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Note

High-performance liquid chromatographic determination of niflumic acid in human plasma and urine

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Niflumic acid, 2-(ααα-trifluoro-*m*-toluidino)nicotinic acid, is a potent analgesic and anti-inflammatory drug widely prescribed in rheumatoid diseases [1]. It is given in usual doses of 250 mg, three times daily by mouth, and shows rapid absorption followed by extensive metabolization, essentially hydroxylation or glucuroconjugation [2].

Several methods for the determination of niflumic acid in biological fluids have been published, using spectrophotometry [3], gas chromatography [4], fluorometry [5] and high-performance liquid chromatography (HPLC) [6–10]. Two of the HPLC methods require sample extraction, which is time-consuming [6,7], two others involved separation and identification in systematic toxicological analysis or for screening programmes [8,9] and another the determination of impurities that appear during the synthesis of niflumic acid [10].

This paper describes a simple and rapid HPLC method that does not involve extraction and is sufficiently sensitive for the determination of niflumic acid in both human plasma and urine, after oral administration of a single dose (detection limit of 0.1 ± 0.007 μg/ml).

EXPERIMENTAL

Materials and apparatus

Niflumic acid was obtained from Farmos (Turku, Finland) and indomethacin (internal standard, I.S.) from Geopharma (Milan, Italy). Solvents for HPLC were purchased from Farmitalia Carlo Erba (Milan, Italy).

A Gilson 802C high-performance liquid chromatograph (Gilson, Villiers-le-Bel, France) was used. It was equipped with a 100-μl loop injector (Rheodyne, Cotati, CA, U.S.A.) and linked to a variable-wavelength UV detector and a Gilson NI chart recorder.

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Method

Separation was performed at room temperature on a stainless-steel column (25 cm × 4.5 mm I.D.) packed with Spherisorb, 5 μm particle size (Perkin-Elmer, Norwalk, CT, U.S.A.). The mobile phase was acetonitrile–0.1 *M* sodium acetate (45·55, v/v), the pH of which was adjusted to 6 4 by the dropwise addition of glacial acetic acid. The flow-rate was 1 ml/min, and the inlet pressure was *ca* 11.72 MPa (1700 p.s.i.). The dectector wavelength was set at 279 nm.

Stock solutions (1 mg/ml) of niflumic acid and indomethacin (I.S.) were prepared in methanol. The niflumic acid stock solution was dissolved in drug-free heparinized plasma and in urine to give standard solutions of final concentration in the range 0.1–100.0 μg/ml. These standard solutions were employed for the preparation of calibration graphs. The I S. stock solution was diluted to a final concentration of 50 μg/ml.

For the determination of niflumic acid, heparinized plasma samples (0.5 ml) and I.S. solution (1 ml) were mixed and centrifuged at 1050 *g* (3100 rpm) for 10 min, and a 100-μl aliquot of the supernatant was injected into the HPLC column.

Urine samples (1.0 ml) were analysed after hydrolysis of the glucuronides of niflumic acid by the addition of 1 *M* sodium hydroxide (100 μl). The alkali-treated samples were vortex-mixed and left to stand for 2 h at room temperature. Then 4 *M* hydrochloric acid (100 μl) was added, followed by the I.S. solution (1 ml) in order to avoid problems associated with the lability of the indomethacin molecules After mixing, 100 μl were injected into the column, since no precipitate was formed.

RESULTS AND DISCUSSION

Typical chromatograms of drug-free plasma and plasma from a volunteer 2 h after the oral ingestion of 250 mg of niflumic acid are shown in Fig. 1A. Chromatograms from the analysis of corresponding urine samples are shown in Fig. 1B.

No interfering peaks were observed due to endogenous constituents in plasma or in urine, either before or after alkali treatment. Under the conditions described above, the peaks were well resolved and consistently symmetrical. The retention times were 4 and 6 min, respectively, for the I.S. and niflumic acid.

The peak-height ratio was used to calculate the concentration of niflumic acid on the basis of a series of calibration graphs obtained by a least-squares method, over the concentration range 0.1–100.0 μg/ml for both plasma and urine. All the calibration graphs were linear and almost passed through the origin. The equations of the regression lines were $x = 66.7y - 0.667$ for plasma and $x = 58.8y - 0.059$ for urine, where x is the niflumic acid concentration (μg/ml) and y is the niflumic acid to indomethacin peak-height ratio. The correlation coefficients were 0.999 for at least eight points.

The reproducibility and precision of the method were examined by the repeated analysis of ten samples spiked with niflumic acid at concentrations of 1 or 5

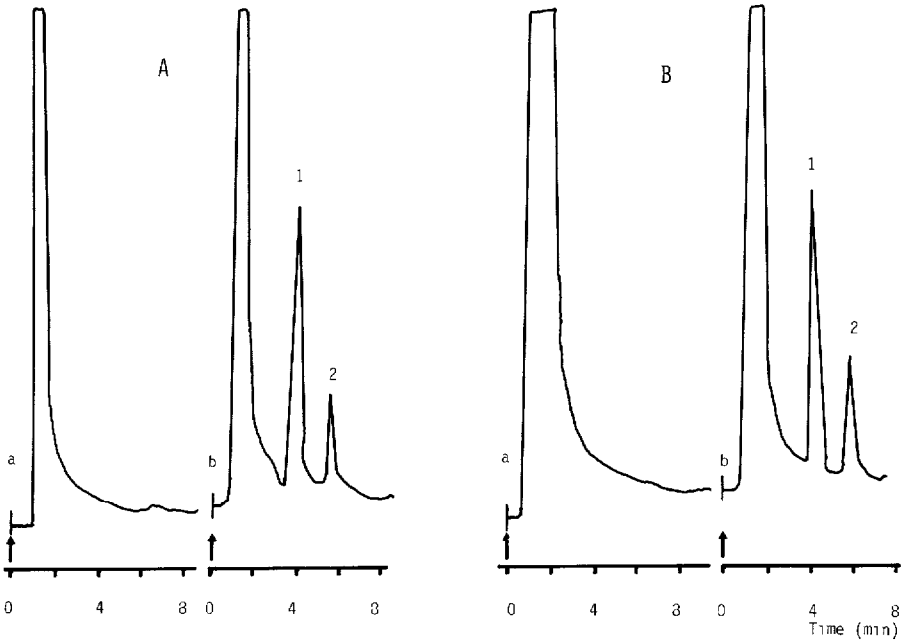

Fig 1 Chromatograms of (A) plasma and (B) urine samples (a) Blank, (b) from a volunteer after oral administration of 250 mg of niflumic acid (20 0 μg/ml in plasma after 2 h and 20 6 μg/ml in urine after 10 h). Peaks 1 = internal standard (indomethacin); 2 = niflumic acid

and 15 or 20 μg/ml for plasma and urine, respectively The results are given in Table I. The detection limit of 0.1 μg/ml and linearity up to 100 μg/ml render the method suitable for monitoring niflumic acid concentrations commonly found in human plasma and urine after therapeutic administration of the drug.

The method was applied to samples from a hospitalized patient A single dose of 250 mg of niflumic acid (one capsule from Military Pharmaceutical Laborato-

TABLE I

REPRODUCIBILITY OF NIFLUMIC ACID DETERMINATION IN PLASMA AND URINE SAMPLES

Sample	Spiked concentration (μg/ml)	Found concentration (mean ± S D) (μg/ml)	Relative standard deviation (n = 10)
Plasma	1 0	1 02 ± 0 05	4 9
	5 0	4 99 ± 0 09	1 8
Urine	15 0	15 07 ± 0 20	1 3
	20.0	20 14 ± 0 30	1 5

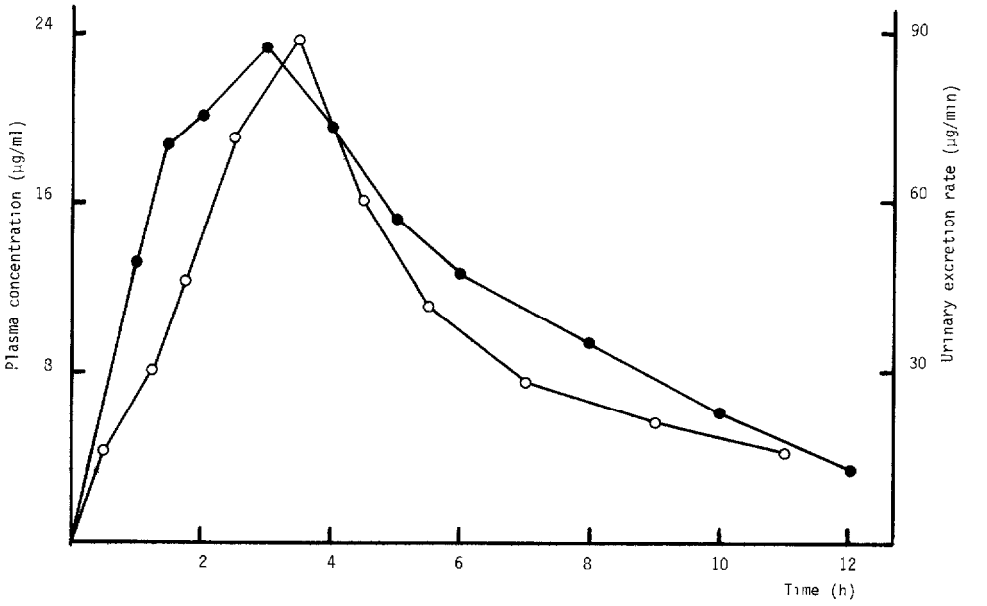

Fig 2 Plasma profile (●) of niflumic acid and urinary excretion rate (○) *versus* time in a volunteer after a single oral dose of 250 mg

ries, Athens, Greece) was administered to the patient after an overnight fast. Blood and urine samples were collected from a forearm vein at scheduled intervals, and the plasma was separated by centrifugation and then frozen. Urine samples (5 ml) were frozen immediately after collection. The plasma concentration–time and urinary excretion rate–time profiles are illustrated in Fig. 2.

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