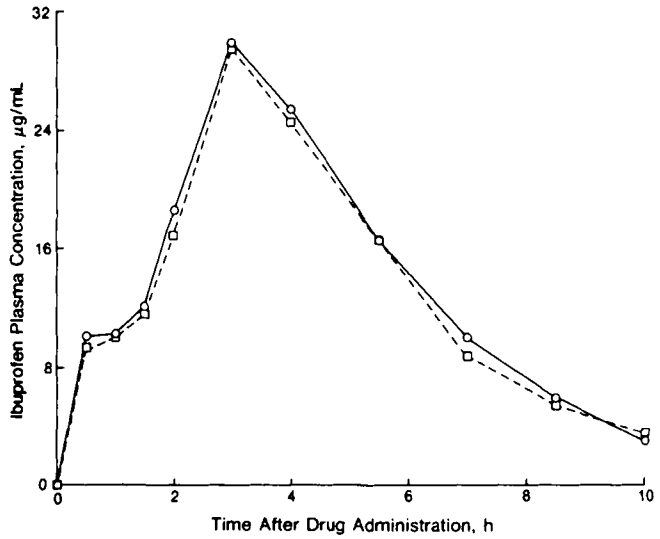

Figure 2—*Average ibuprofen plasma level curves after a single oral 400-mg dose of ibuprofen tablets. Key: (○) capillary plasma; (□) venous plasma.*

[10] N-Evap nitrogen evaporator; Organomation Associates, South Berlin, Mass.
[11] Model 3356 data system integrator; Hewlett-Packard, Avondale, Pa.
[12] Informed written consent was obtained from each subject prior to participation.

Table II—Evaluation of Between- *versus* Within-Day Variation *via* Analysis of Variance

Source	DF[a]	Mean Square	F	p
Low Calibration Curve (1–10 μg/mL)				
Nominal concentration	3	92.8	2546	<0.0001
Day	2	0.0384	1.05	0.37
Error	18	0.0365		
High Calibration Curve (10–100 μg/mL)				
Nominal concentration	3	9166[b]	1593	<0.0001
Day	2	0.0466[b]	0.00811	0.99
Error	17	5.75		

[a] Degrees of freedom. [b] Adjusted for missing day 3, a 60-μg/mL value.

9 h prior to drug administration, then received a 400-mg dose of ibuprofen[13] with 180 mL of water. Food was withheld for an additional 4 h after dosing.

Blood specimens (venous and capillary) were withdrawn at predetermined times over a 10-h period after dosing. Seven milliliters of venous blood was collected at each interval from a forearm vein into evacuated tubes containing sodium heparin[14]. Approximately 0.5 mL of capillary blood was simultaneously collected by fingertip puncture with a sterile blood lancet into heparinized capillary collection tubes[15]. The plasma was harvested and stored frozen until the time of assay.

Saliva samples also were collected at each sampling time and frozen immediately. The production and collection of saliva was facilitated by allowing the subjects to chew on a piece of flexible thermoplastic sheeting[16]. Plasma and saliva samples were assayed using a seven-point standard curve, a blank plasma control, and 5.0- and 50.0-μg/mL positive plasma controls.

RESULTS AND DISCUSSION

A typical chromatogram (Fig. 1) of a plasma extract with and without ibuprofen and the internal standard, flurbiprofen, shows well-resolved sharp peaks free from endogenous interferences. The precision of the assay was assessed by evaluating 14 sets of calibration curve data. Six sets were generated from duplicate curves run on three consecutive days, and eight sets were generated during the analysis of plasma levels of ibuprofen from the four subjects who were administered a 400-mg dose of ibuprofen tablets. The *RSD* ranged from 1.91 to 11.6% (Table I). Excluding the 1-μg/mL concentration, the mean *RSD* was 3.7%. Assay sensitivity was 1 μg/mL for a 0.1-mL sample. The mean correlation coefficient for the low curve was 0.9987, while that for the high curve was 0.9992.

An estimate of within-day *versus* between-day variability was obtained by statistically evaluating the duplicate calibration curve data using a two-way ANOVA with nominal concentrations and days as blocking variables. In this analysis, the mean square associated with days represents between-day variation, while that for the error term represents within-day variation. No significant differences were observed for either the low or high calibration curves (Table II) indicating that between- *versus* within-day variabilities were comparable.

The accuracy of the method was estimated by assaying the two positive plasma controls on 14 separate occasions. The results show that excellent accuracy was obtained at both low and high concentrations; the mean *RSD* was 5.1%. Recoveries were 59.2 and 57.2% for the internal standard and ibuprofen, respectively.

Results from the measurement of ibuprofen in capillary and venous plasma in four normal human volunteers after a single oral 400-mg dose of ibuprofen (tablets) are shown in Fig. 2. Capillary and venous plasma level curves were superimposable. No significant differences between capillary and venous concentrations were observed using the nonparametric Wilcoxon signed-rank test.

In Table III the pharmacokinetic parameters obtained from capillary and venous plasma for the four subjects are compared. Values from the two sampling techniques were virtually identical, demonstrating the utility of capillary sampling for bioavailability/pharmacokinetic studies where small volumes of blood are prerequisites.

Saliva samples were also assayed for ibuprofen; calibration curves, positive controls, and blank (negative) controls were prepared in drug-free saliva rather

[13] Motrin tablets, 400 mg, Lot 270PF; The Upjohn Manufacturing Co., Barceloneta, P.R.
[14] Becton, Dickinson and Co., Rutherford, N.J.
[15] Caraway tubes; Scientific Products, McGaw Park, Ill.
[16] Parafilm M; American Can Co., Greenwich, Conn.

Table III—Comparison of Ibuprofen Pharmacokinetics in Venous and Capillary Plasma Following the Administration of One 400-mg Ibuprofen Tablet to Four Normal Healthy Subjects

Subject	C_{max}, μg/mL		t_{max}, h		AUC_{10}, μg/mL · h		$t_{1/2}$, h	
	Capillary	Venous	Capillary	Venous	Capillary	Venous	Capillary	Venous
1	32.8	32.1	4.0	4.0	180	162	1.65	2.54
2	35.2	31.4	2.0	2.0	151	148	1.95	2.01
3	33.9	32.6	3.0	3.0	107	106	1.50	1.45
4	33.1	32.1	3.0	3.0	138	132	2.49	2.76
Mean	33.7	32.1	3.0	3.0	144	137	1.90	2.19
SD	1.07	0.493	0.816	0.816	30.3	24.0	0.439	0.585

than drug-free plasma[17]. All saliva concentrations were below assay sensitivity, indicating the unsuitability of saliva sampling as a noninvasive alternative to the collection of blood samples.

[17] The precision, sensitivity, and accuracy of the method were comparable whether the biological matrix was plasma or saliva.

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A New Method for High-Performance Liquid Chromatographic Determination of Drotaverine in Plasma

J. MEZEI ˣ, S. KÜTTEL, P. SZENTMIKLÓSI, S. MARTON, and I. RÁCZ

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Abstract □ A sensitive, specific high-performance liquid chromatographic procedure was developed for the determination of plasma drotaverine levels. Basic plasma samples were adjusted to pH 1.5 and extracted with chloroform. HPLC [*n*-heptane–dichloromethane–diethylamine (50:25:2)] on a microporous silica column, with a variable-wavelength UV detector set at 302 nm allowed the measurement of drotaverine at the 50-ng/mL level. The utility of this method for determination of drotaverine in dog and rat plasma was demonstrated.

Keyphrases □ Drotaverine—liquid chromatography, plasma □ Liquid chromatography—determination of plasma drotaverine levels

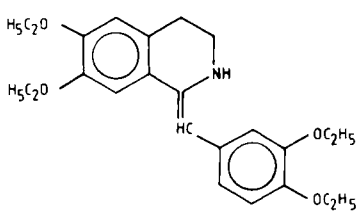
Drotaverine, [1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline] (I), an effective spasmolytic drug (1), is also marketed as a salt developed with theophylline-7-acetic acid (2).

There are only a few reports in the literature describing the measurement of drotaverine in plasma. ^{14}C-Labeled drotaverine was used to study the pharmacokinetics of the drug in mice (3) and humans (4). The spectrophotometric method (5) and the procedure suggested for the assay of papaverine (6) was found to be neither specific nor sensitive. This paper describes a modification of the high-performance liquid chromatographic method (HPLC) which has been reported for the measurement of papaverine in plasma (7).

EXPERIMENTAL SECTION

Reagents—Drotaverine[1], papaverine hydrochloride[1], drotaveraldine[1] (II), chloroform[2], hydrochloric acid[3], *n*-heptane[4], diethylamine[3], dichloromethane[3], glacial acetic acid[5], and anhydrous sodium sulfate[3] were used without any further purification.

Apparatus—The liquid chromatograph[6] was fitted with a stopped-flow injector[6], a variable-wavelength UV detector[7] (set at 302 nm), and a microporous silica column[8]. The flow rate of the mobile phase was 1.6 mL/min. Chromatograms were recorded[9] at 1 cm/min chart speed.



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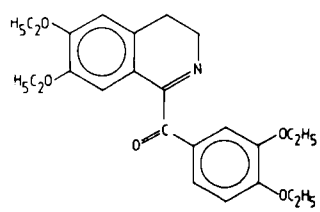

II

[1] Chinoin Chemical Works Ltd., Budapest, Hungary.
[2] Merck, Darmstadt, Federal Republic of Germany.
[3] Reanal Chemical Works, Budapest, Hungary.
[4] Reachim, Soviet Union.
[5] Erdökémia Chemical and Industrial Co., Budapest, Hungary.
[6] Model Liquochrom 307; Labor MIM, Budapest, Hungary.
[7] Model Liquodet 308; Labor MIM, Budapest, Hungary.
[8] Chromspher-Sil 10 μm; 3.9 × 250 mm; Labor MIM, Budapest, Hungary.
[9] NE-230 recorder; EMG, Budapest, Hungary.

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