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QUANTITATIVE DETERMINATION OF NIFLUMIC ACID AND ITS β-MORPHOLINOETHYL ESTER IN HUMAN PLASMA BY GAS—LIQUID CHROMATOGRAPHY

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SUMMARY

Niflumic acid and its β-morpholinoethyl ester are extracted from plasma with diethyl ether. After methylation with diazomethane the solution is evaporated to dryness and the residue dissolved in methanol before injection in the chromatographic column. Using a nitrogen-sensitive detector the method permits the determination of 100 ng of each compound in 1 ml of plasma. The coefficient of variation is 5.3% and 4.8% for the acid and the ester, respectively, at the 2-μg level.

INTRODUCTION

Niflumic acid (Nifluril®) is a potent anti-inflammatory drug, and pharmacokinetic studies using [^{14}C] niflumic acid [1] have shown rapid absorption followed by extensive metabolization, essentially hydroxylation and glucuroconjugation. Several methods [2—4] have been published, using spectrophotometry, gas—liquid chromatography (GLC) and high-performance liquid chromatography, but none of them was effective for the analysis of the β-morpholinoethyl ester of niflumic acid, which may be used as a pro-drug of niflumic acid. In order to determine the pharmacokinetics and bioavailability of these two substances, a GLC method has been developed that allows the simultaneous chromatography of the acid and the ester after a single-step extraction.

EXPERIMENTAL

Reagents

Niflumic acid (UP 83) and its β-morpholinoethyl ester (UP 164) (Fig. 1)

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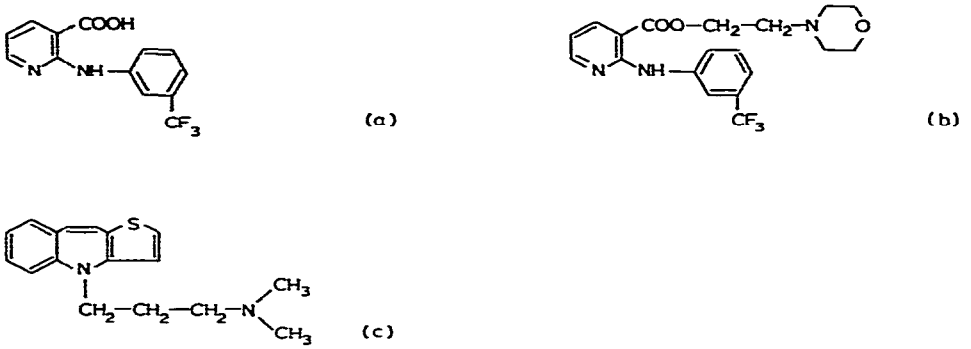

Fig. 1. Chemical structure of (a) niflumic acid, (b) its β-morpholinoethyl ester and (c) the internal standard.

were obtained from UPSA Labs. (Rueil-Malmaison, France). Solutions of these drugs were prepared in methanol.

All reagents were of analytical grade. Methanol, potassium chloride, acetic acid and sodium acetate were obtained from Merck (Darmstadt, G.F.R.). N-Methyl-N-nitroso-*p*-toluenesulphonamide, diethyl ether, ethanol (Merck) and potassium hydroxide (Prolabo, Paris, France) were used as described by Tuong and Tuong [5] for the preparation of diazomethane, which was kept at −20°C until used.

OV-17 and Gas-Chrom Q were obtained from Applied Science Labs. (State College, PA, U.S.A.).

Equipment

A Gilford Model 300 gas chromatograph equipped with a nitrogen-sensitive detector and a Servoriter recorder was used. The glass column (2 m × 2 mm I.D.) was packed with 3% (w/w) OV-17 on Gas-Chrom Q (100—120 mesh) and conditioned overnight at 300°C with nitrogen before use. The injector and detector temperatures were 260 and 305°C, respectively. The column temperature was held at 200°C for 5 min, then raised at 25°C/min for 3.6 min up to 290°C and finally held at that temperature for 5 min. The nitrogen, hydrogen and air flow-rates were 350, 25 and 100 ml/min, respectively.

Preparation of standard solutions

Standard solutions of 1 mg/ml niflumic acid, UP 164, and 5-(3-dimethylaminopropyl)benzazepinethiophene (internal standard, Fig. 1) were prepared in methanol for each series of analyses. The standard solutions of niflumic acid and its ester were then dissolved in drug-free plasma to give final concentrations of 0.1—20 μg/ml. The internal standard solutions were diluted to a final concentration of 0.5 mg/l.

Assay procedure

Plasma (1 ml), 20 μl of internal standard solution and 1 ml of acetate buffer are pipetted into a 20-ml glass-stoppered centrifuge tube. After gentle shaking, 5 mg of potassium chloride are added and mixed carefully. Diethyl ether (6 ml) is added and the tube is shaken for 20 min at 4°C. After centrifugation (7 min

at 3500 *g*), 5 ml of organic phase are transferred into a 5-ml glass tube and carefully evaporated to dryness. A 100-μl volume of an ethanolic solution of diazomethane is added and the tube is kept for 10 min in an ice-bath. The solution is evaporated to dryness and the residue dissolved in methanol (50 μl) with vigorous shaking (Vortex mixer). A 4—5-μl volume of the methanol solution is then injected into the column.

RESULTS AND DISCUSSION

A typical chromatogram of a plasma extract containing niflumic acid, its ester and the internal standard is shown in Fig. 2. The retention times are 4.8,

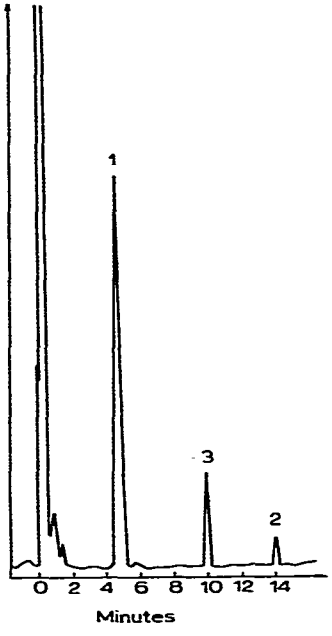

Fig. 2. Typical chromatogram of plasma extract containing 0.72 mg/l of niflumic acid (1), 0.07 mg/l of its ester (2) and 0.01 mg/l of internal standard (3).

14 and 10 min, respectively. Calibration graphs were obtained by a least-squares method of fitting between the peak-height ratio of the sample substance and the internal standard versus amount of substance added. The equations of these regression lines were

$$y_a = 1.20x_a - 0.012 \quad (1)$$

and

$$y_e = 0.40x_e - 0.04 \quad (2)$$

with correlation coefficients of 99.85 and 99.16% for the acid (eqn. 1) and the ester (eqn. 2), respectively. This indicates reasonable linearity for both products between the detector response and amount added to plasma in the range of concentrations tested, i.e., 0.1—20 mg/l.

TABLE I

APPARENT RECOVERIES OF NIFLUMIC ACID AND ITS ESTER

Sample	Niflumic acid		Ester	
	Internal std.		Internal std.	
	Without extraction	After extraction	Without extraction	After extraction
1	0.750	0.335	0.800	0.557
2	0.745	0.377	0.792	0.580
3	0.746	0.325	0.795	0.525
4	0.752	0.343	0.795	0.548
5	0.749	0.370	0.794	0.602
6	0.750	0.380	0.798	0.575
7	0.754	0.375	0.796	0.585
8	0.750	0.330	0.795	0.542
9	0.748	0.345	0.797	0.580
10	0.749	0.354	0.794	0.592
$\bar{m}$	0.749	0.353	0.796	0.569
S.D.	0.003	0.021	0.002	0.035
C.V. (%)	0.4	5.9	0.3	6.2

Apparent recoveries of the acid and ester are indicated in Table I. Comparison of the peak-height ratio after and without extraction for samples containing 2 mg/l of each drug gives apparent recoveries of 47.1% for niflumic acid and 71.5% for its ester.

The detector sensitivities for niflumic acid and its ester are 1 and 1.5 ng, respectively. According to apparent recovery, the overall sensitivity is 100 ng/ml for each substance when 5 μl of a methanolic solution of the sample are injected.

Replicate analyses of plasma samples to which known amounts of acid or ester had been added demonstrated that the method has acceptable accuracy and precision (Table II).

TABLE II

PRECISION AND ACCURACY OF ANALYSIS OF NIFLUMIC ACID AND ITS ESTER

The mean ± standard deviation in mg/l for 10 determinations is given, followed by the coefficient of variation.

Concentration added (mg/l)	Concentration found using internal standard	
	Niflumic acid	Ester
0.1	0.119 ± 0.029 (24.4%)	0.111 ± 0.028 (25%)
0.2	0.190 ± 0.024 (13.0%)	0.204 ± 0.024 (11%)
0.5	0.492 ± 0.03 (7%)	0.488 ± 0.055 (11%)
1.0	1.01 ± 0.12 (12%)	1.00 ± 0.10 (10%)
2.0	1.958 ± 0.1165 (5.9%)	1.986 ± 0.095 (4.8%)

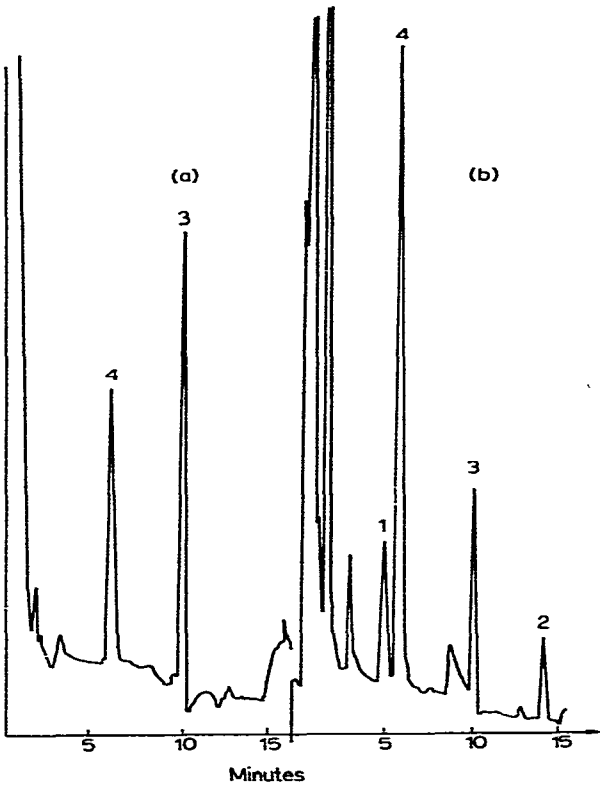

Fig. 3. Gas chromatograms from volunteers samples: (a) sample obtained prior to the oral administration of the ester; (b) sample obtained 2 h later. Peaks: 1 = niflumic acid; 2 = ester; 3 = internal standard; 4 = impurity from plasma.

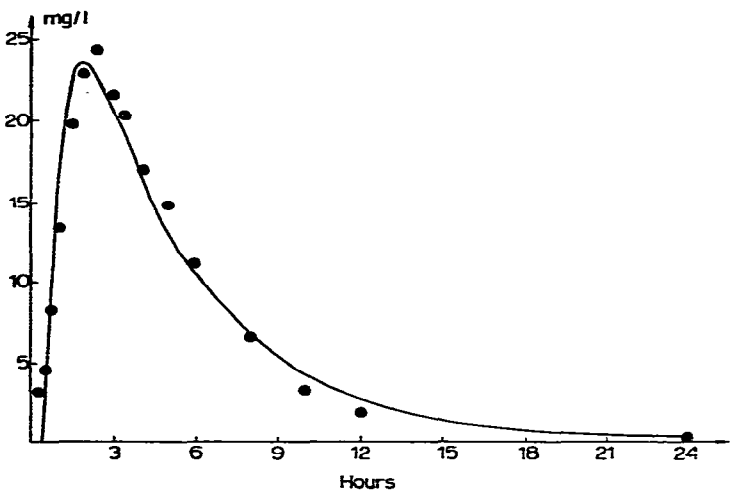

Fig. 4. Plasma profile of niflumic acid in a volunteer after a 350-mg oral dose of the ester.

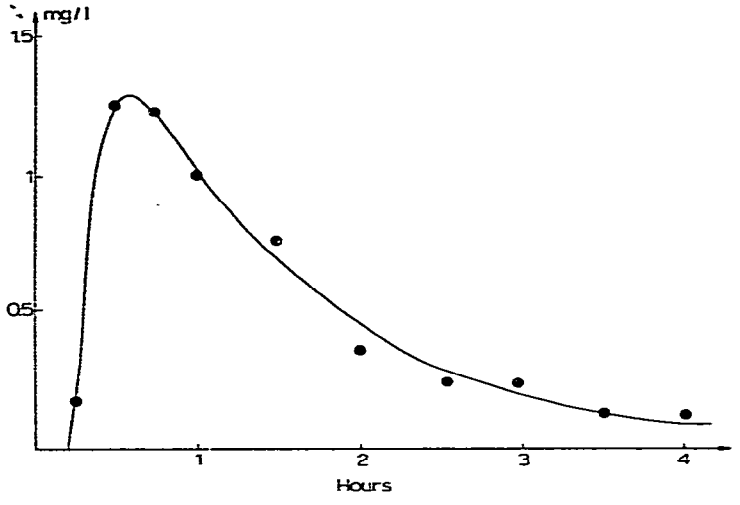

Fig. 5. Plasma profile of β-morpholinoethyl ester in a volunteer after a 350-mg oral dose of the ester.

The method was applied to the determination of niflumic acid and its ester in plasma using twelve healthy volunteers in order to estimate the bioavailability of commercial tablets. None of them showed any contaminant in the zero-time plasma sample corresponding to the retention times of the tested drugs (Fig. 3).

Plasma profiles of niflumic acid and its ester over 24 h for one of the subjects after a single oral dose of 350 mg of β-morpholinoethyl ester are shown in Figs. 4 and 5, respectively. The maximum niflumic acid plasma level occurred after 3 h and that of the ester after about 1 h, with apparent half-lives of 2.87 and 0.89 h, respectively. These figures show that the described technique is sufficiently sensitive for the determination of plasma levels of niflumic acid and its ester in humans, allowing pharmacokinetic or drug monitoring studies after therapeutic doses.

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