Determination of Flurbiprofen in Human Serum by Reverse-Phase High-Performance Liquid Chromatography with Fluorescence Detection

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Abstract □ A reverse-phase high-performance liquid chromatographic method is described for the determination of flurbiprofen in human serum. Flurbiprofen is extracted from hydrochloric acid-acidified serum with pentane–ether (80:20). An octadecylsilane column was used with a mobile phase of acetonitrile–water–phosphoric acid (650:350:0.5, v/v/v). A fluorescence detector with excitation at 250 nm and emission at 315 nm provided a quantifiable peak for 0.1 μg/mL of flurbiprofen in 0.5 mL of plasma. A comparison between UV and fluorescence detection systems is presented. The method is applicable to human bioavailability and pharmacokinetic studies with flurbiprofen.

Keyphrases □ Flurbiprofen– HPLC detection compared with fluorescence detection, human serum

Flurbiprofen [(±)-2-(2-fluoro-4-biphenylyl)propionic acid] is an orally active, nonsteroidal, anti-inflammatory drug found to be effective in the treatment of rheumatoid arthritis (1–3). We have developed a simple, sensitive, specific, and rapid analytical procedure to measure levels of flurbiprofen (0.1–10 μg/mL) in serum generated in clinical studies. Previous methods either required specialized sample preparation apparatus (4) or time-consuming TLC separation followed by derivatization and GC analysis (5). Investigations into the fluorescence of flurbiprofen suggested that sufficient sensitivity and selectivity could be achieved by employing reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection. Liquid–liquid extraction proved to be a satisfactory sample preparation technique and 4-biphenylacetic acid exhibited partition and fluorescence characteristics similar enough to flurbiprofen to be used as an internal standard. A method for measuring up to 0.1 μg/mL in 0.5 mL of plasma is described herein.

EXPERIMENTAL SECTION

Reagents and Materials—Reagents were at least analytical reagent grade quality. Acetonitrile[1], methanol[1], and pentane[1] were distilled in glass. Deionized water was supplied[2]. Stock solutions of 4-biphenylacetic acid[3] and flurbiprofen[4] were prepared at 1 mg/mL in methanol. Anhydrous ether[5], phosphoric acid[5] and 1 M hydrochloric acid[6] were used as supplied. The ether was monitored daily for peroxide formation.

Instrumentation—A fluorescence detector[7], solvent pump[8], and an autoinjector[8] were used for the routine chromatographic analysis. A variable-wavelength UV detector[9] was used for comparison with the fluorescence detector. A recorder[10] and an integrator[11] were used for data acquisition. A two-speed reciprocating shaker[12] and centrifuge[13] were used in the sample preparation.

Chromatographic Conditions—Chromatography took place on a 0.46 cm i.d. × 25-cm column packed with octadecylsilane bonded to microparticulate silica (6 μm)[14]. A guard column[15], 0.46 cm × 3 cm, packed with octadecylsilane bonded to microparticulate silica (5 μm), preceded the analytical column. The mobile phase was acetonitrile–water–phosphoric acid (650:350:0.5, v/v/v) filtered through a 0.45-μm membrane[16].

The flow rate was 1.0 mL/min, the column temperature was ambient, and the column back pressure was ~1000 psi. The injection volume was 20 μL, and the run time was 8 min. The retention times for flurbiprofen and 4-biphenylacetic acid were 5.0 min and 6.1 min, respectively. The settings for the fluorescence detector were: excitation wavelength, 250 nm; emission wavelength, 315 nm; slit width, 5 nm; range, 3.0; photomultiplier gain, normal; response, normal; output to recorder, 1 V; output to integrator, 10 mV.

Sample Preparation—Appropriate spiking solutions of flurbiprofen in methanol were prepared from the 1-mg/mL stock solution. A methanolic solution (10 μg/mL) of the internal standard (4-biphenylacetic acid) was prepared from the 1-mg/mL stock solution.

The appropriate standard spiking solution (50 μL for standard tubes) or 50 μL of methanol (for controls, samples, and the blanks) was added to culture tubes (16 × 125 mm) equipped with polytef-lined screw caps. Internal standard (50 μL), 0.5 mL serum, and 0.5 mL of 1 M hydrochloric acid was added to the tube and the contents were mixed on a vortex mixer. Pentane–ether (80:20, 10 mL) was added, the tube was shaken at high speed for 10 min and then centrifuged at 2000 rpm for 10 min. The organic layer was transferred to an empty culture tube (16 × 125 mm), and it then was evaporated under nitrogen in a water bath maintained at 30°C. When dry, the residue was dissolved in 5.0 mL of acetonitrile by mixing on a vortex mixer. A portion of the solution was transferred to a vial for autoinjection.

Two normal healthy male adult volunteers were administered flurbiprofen. These volunteers fasted 9 h before and 4 h after administration. One 50-mg film-coated flurbiprofen tablet was administered with ~188 mL of water, and blood (7 mL) was drawn by venipuncture into blood collection tubes[17] just before drug administration (0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, and 30 h. Serum was harvested from the blood samples immediately after collection and kept frozen until assayed by the above procedure.

Calculations—Data were processed by a data system[11] using computer-generated, baseline-corrected peak heights. Peak height ratio curves (concentration flurbiprofen/concentration internal standard) were established and checked daily with standards to ensure acceptable slope and y-intercept values.

The apparent elimination rate constant for each subject, λ_z, was determined from a least-squares regression fit of the terminal log-linear region of the semilogarithmic plasma concentration–time curves. Area under the concentration–time curve, AUC_∞, was estimated by the trapezoidal rule with extrapolation to infinity. Apparent clearance after oral dosing, CL/F, was calculated from the expression:

$$CL/F = \frac{\text{Dose}}{AUC_\infty} \qquad \text{(Eq. 1)}$$

Apparent volume of distribution, Vd/F was calculated according to:

$$Vd/F = \frac{\text{Dose}}{\lambda_z AUC_\infty} = \frac{CL/F}{\lambda_z} \qquad \text{(Eq. 2)}$$

[1] Burdick and Jackson Laboratories, Muskegon, Mich.
[2] Milli-Q; Millipore Corp., Bedford, Mass.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Supplied by The Upjohn Co., Kalamazoo, Mich.
[5] Mallinckrodt, Inc., St. Louis, Mo.
[6] Fisher Scientific, Pittsburgh, Pa.
[7] Model 650-105; Perkin-Elmer, Norwalk, Conn.
[8] Model 1081B; Hewlett Packard, Palo Alto, Calif.
[9] Model LDC Spectromonitor III; Milton Roy Co., Riviera, Fla.
[10] Linear Instruments Corp., Irvine, Calif.
[11] Model 3356; Hewlett Packard, Avondale, Pa.
[12] Eberback Corp., Ann Arbor, Mich.
[13] International Equipment Corp., Needham, Mass.
[14] Zobax ODS; DuPont, Wilmington, Del.
[15] Brown Laboratories, Santa Clara, Calif.
[16] Millipore Corp.
[17] Vacutainer without anticoagulant.

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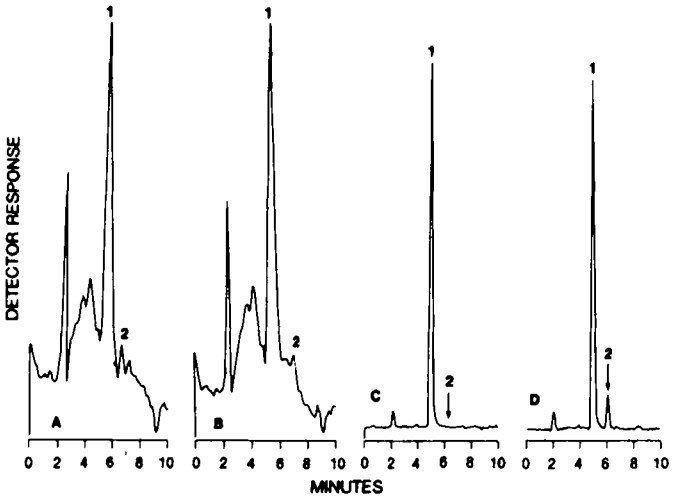

Figure 1—*Reverse-phase high-performance liquid chromatograms of serum extracts using UV (A,B) and fluorescence (C,D) detection. Serum was spiked with 4-biphenylacetic acid (A,C) or 4-biphenylacetic acid and flurbiprofen (0.1 μg/mL serum concentration) (B,D). Key: (1) 4-biphenylacetic acid; (2) flurbiprofen.*

Table I—Flurbiprofen Data Used in Constructing Standard Curves

Theoretical Conc., μg/mL	Mean Experimental Value, μg/mL[a]	*SD*	*RSD*, %
0	< 0.10		
0.10	0.099	0.0107	10.8
0.30	0.301	0.0162	5.38
0.60	0.597	0.0343	5.74
1.00	0.993	0.0395	3.98
3.00	3.020	0.156	5.17
6.00	5.980	0.075	1.25
10.0	10.00	0.230	2.28
Curve	Slope	Intercept	*r*
1	0.8562	−0.0090	0.9999
2	0.8289	−0.0205	0.9998
3	0.8336	−0.0026	0.9997
4	0.8479	0.0145	0.9989
Mean	0.8417	−0.0044	0.9996
SD	0.0126	0.0146	0.00046

[a] Mean of eight values, *i.e.*, samples analyzed in duplicate on four successive days.

Table II—Recoveries of 4-Biphenylacetic Acid and Flurbiprofen from Human Serum

Amount Added, μg/mL	Amount Found, μg/mL	Recovery, %
	4-Biphenylacetic Acid	
1.0	0.967	96.7
1.0	0.906	90.6
1.0	1.003	100.3
1.0	0.965	96.5
1.0	0.952	95.2
1.0	0.903	90.3
Mean		94.9
SD		3.9
	Flurbiprofen	
0.10	0.093	92.6
0.10	0.095	94.6
1.00	1.05	104.5
1.00	1.01	101.1
10.0	9.77	97.7
10.0	9.36	93.6
Mean		97.4
SD		4.7

RESULTS AND DISCUSSION

A comparison of chromatograms with UV and fluorescence detection (Fig. 1) of blank and flurbiprofen-spiked serum illustrates the sensitivity and selectivity of the fluorescence assay. The blank (Fig. 1C) reveals no interfering peaks and the peak height of the flurbiprofen at required limit of sensitivity is adequate for quantitation. Because of the interferences evident in the chromatograms from UV detection, fluoresence is the preferred detection technique.

The precision and linearity of the method was investigated. A standard curve (eight points) was analyzed on 4 consecutive days. Table I summarizes the experimental data. The low deviation from linearity within each curve and the low relative standard deviation for the different runs demonstrates the efficacy of the method.

Table II summarizes the recovery results for flurbiprofen and internal standard (4-biphenylacetic acid) from spiked serum as compared with direct injection of standards prepared from stock solutions. The integrator peak height information was used in the calculations (*i.e.*, no internal standards were used). The results of 97.4 + 4.7% for flurbiprofen and 94.9 + 3.9% for 4-biphenylacetic acid show the essential quantitative recovery from serum.

The strong native fluorescence of flurbiprofen allowed achievement of the desired sensitivity of 0.1 μg/mL without approaching the design capability limits of the detector. Likewise, the amount of acetonitrile added to dissolve the extract residue in the sample preparation was chosen for convenience and can be decreased significantly, thus increasing the assay sensitivity. The limit of quantitation might also be improved by increasing the volume of solution injected.

The serum concentration–time profiles resulting from the administration of single oral 50-mg doses of flurbiprofen to two human subjects are shown in Fig. 2. Estimates of selected pharmacokinetic parameters are presented in Table III. The results are consistent with previously published reports describing flurbiprofen pharmacokinetics (6).

In conclusion, a sensitive, precise, and specific HPLC method with fluorescence detection has been described for the detection of 0.1 μg/mL of flurbiprofen in 0.5 mL of plasma. The method is readily applicable to routine analysis of plasma samples from bioavailability–pharmacokinetic studies in humans. Moreover, because of the strong native fluoresence of flurbiprofen,

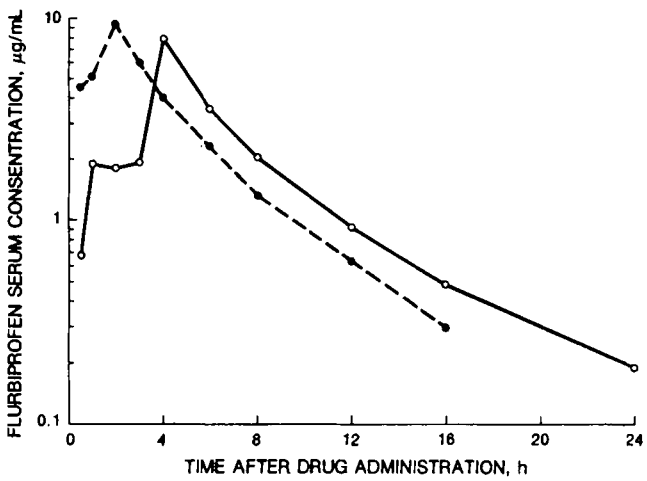

Figure 2—*Flurbiprofen serum concentrations following the administration of single oral 50-mg doses to two adult volunteers. Key: (○) subject 1; (●) subject 2.*

Table III—Flurbiprofen Pharmacokinetics Following the Administration of Single Oral 50-mg Doses to Two Adult Volunteers

	Subject 1	Subject 2
Age, years	26	30
Body weight, kg	80.7	62.6
Peak concentration, μg/mL	8.01	9.44
Peak time, h	4	2
Area under the concentration–time curve, μg·h/mL	39.8	41.2
Apparent clearance, mL/h/kg	15.6	19.4
Apparent distribution volume, mL/kg	131	104
Apparent elimination rate constant, h^{-1}	0.118	0.186

the sensitivity of the method can no doubt be extended making it an attractive technique for studying the bioavailability and pharmacokinetics of flurbiprofen in pediatric populations where small sample volumes and lower doses are required.

REFERENCES

(1) S. S. Adams and K. F. McCullough, 7th European Rheumatology Congress, London, England, Abstr. 32.2 (1971).

(2) I. M. Chalmers, B. J. Cathcart, E. B. Kumar, W. Carson Dick, and W. W. Buchanan, *Ann. Rheum. Dis.*, **31**, 319 (1972).

(3) I. M. Chalmers, M. A. Bell, and W. W. Buchanan, *Ann. Rheum. Dis.*, **32**, 58 (1973).

(4) B. G. Snider, L. J. Beaubein, D. J. Sears, and P. D. Rahn, *J. Pharm. Sci.*, **70**, 1347 (1981).

(5) D. G. Kaiser, S. R. Shaw, and G. J. Vangiessen, *J. Pharm. Sci.*, **63**, 567 (1974).

(6) P. C. Risdall, S. S. Adams, E. L. Crampton, and B. Marchant, *Xenobiotica*, **8**, 691 (1978).

Direct, Simultaneous Determination of Propranolol and Its 4-Hydroxy Metabolite by Liquid Chromatography

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Abstract □ Propranolol and its active 4-hydroxy metabolite are determined by direct injection of spiked plasma samples onto a protein-coated ODS liquid chromatographic column. The recovery of propranolol and the 4-hydroxy metabolite is essentially quantitative and the reproducibility is good. The reproducibility and simplicity may be superior to the conventional HPLC analyses which include pretreatment, *i.e.*, solvent extraction or deproteinization.

Keyphrases □ Protein-coated ODS column—reverse-phase, direct injection analysis of plasma sample □ Propranolol—analysis and its 4-hydroxy metabolite, plasma

Propranolol, a β-adrenergic blocking drug, is widely used for the treatment of cardiac arrhythmia, sinus tachycardias, hypertension (1), and other pathological states (2, 3). The therapeutic drug level of propranolol is considered to be in the range of 50–100 ng (193–386 pmol)/mL of plasma (4). The 4-hydroxy metabolite, formed by hydroxylation of propranolol (5), has been reported to be an equipotent β-adrenergic blocker (6). Thus, for drug level monitoring, both propranolol and the 4-hydroxy derivative should be determined. Determination of propranolol (7, 8) and the 4-hydroxy metabolite using a reverse-phase HPLC method including sample pretreatment, such as solvent extraction or deproteinization has been reported previously (9–11). We reported that the protein-coated ODS column had no affinity for proteins, but did have an affinity for small hydrophobic molecules (12). The simultaneous plasma determination of propranolol and the 4-hydroxy metabolite reported herein is rapid and reproducible, and deproteinization can be carried out by the direct injection of plasma samples onto a protein-coated ODS liquid chromatographic column.

EXPERIMENTAL SECTION

Chemicals and Reagents—Propranolol hydrochloride[1], the 4-hydroxy metabolite (as the hydrochloride)[2], and bovine serum albumin[3] were obtained commercially. All other chemicals were analytical reagent grade.

Preparation of the Protein-Coated Octadecylsilane Column—An ODS column (pore size, 10-nm) was packed by the balanced-slurry method (slurry solvent, chloroform; purge solvent, 50% methanol). After the ODS column was equilibrated with phosphate-buffered saline (pH 7.4), 6% bovine serum albumin solution was injected to saturate the column. After the column was equilibrated with 0.1 M phosphate solution (pH 3.0), bovine serum albumin solution (6%) was again injected, and the column was then washed with absolute methanol. This procedure was repeated several times. The same procedure was repeated, using dialyzed rabbit plasma instead of the bovine serum albumin solution. The column treated by the aforementioned procedure is referred to as the protein-coated ODS column below.

Liquid Chromatographic Apparatus—An HPLC apparatus with a single plunger pump[4] and a sample injector[5] was assembled in our laboratory. For stepwise elution, solvent switching was performed by a solvent selector[6] and controlled by a sequence programmer[7]. For double-column analysis, a flow-direction switch valve[8] was placed between the precolumn and the analytical column. The valve was controlled by the sequence programmer[7]. Two fluorometers[9] were used in series: one for detection of propranolol (Ex, 297 nm; Em, 347 nm); the other for the 4-hydroxy metabolite (Ex, 327 nm; Em, 427 nm).

Preparation of Standard and Sample Solutions—A stock solution of propranolol and the 4-hydroxy metabolite containing 2% sodium metabisulfite (13) was prepared in distilled water at 10^{-5} M. Heparinized human and rabbit plasma samples were obtained by centrifuging whole blood (1000×*g*, at 25°C, for 10 min) and filtering[10] to remove solid materials. A plasma sample (0.25 mL) with or without propranolol and the 4-hydroxy derivative at the drug monitoring level, was injected onto the column. If the pressure of the column increased during successive analyses, the inlet filter of the column was washed with 1 M NaOH.

Single-Column Analysis—An ODS column of 100 mm × 6 mm i.d.[11] (particle size 5 μm, pore size 10 nm) was protein-coated and then used at room temperature. The protein-coated ODS column was equilibrated with phosphate-buffered saline (flow rate, 2 mL/min). The plasma sample spiked with propranolol and its 4-hydroxy derivative (0.25 mL) was injected onto the column. After eluting plasma proteins and hydrophilic components with phosphate-buffered saline for 5 min, the column was washed with phosphate-buffered saline containing 22% acetonitrile for 4 min. Propranolol and the 4-hydroxy derivative were then eluted with 0.1 M citrate buffer (pH 4.0) containing 22% acetonitrile.

[1] Supplied by Sumitomo Chemicals, Osaka, Japan.
[2] Supplied by I.C.I. Pharmaceutical Ltd., Osaka, Japan.
[3] Fraction V Powder; Sigma, St. Louis, Mo.
[4] Model INTD18-40-3S2K; Toyo Soda, Tokyo, Japan.
[5] Model 7125; Rheodyne, Calif.
[6] Model 8V; Kyowa Seimitsu, Tokyo, Japan.
[7] Model SCY-PO; Omron, Tokyo, Japan.
[8] Model 7010; Rheodyne.
[9] Model RF-530; Shimadzu, Kyoto, Japan.
[10] Millex-HA; Millipore Corp., Bedford, Mass.
[11] Shodex ODSpak; Showa Denko, Tokyo, Japan.

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