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Technical Note

Stereospecific High-Performance Liquid Chromatographic (HPLC) Assay of Flurbiprofen in Biological Specimens[1]

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KEY WORDS: flurbiprofen; stereospecific high-performance liquid chromatographic (HPLC) assay; biological fluids; enantiomers; nonsteroidal antiinflammatory.

INTRODUCTION

Flurbiprofen (Froben, Boots; Ansaid, Upjohn), 2-(2-fluoro-4-biphenylyl)-propionic acid, is a nonsteroidal analgesic/antiinflammatory drug recently introduced to the North American market. As with all 2-arylpropionic acid (2-APA) derivatives, flurbiprofen (FL) contains a chiral center and consequently is marketed as a racemate. Investigations with similar drugs have revealed differences in the pharmacokinetic behavior and potency of the individual isomers (1–4).

Only a limited number of the reported FL assays have been stereospecific. Maitre *et al.* (5) reported a general enantiospecific high-performance liquid chromatographic (HPLC) method for the 2-APAs for use *in vitro,* requiring a time-consuming derivatization procedure and necessitating cleanup extractions before injection. A stereospecific gas chromatographic (GC) method was reported by Singh *et al.* (6) which required long incubation times for derivatization. It, therefore, became necessary to develop a rapid and sensitive assay capable of analyzing the individual isomers of FL as reported here. This method, based on the derivatization procedure of Bjorkman (7) with some modifications, has previously been applied to the stereospecific assay of ketoprofen (8) and fenoprofen (9).

MATERIALS AND METHODS

Materials. All solvents were HPLC or analytical grade (8,9). L-Leucinamide was purchased from Sigma Chemical Co. (St. Louis, MO.). Flurbiprofen racemate, individual (−) and (+) isomers were kindly supplied by Organon Canada Ltd. (Westhill, Ontario, Canada). Internal standard (IS) ketoprofen was supplied by Rhone-Poulenc Pharma Inc. (Montreal, Quebec, Canada).

Chromatography. A Waters HPLC system (Rexdale, Canada) consisting of a Model 6000A pump, a 710B WISP auto-injector, and a 490 multiple-wavelength UV detector was operated at ambient temperature. Flurbiprofen and IS were detected at 250 and 275 nm, respectively. The column was a 10-cm stainless-steel (4.6-mm-i.d.) octadecyl-bonded silica (5-μm Partisil 5 ODS-3, Whatman Inc. Clifton, N.J.) along with a 5-cm guard column of the same material with a particle size of 10 μm. The peak-area ratio of flurbiprofen to IS was used for all quantitation using an integrator-recorder (3390A, Hewlett-Packard, Edmonton, Canada). The mobile phase consisted of acetonitrile:0.067 *M* KH_2PO_4:triethylamine (35:65:0.02, v/v) and the flow rate was 1 ml/min.

Extraction Procedure. To 0.5 ml of plasma was added 200 μl of 0.6 *M* H_2SO_4. To hydrolyze conjugated FL in urine, 100 μl of 1 *M* NaOH preceded the acid, vortexing between additions. To this was added 50 μl of IS (100 mg of racemic ketoprofen/liter of HPLC-grade water) followed by 3 ml of isopropanol:isooctane (5:95, v/v). This mixture was mixed vigourously for 45 sec then centrifuged at 3000 rpm on a Clay-Adams centrifuge for 5 min. The top layer was transferred to a clean tube and 3 ml HPLC-grade water was added. The mixture was again mixed vigourously and centrifuged for 5 min. The organic layer was aspirated off and 350 μl of 0.6 *M* H_2SO_4 was added to the remaining aqueous layer. This was mixed with 3 ml of chloroform and centrifuged for 5 min. The aqueous layer was aspirated off and the remaining chloroform layer was evaporated by dryness using a SVC100H Savant Speed Vac Concentrator and Refrigerated Condensation Trap (Emerston Instruments, Scarborough, Canada).

The extraction efficiency was determined in spiked samples of human urine and plasma by comparing the peak-area ratios of FL (1.0 and 5.0 mg/liter, $N = 3$) before and after extraction. For this purpose, the samples were analyzed by a nonstereospecific HPLC assay (10).

Derivatization. To the evaporated residue was added 100 μl of 50 m*M* triethylamine in acetonitrile, followed by, at 30-sec intervals, 50 μl of 60 mM ethylchloroformate in acetonitrile, then 50 μl of 1 *M* L-leucinamide. After 2 min 50 μl of HPLC-grade H_2O was added. A 10- to 50-μl aliquot of this mixture was used for analysis.

The intraday ($N = 4$) and interday ($N = 6$) variations of the assay were examined for solutions containing 0.1, 1, 10, and 20 mg/liter.

Standard Curves. Racemic FL, 10 mg, was dissolved in

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20 ml methanol and made up to 100 ml with water. A 10-ml aliquot of this solution was further diluted to 100 ml with 20% methanol. To 0.5 ml of plasma or urine were added different aliquots of the above solutions to produce final concentrations of 0.1 to 20 mg/liter (6 points) and 0.25 to 100 mg/liter (9 points), respectively. The standard samples were then treated in the same manner as described above for plasma and urine samples.

RESULTS AND DISCUSSION

Peaks representing the diastereoisomers of (−) and (+) FL appeared 17 and 21 min after injection, respectively (Fig. 1). Retention times of the derivatized isomers of (−) and (+) IS were 8 and 10 min, respectively. The enantiomers' order of elution was verified by subjecting individual enantiomers to the same procedure as the racemates. There were no interfering peaks from the normal components of plasma.

In urine, FL appeared almost completely as readily hydrolyzable conjugates (Fig. 2). In addition to the peaks representing hydrolyzed conjugated enantiomers of FL, two additional major peaks were noted which are presumed to be enantiomers of a metabolite.

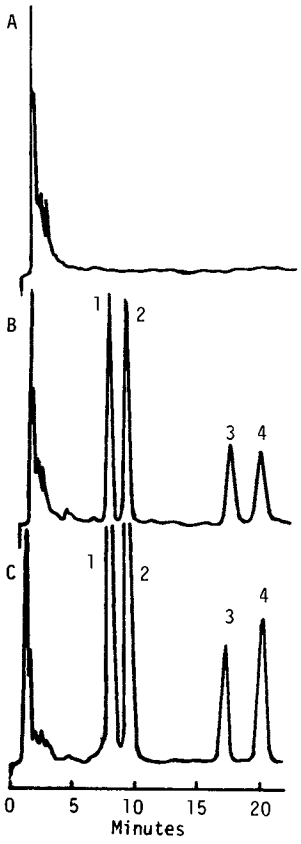

Fig. 1. Chromatograms of drug-free plasma (A), plasma spiked with 1 mg/liter FL (B), and a healthy subject's plasma sample at 12 hr after a 100-mg oral dose (C) depicting (1) (−)- and (2) (+)-ketoprofen and (3) (−)- and (4) (+)-flurbiprofen.

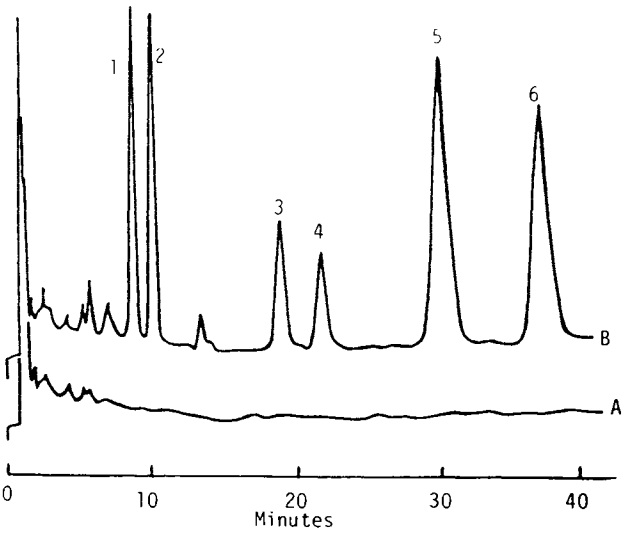

Fig. 2. Chromatograms of drug-free urine (A) and a healthy subject's 12- to 24-hr urine sample after a 100-mg dose of racemic flurbiprofen (B). (1) (−)- and (2) (+)-ketoprofen; (3) (−)- and (4) (+)-flurbiprofen; (5, 6) presumed enantiomers of a metabolite.

Excellent linear relationships ($r^2 > 0.996$) were found between the peak-area ratios and the concentrations. Typical regression lines through the data points could be described by $Y = 0.71X - 0.02$ and $Y = 0.71X - 0.03$ for both enantiomers in plasma and urine, respectively. The extraction efficiency of (−) and (+) enantiomers of flurbiprofen in plasma and urine was greater than 90%. The efficiency of the derivatization determined by measurement of the peak representing underivatized FL was approximately 100%. The intra- and interday variations of the assay ranged from 1.1 to 8.9%. The limit of reliable quantitation was set at 0.1 and 0.25 mg/liter for each enantiomer in plasma and urine, respectively, which encompasses the concentrations observed in a healthy volunteer (Fig. 3).

The assay was found to be suitable for the determination of flurbiprofen enantiomers in human plasma and urine following the administration of recommended doses.

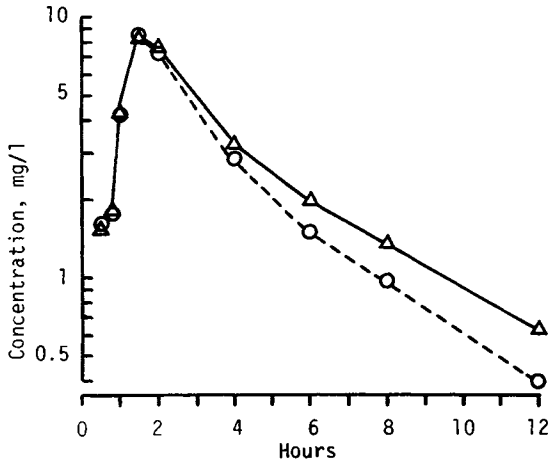

Fig. 3. Flurbiprofen [(+), △; (−), ○] plasma concentration versus time profile for a single healthy subject after a 100-mg oral dose of racemic drug.

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