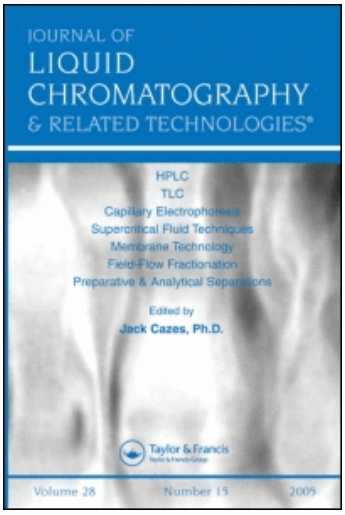


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# Assay of Flurbiprofen in Rat Plasma Using HPLC with Fluorescence Detection

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# ASSAY OF FLURBIPROFEN IN RAT PLASMA USING HPLC WITH FLUORESCENCE DETECTION

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## ABSTRACT

A high performance liquid chromatographic (HPLC) method was developed for the determination of flurbiprofen in rat plasma. The method employs a smaller sample volume (0.05 mL) and involved deproteinization of the biological sample with 2.5 volumes of acetonitrile for the determination of flurbiprofen. Ten microliters of the supernatant was injected onto a $C_{18}$ reverse phase column. The mobile phase employed was acetonitrile-water-phosphoric acid (600:400:0.5, v/v/v). The flow rate was 1.5 mL/min. The column effluent was monitored by fluorescence detection at excitation wavelength of 250 nm and emission wavelength of 285 nm. The retention time was 3.4 min. The detection limit in rat plasma was 50 ng/mL. The mean percentage recovery of the drug in the concentration range of 0.05-5 μg/mL was 95.14% while the mean of the inter-day coefficient of variation of the same concentration range was 1.37%. The method was simple, rapid and accurate for quantitation of flurbiprofen in rat plasma.

## INTRODUCTION

Flurbiprofen, dl-2-(2-fluoro-4-biphenylyl) propionic acid, is a potent nonsteroidal anti-inflammatory drug used for the treatment of rheumatoid arthritis[1] and its related conditions.

Gas chromatographic[2] and high performance liquid chromatographic[3-7] methods have been developed for the quantitation of flurbiprofen. The previously developed gas chromatographic method required time-consuming TLC separation followed by derivatization prior to analysis.[2] In the reported HPLC methods, flurbiprofen in human plasma, dog serum, and urine was determined. However, one of the previous methods required special apparatus for sample preparation[3] and in most of the previous work, a tedious extraction procedure[4-6] and more than 0.5 mL of serum[4] were employed. This paper describes HPLC methods with simple preparation procedures for the determination of flurbiprofen in plasma of small volume (0.05 mL) to study pharmacokinetics for flurbiprofen in rats.



## MATERIALS AND METHODS

### Materials

Flurbiprofen was kindly supplied by Samil Pharm. Co. (Seoul, Korea). HPLC grade acetonitrile was purchased from Merck Co.(Darmstadt, Germany) Phosphoric acid was received from Kokusan Chemical Works Ltd. (Tokyo, Japan). Water was distilled, deionized and filtered in house.

### Preparation of Standard Solutions

Stock solution of flurbiprofen was dissolved in methanol(1 mg/mL). Standard solutions of flurbiprofen in water or rat plasma were prepared by spiking the appropriate volume(less than 10 μL per mL) of variously diluted stock solutions giving final concentrations of 0.05, 0.1, 0.5, 1, 2 or 5 μg/mL.

Recoveries from plasma were calculated by dividing the peak heights of the drug in rat plasma by those in water. Response factors were calculated by dividing the peak height of the drug by their concentrations (μg/mL).

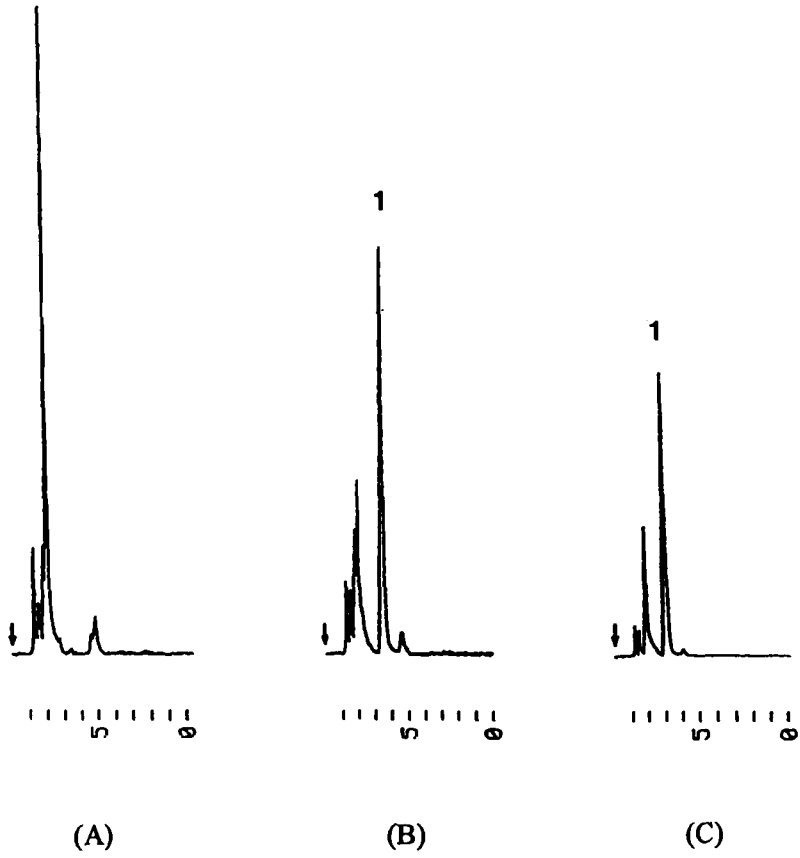


**Figure 1**. Chromatograms of (A) drug-free rat plasma, (B) rat plasma spiked with 0.5 μg/mL of flurbiprofen, (C) plasma obtained from a rat 480 min after intravenous administration of flurbiprofen at 2.5 mg/kg. Peak 1, flurbiprofen (3.4 min).

## Sample Preparation

To 50 μL of rat plasma, 125 μL of acetonitrile were added for the deproteinization of the samples. After vortex mixing and centrifugation at 9000 *g* for 10 min, 10 μL of the supernatant were injected directly onto the HPLC column.

**Table 1**

**Recoveries at Various Concentrations of Flurbiprofen in Rat Plasma**

| Concentration (μg/mL) | Response Factor[a] Mean ± S.D. (n=5) | Recovery (%) |
|---|---|---|
| 0.05 | 1.359 ± 0.0092 | 98.39 ± 2.38 |
| 0.1 | 1.335 ± 0.0087 | 96.88 ± 3.38 |
| 0.5 | 1.205 ± 0.0100 | 91.63 ± 1.87 |
| 1 | 1.208 ± 0.0528 | 96.90 ± 3.78 |
| 2 | 1.187 ± 0.0168 | 94.08 ± 2.58 |
| 5 | 1.165 ± 0.0028 | 92.97 ± 2.87 |
| **Mean** | 1.243 ± 0.0822 | 95.14 ± 2.64 |

*peak height ($10^{-1}$ mV)/concentration (μg/mL)



**Table 2**

**Intra- and Inter-day C.V.s at Various Concentrations of Flurbiprofen in Rat Plasma**

| Concentration (μg/mL) | Intra-day C.V. (%) | Inter-day C.V. (%) |
|---|---|---|
| 0.05 | 1.226 | 0.679 |
| 0.1 | 3.953 | 0.654 |
| 0.5 | 3.383 | 0.827 |
| 1 | 3.994 | 4.372 |
| 2 | 4.318 | 1.417 |
| 5 | 1.920 | 0.243 |
| **Mean** | 3.132 | 1.365 |

## HPLC Apparatus

The HPLC system consisted of a Model 7725i injector (Rheodyne, Cotati, CA, USA), a Model 6200 intelligent pump (Hitachi, Tokyo, Japan), a guard column ($C_{18}$, 5 μm, Brownlee™, Alltech, Deerfield, IL, USA), a reverse phase

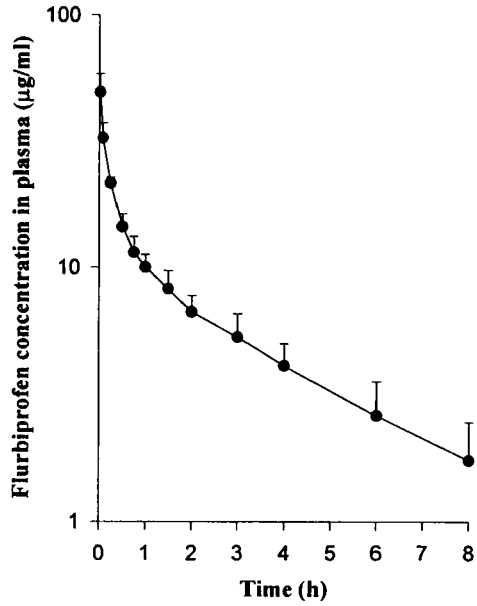


**Figure 2**. Plasma concentration-time profile of flurbiprofen after intravenous administration of flurbiprofen at 2.5 mg/kg to Sprague-Dawley rats. Bars represent standard deviations. Each point represents the mean S.D. (n=5).



column ($C_{18}$, 25 cm 4.6 cm I.D., particle size 5 μm, Hibar, Merck, Germany), a fluorescence spectrophotometer (F-1050, Hitachi, Tokyo, Japan) and a Model D-2520 integrator (Hitachi, Tokyo, Japan). The mobile phase, acetonitrile-water-phosphoric acid (600:400:0.5, v/v/v), was run at a flow rate of 1.5 mL/min and the column effluent was monitored by fluorescence detection at excitation wavelength of 250 nm and emission wavelength of 285 nm. The column temperature was ambient and the column back pressure was 120 $kg/cm^2$.

## RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms of drug-free rat plasma(A), drug standards in rat plasma(B) and plasma(C) collected at 8 h after intravenous administration of 2.5 mg/kg of flurbiprofen to a rat. No interferences from endogenous substances were observed in any of the biological samples. The

retention time for flurbiprofen was 3.4 min. The peaks were sharp and symmetrical, thus making the peak quantitation highly reliable. The detection limit for flurbiprofen in rat plasma was 50 ng/mL (Table 1), based on a signal-to-noise ratio of 3.0. The mean intraday coefficient of variation (C.V.s) of flurbiprofen in rat plasma was 3.13% (Table 2). The mean interday C.V.s for the analysis of the same samples on three days was 1.37% (Table 2). Mean percent recoveries of spiked flurbiprofen from plasma was 95.14% (Table 1).

Flurbiprofen was administered intravenously to 5 rats at the dosage of 2.5 mg/kg. Blood samples were collected from the femoral artery after 0.0167, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6 and 8 hr. Fifty microliters of plasma sample were stored in a freezer prior to the HPLC assay. The mean plasma concentration-time profile of flurbiprofen is shown in Figure 2. The mean terminal half-life, total body clearance, apparent volume of distribution at the steady state and mean residence time of flurbiprofen was 2.78 hr, 47.94 mL/hr/kg, 156.78 mL/kg and 3.40 hr, respectively.

It can be seen that this simple and reproducible HPLC method has enough sensitivity for in vivo studies to evaluate flurbiprofen pharmacokinetics.



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