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Note

Flurbiprofen analysis in plasma and breast milk by high-performance liquid chromatography

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Flurbiprofen is a non-steroidal anti-inflammatory, analgesic, antipyretic drug with an assumed mode of action to inhibit prostaglandin synthesis, as found with other non-steroidal anti-inflammatory agents [1]. The drug is a phenylalkanoic acid [2-(2-fluoro-4-biphenylyl)propionic acid], exhibits weakly acidic properties, and has a pK_a of 4.13 [1, 2]. In man, flurbiprofen is rapidly absorbed after oral administration. Only the unchanged drug has been detected in human plasma, and it is greater than 99% plasma protein bound [1, 2].

Several methods have been reported for the detection of flurbiprofen in plasma, including thin-layer chromatography, gas chromatography, and high-performance liquid chromatography (HPLC) [2—4]. The HPLC technique allows sufficient sensitivity for the detection of plasma levels of flurbiprofen with the least complicated sample preparation. A method for analysis of the drug in milk has not been described. The unique nutritional features of human breast milk and the meager state of knowledge about drugs in human milk have been emphasized [5—8]. Accordingly, a method of sample preparation and HPLC suitable for the assay of flurbiprofen in both plasma and human breast milk is reported here.

EXPERIMENTAL

Materials

Flurbiprofen and the internal standard 2-(2′-chloro-4-biphenylyl)propionic acid were supplied by Boots Pharmaceuticals (Shreveport, LA, U.S.A.). Acetonitrile, methanol, and acetic acid were HPLC grade from J.T. Baker.

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Reagent-grade perchloric acid and sodium hydroxide were used. Water was double distilled and deionized. Mobile phase was filtered through a 0.45-μm membrane (Millipore) prior to use. Stock solutions (1 mg/ml) of drug and internal standard were prepared in methanol and working standard solutions were made by dilution of these with water. Blank plasma and milk samples for standard curve and recovery data (%) were obtained from drug-free lactating women. One patient received 50 mg flurbiprofen four times a day for a total of nine doses over three days. Paired plasma and breast milk samples were obtained.

Chromatography

Analyses were performed at room temperature (23°C) on an HPLC system consisting of a Waters M6000A solvent delivery system, WISP 710A injector, RCM 100 radial compression separation system, Model 440 absorbance detector, Waters 730 data module and 720 system controller. The column was a Waters 10 cm × 8 mm μBondapak C_8 Radial Pak cartridge, particle size 10 μm, with a C_{18} Guard Pack insert. Mobile phase was acetonitrile—methanol—1% acetic acid (30:30:40), flow-rate 2.0 ml/min. Detector wavelength was 254 nm with sensitivity 0.005 a.u.f.s. Injection volume was 50 μl. Under these conditions flurbiprofen eluted at 6 min and internal standard at 7 min. Interference from endogenous compounds in plasma and milk samples was not observed (see Fig. 1).

Preparation of samples

A 200-μl sample of milk or plasma was prepared for chromatography by addition of 20 μl of 30% perchloric acid to precipitate protein, followed by 200 μl methanol containing 2 μg/ml internal standard. This sample was mixed by vortexing for 2 min to remove trapped or bound drug from the precipitate. The sample was then made basic by adding 20 μl of 5 *M* sodium hydroxide, and it was centrifuged at 5000 *g* to remove the precipitate. A 200-μl aliquot of the supernatant fraction was diluted with an equal volume of mobile phase, mixed, and centrifuged if further precipitation was found. The resultant supernatant fraction was placed in a WISP (Waters) vial for injection into the HPLC system.

Preparation of standards

Water standards for recovery determinations were prepared by adding 20 μl of flurbiprofen standard to 180 μl of water, followed by 20 μl of 30% perchloric acid, 200 μl of methanol containing internal standard, and 20 μl of 5 *M* sodium hydroxide. This was diluted 1:1 with mobile phase and placed in a WISP vial for injection into the HPLC system.

Plasma or milk standards, made by adding 20 μl of flurbiprofen standard to 180 μl of blank patient plasma or milk, were prepared as described for the samples.

Data analysis

Data from the chromatography were reported as ratios of integrated peak areas from drug peak area versus peak area from internal standard for each

sample. Concentrations were determined by comparing these ratios from plasma or milk unknowns to those generated from the least-squares fit linear regression analysis of the standard curve data.

Standard curves were done separately for both plasma and milk by using standards prepared in each medium as described above. Concentrations of flurbiprofen used were 0.05—2.0 μg/ml. The recovery (%) was determined by comparing the peak-area ratios of a water standard versus a plasma or milk standard of the same concentration.

RESULTS

Both plasma and milk standard curves showed linearity in the range 0.05—2.0 μg/ml. For plasma the equation of the least-squares fit line is $y = 0.7096x + 0.034$, $r = 0.998$; for milk, $y = 0.8000x + 0.0145$, $r = 0.999$, where x is the drug concentration in μg/ml and y is the ratio of the area of drug peak to internal standard peak.

Accuracy and precison of the method was assessed by analyzing replicate samples of plasma and milk at a concentration of 1 μg/ml. For plasma the mean value of nine analyses was 1.04 ± 0.03 μg/ml, coefficient of variation (C.V.) = 2.8%. For milk the mean value of ten analyses was 0.96 ± 0.03 μg/ml, C.V. = 3.1%.

The recovery of 1.0 μg/ml flurbiprofen in plasma was 96.8% (n=8). The recovery of 1.0 μg/ml flurbiprofen in milk was 106% (n=5). Recovery was determined by comparing the peak-area ratio of drug to internal standard in rep-

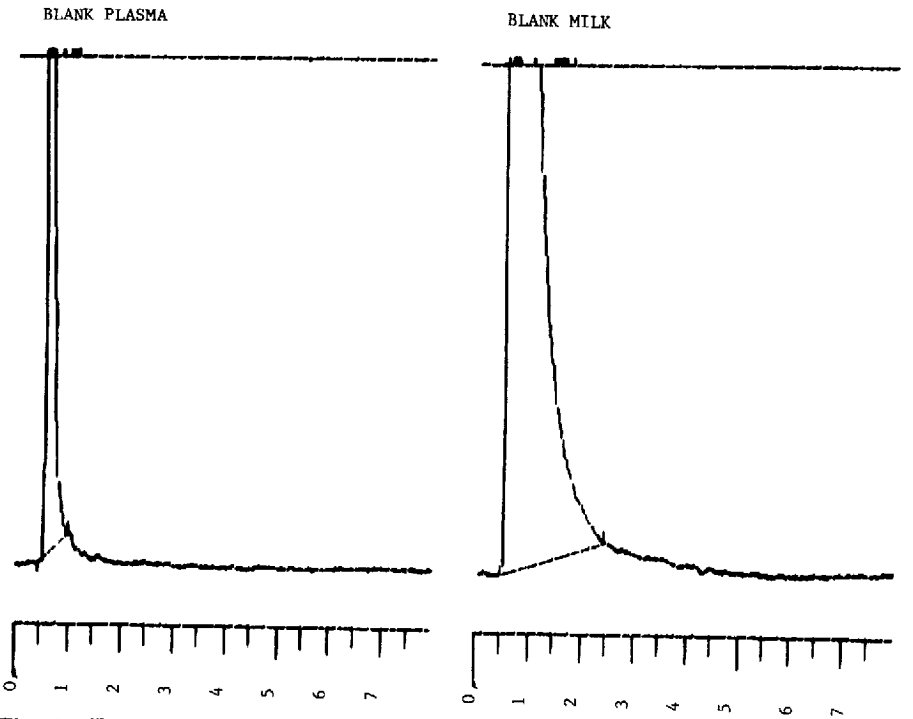

Fig. 1. Chromatograms of blank plasma and milk without internal standard. Sample preparation and chromatographic conditions are as described in the text.

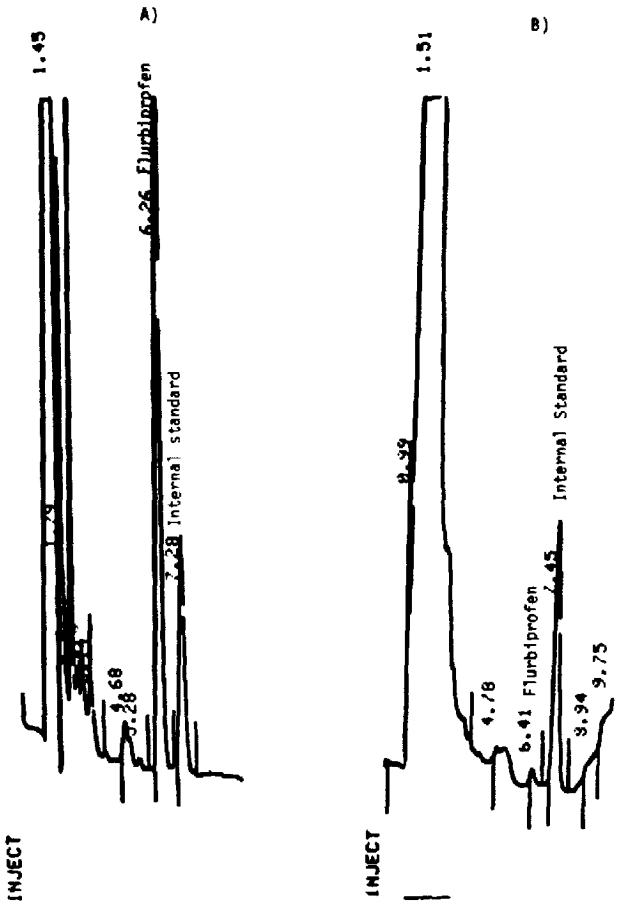

Fig. 2. Chromatograms of (A) plasma and (B) milk showing separation of flurbiprofen and internal standard. Sample preparation and chromatographic conditions are as described in the text. Plasma concentration of flurbiprofen is 5.07 μg/ml. Milk concentration of flurbiprofen is 0.07 μg/ml.

licate plasma and milk to that of an equal amount of drug injected in water.

Fig. 2 contains typical chromatograms showing separation of flurbiprofen and internal standard in both plasma and milk samples obtained from a patient 2 h after oral dosing with flurbiprofen. This method allows sufficient sensitivity to detect flurbiprofen at 0.05 μg/ml in both media.

DISCUSSION

Flurbiprofen is a weakly acidic drug, pK_a = 4.13, which is highly protein-bound and, in the unionized form, likely to be highly lipid-soluble due to its biphenyl moiety. Analysis of samples containing this drug is guided by these characteristics. For initial sample preparation, protein precipitation followed by methanol wash with a 2-min mix by vortex was sufficient for liberation of drug bound to precipitated protein. Similar results were found for recovery of drug from water or plasma.

In a medium such as milk, where lipid concentrations are higher than plasma, precautions must be taken to ensure that the drug is not trapped and therefore lost from the assay.

The weakly acidic flurbiprofen exists more in the unionized state in milk. It is highly susceptible to loss in the lipid component of milk after addition of acid to precipitate protein from the samples. In order to circumvent this potential loss, samples are made basic by addition of sodium hydroxide after the methanol wash. The drug then exists in its ionized form and is solubilized in the aqueous portion of the sample preparation. Although some protein is redissolved, this problem is minimized by the dilution of sample supernatant fraction with mobile phase containing 60% acetonitrile–methanol (50:50). This dilution causes precipitation of remaining protein thus obviating problems for injection on the column. The drug, along with any lipid which may have been carried over in the supernatant fraction, remains in the fraction diluted with mobile phase. In addition, the pH of the sample solution is then similar to that of the mobile phase so that the samples injected into the HPLC system are not basic. This prolongs column usefulness.

In summary, we have reported a method of sample preparation and HPLC which may be similarly applied to plasma and breast milk samples for determination of concentrations of the non-steroidal anti-inflammatory drug flurbiprofen. This method exhibits sufficient accuracy and precision for determination of drug levels to 0.05 μg/ml in either fluid. Sample preparation with this method is direct, avoids time-consuming extraction procedures and requires only a small sample volume. Because the plasma and milk methods are identical, samples may be analyzed concomitantly without changes in preparation or chromatography conditions. To date, over 500 plasma and milk samples containing flurbiprofen have been analyzed in this laboratory. The principles employed for sample preparation and chromatography of this drug are likely suitable for other drugs in breast milk.

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