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Note

Determination of the non-steroidal anti-inflammatory drug flunoxaprofen, *S*(+)-2-(4-fluorophenyl)-α-methyl-5-benzoxazoleacetic acid, in blood and urine by high-performance liquid chromatography

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A considerable amount of evidence has accumulated on the anti-inflammatory, analgesic and antipyretic properties of the non-steroidal anti-inflammatory drug *S*(+)-2-(4-fluorophenyl)-α-methyl-5-benzoxazoleacetic acid (flunoxaprofen; FLX), both in animal tests [1–3] and in several human pathological conditions, such as rheumatoid arthritis, osteoarthritis [4–6] and gynaecological [7] and venous [8] inflammatory diseases.

The development of a sensitive and selective assay method for the determination of FLX was critical for the measurement of drug levels in pharmacokinetic studies [9]. In order to study the absorption and the excretion of this drug in animals and man, we have developed a high-performance liquid chromatographic (HPLC) procedure for the determination of FLX in blood and urine.

EXPERIMENTAL

Apparatus

Chromatography was performed with a Perkin-Elmer (Norwalk, CT, U.S.A.) Series 3B pump fitted with a Rheodyne 7105 injection valve (Perkin-Elmer) and a 175-μl sample loop.

A 25 cm×0.46 cm I.D. reversed-phase LiChrosorb RP-18 (10 μm) column (Merck, Darmstadt, F.R.G.) was used. A Perkin-Elmer Model LC 75 variable-wavelength UV detector with autocontrol was operated at 300 nm. A Sigma 10B Chromatography Data Station computer (Perkin-Elmer) was used to record, store and analyse the chromatograms.

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Reagents and chemicals

Analytical-reagent-grade diethyl ether, acetonitrile, phosphoric acid, sodium hydroxide, monobasic potassium phosphate and sodium chloride were obtained from Merck and FLX and (*R,S*)-2-(4-chlorophenyl)-α-methyl-5-benzoxazole-acetic acid (benoxaprofen, BNX) were synthesized in our laboratories.

Water was passed through an SM 11607 0.2-μm membrane filter (Sartorius, Göttingen, F.R.G.) before use.

Stock standard solutions of FLX and BNX (100 μg/ml) were prepared in 10 m*M* sodium hydroxide solution.

Procedure

The mobile phase consisted of 4 m*M* phosphoric acid and 10 m*M* monobasic potassium phosphate in acetonitrile–water (60:40, v/v). The flow-rate was 1 ml/min. Serum and urine standards for calibration graphs were prepared by spiking 1-ml aliquots of serum and urine with stock solution of FLX to give standards ranging from 0.150 to 10 μg/ml. The concentration of the internal standard (BNX) was 5 μg/ml in each serum and urine sample.

For the extraction of serum, 1 ml of sample (standard, control or patient serum) was pipetted into a glass tube and exactly 50 μl (5 μg) of internal standard (BNX), 500 μl of 2 *M* phosphoric acid and 5 ml of diethyl ether were added. The tubes were then gently vortexed for 10 min, and after centrifuging the aqueous layer was separated and 5 ml of diethyl ether were added again following the above procedure. The organic layers were evaporated to dryness under a stream of nitrogen. The residue was dissolved in 500 μl mobile phase and a 50-μl aliquot of this solution was injected into the HPLC column.

For the extraction of the free compound from urine 1 g of sodium chloride and exactly 50 μl (5 μg) of internal standard (BNX) were added to 1 ml of urine, which was then acidified to pH 2 with 2 *M* phosphoric acid. The subsequent extraction procedure was carried out as described above for serum.

For the free and bound compound (glucuronide derivative), 1 g of sodium chloride, 1 ml of phosphate buffer (pH 6.8) and 100 μl of β-glucuronidase–arylsulphatase (12 and 60 U/ml, respectively) (from *Helix pomatia*; Merck) were added to 1 ml of urine. Each sample was mixed briefly following each addition using a vortex-type mixer. The tubes were allowed to react for 40 h at 37°C, then the described procedure was followed.

RESULTS AND DISCUSSION

Fig. 1a is a representative chromatogram of FLX and the internal standard (BNX). FLX was identified by comparing the retention times of peaks from standard solutions of FLX and BNX; under our experimental conditions the retention times were 3.4 min (FLX) and 4.5 min (BNX). Excellent linearity of the calibration graph was achieved for the concentration range 0.150–10 μg/ml ($r = 0.9998$; intercept = 0.057).

The HPLC method described for the assay of FLX in urine and serum was applied to samples collected from human volunteers after an oral dose of 100 mg

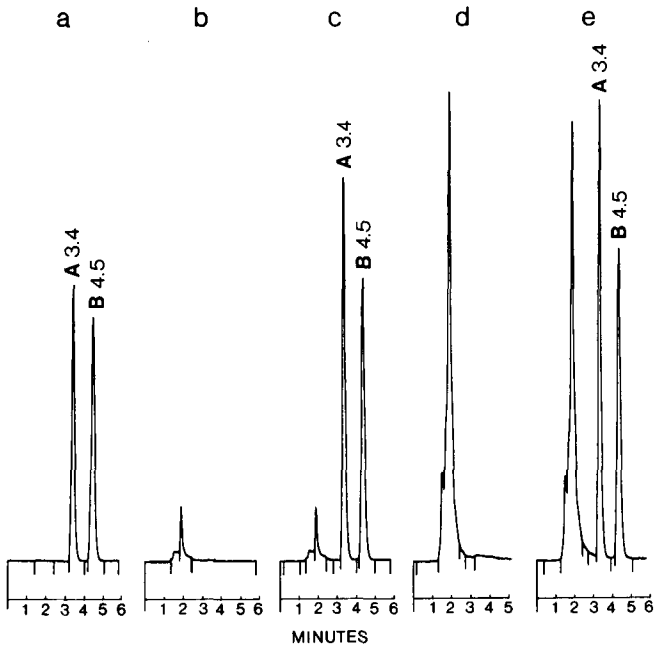

Fig. 1. Chromatograms of (a) standard solutions of FLX (5 μg/ml) and BNX internal standard (5 μg/ml); (b) extracts from 1 ml of blank serum; (c) serum obtained from a man 1 h after the administration of 100 mg of flunoxaprofen orally (the concentration of FLX was found to be 5.9 μg/ml); (d) extracts from 1 ml of blank urine; (e) 24-h urine samples obtained from a man (a concentration of 6.5 μg/ml was found). Peaks: A = flunoxaprofen, FLX (retention time 3.4 min); B = benoxaprofen, BNX, as internal standard (retention time 4.5 min).

of the drug. Fig. 1b–d shows the absence of interfering peaks from extract blanks of serum and urine; Fig. 1c and e represents chromatograms of FLX and BNX in 1 ml of serum and 1 ml of urine from a patient treated with flunoxaprofen (100 mg).

The limit of detection is 50 ng/ml both in serum and urine. The reproducibility for FLX is indicated in Table I. Average recoveries of FLX when extracted from spiked serum and urine were 93.0–103.8% and 95.2–102.7%, respectively (Table II), i.e., essentially quantitative.

The method presented is precise, selective and sensitive for the assay of FLX in serum and urine from humans who have received therapeutic doses of the drug,

TABLE I

REPRODUCIBILITY OF FLUNOXAPROFEN ASSAY ($n=4$)

Nominal concentration (μg/ml)	Concentration found (mean ± S.E.) (μg/ml)	Coefficient of variation (%)
0.15	0.15 ± 3.71	4.85
10.00	9.99 ± 4.61	0.92
15.00	14.9 ± 5.11	0.68

TABLE II

RECOVERY OF FLUNOXAPROFEN FROM HUMAN SERUM AND URINE SPIKED WITH VARIOUS CONCENTRATIONS OF THE DRUG ($n=6$)

Nominal concentration (μg/ml)	Serum			Urine		
	Mean found (μg/ml)	Coefficient of variation (%)	Recovery (%)	Mean found (μg/ml)	Coefficient of variation (%)	Recovery (%)
0.15	0.14	9.5	99.8	0.14	7.7	95.2
3.00	2.78	4.3	99.0	2.95	4.3	98.3
7.50	7.78	5.1	103.8	7.70	3.4	102.7
10.00	10.01	1.2	100.2	10.09	2.2	100.9

and it has been used to determine FLX levels in animal and clinical pharmacokinetic studies [9].

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