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QUANTITATIVE DETERMINATION OF NAPROXEN IN PLASMA BY A SIMPLE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

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SUMMARY

A high-performance liquid chromatographic method for the determination of naproxen in plasma is described. The technique is based on the single extraction of the drug from acidified plasma with chloroform using 2-naphthalene acetic acid as internal standard. The chromatographic system consisted of a column packed with Spherisorb ODS (5 μm); the mobile phase was acetonitrile—phosphoric acid (pH 3) (45 : 55, v/v).

The method can accurately measure plasma naproxen concentrations down to 1 μg/ml using 100 μl of sample, with no interference from endogenous compounds. The coefficients of variation of the method at 120 μg/ml and 1 μg/ml are 2.8 and 21.6%, respectively, and the calibration curve is linear. The method described is very suitable for routine clinical and pharmacokinetic studies.

INTRODUCTION

Naproxen [(+)-6-methoxy-α-methyl-2-naphthalene acetic acid] is a potent non-steroidal anti-inflammatory, analgesic and antipyretic drug in widespread clinical use in the treatment of rheumatism and osteorarthritis. The drug is normally administered in a daily dose of 250—750 mg [1]. Naproxen is readily absorbed from the gastrointestinal tract and, after a single oral dose of 500 mg, peak plasma drug concentrations are in the order of 100 μg/ml [2]. With the increasing clinical use of naproxen, there is a need for a rapid, sensitive and (because of the large number of drugs which may be concomitantly prescribed) selective assay of naproxen in plasma.

Several methods for the measurement of naproxen in plasma have already

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been reported. Gas chromatographic methods [3, 4] using derivatization and flame ionization detection are relatively insensitive. Fluorimetric assays [5, 6], although sensitive, are laborious to perform and are not entirely specific. A few high-performance liquid chromatographic (HPLC) methods for the measurement of naproxen have been described but these do not appear to be entirely satisfactory for routine use. The system reported by Slattery and Levy [7] using a CN-bonded phase is relatively insensitive and tedious for routine operation. The method described by Westerlund et al. [8] either lacks specificity when reversed-phase HPLC is used or is tedious to perform and requires a change in the detector attenuation when an ion-pair solvent mixture is used to determine desmethylnaproxen at the same time. Upton et al. [9] proposed a very sensitive reversed-phase method which unfortunately used ketoprofen as the internal standard and is therefore subject to error. The most recently described method of Burgoyne et al. [10], although satisfactory in some aspects, may be criticized for lack of internal standardization and use of a two-stage extraction which is tedious to perform and greatly increases the time taken for each analysis.

In the present study, a new method has been developed using a single-stage extraction of naproxen and internal standard followed by reversed-phase HPLC. The optimization of the mobile phase avoided possible interference with naproxen by some of the most commonly prescribed anti-inflammatory agents. This method is equally as sensitive as previously described fluorimetric [5, 6] or liquid chromatographic techniques [7—10] but because of its simplicity and greater specificity it can easily be applied to routine clinical or pharmacokinetic studies.

MATERIALS

Solvents

Reagent grade chloroform, used for the extraction, analytical grade orthophosphoric acid and LiChrosolv acetonitrile, used for the mobile phase, were all purchased from Merck (Darmstadt, G.F.R.).

Standards

Pure naproxen was extracted from a commercial formulation, Naprosyn®, in the laboratories of Synthélabo—L.E.R.S. Its purity was checked by measuring the melting point and optical activity. The internal standard used in the study (2-naphthalene acetic acid) was purchased from Aldrich Europe (Beerse, Belgium). Standard solutions were prepared by dissolving naproxen and its internal standard in pure acetonitrile.

Equipment

Analyses were carried out on a Micromeritics 7000B liquid chromatograph (Norcross, GA, U.S.A.) equipped with a Micromeritics 730 universal injector and a Micromeritics 785 UV—visible spectrophotometer.

METHODS

Chromatographic analysis

The mobile phase [acetonitrile—aqueous orthophosphoric acid (pH 3) (45:55, v/v)] was adjusted to a flow-rate of 1.00 ± 0.01 ml/min through a stainless-steel column, 15 cm × 4.6 mm I.D., packed in the laboratory [11] with Spherisorb ODS (5 μm, batch 17/49, Phase Separations, Queensferry, Great Britain). The detector wavelength was set at 230 nm, which corresponds to the highest optical absorption of naproxen dissolved in the mobile phase.

Extraction procedure

The procedure used for the extraction of naproxen in plasma is schematically outlined in Fig. 1. A 100-μl sample of plasma together with 1 ml of KCl solution (1 *M*, pH 2) were introduced into a tapered tube containing 10 μg of the internal standard (10 μl of a 1 μg/μl solution in acetonitrile). This mixture was shaken on a Vortex mixer, then extracted with chloroform (6 ml) on a "rock and roll" shaker for 20 min. The two phases were then separated by centrifugation (1000 *g* for 5 min at 4°C) and the aqueous phase was discarded.

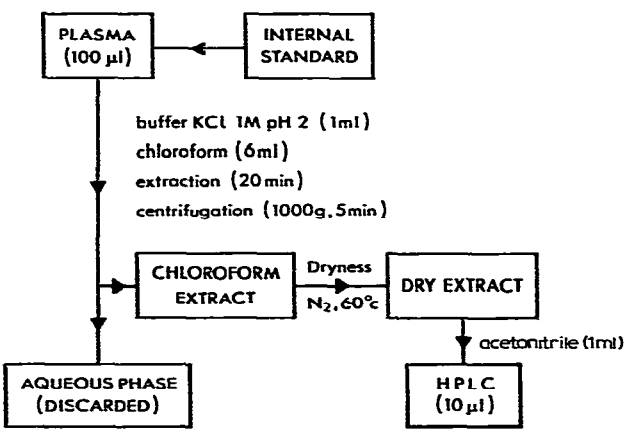

Fig. 1. Extraction scheme for naproxen from plasma.

Then 5 ml of the chloroform extract were transferred into a second tube and evaporated to dryness at 60°C under a gentle stream of nitrogen. The dry extract was then dissolved in 1 ml of pure acetonitrile by agitation on a Vortex mixer; 10 μl of this solution were injected into the column with the detector attenuation set at 0.1 a.u.f.s. or alternatively a 1-μl injection with the detector attenuation at 0.01 a.u.f.s.

RESULTS

Two chromatograms, one obtained from blank plasma and the other from a plasma sample of a patient, are presented in Fig. 2. The peaks corresponding to internal standard and naproxen under the conditions described above were well resolved and no endogenous compound extracted at the same time interfered

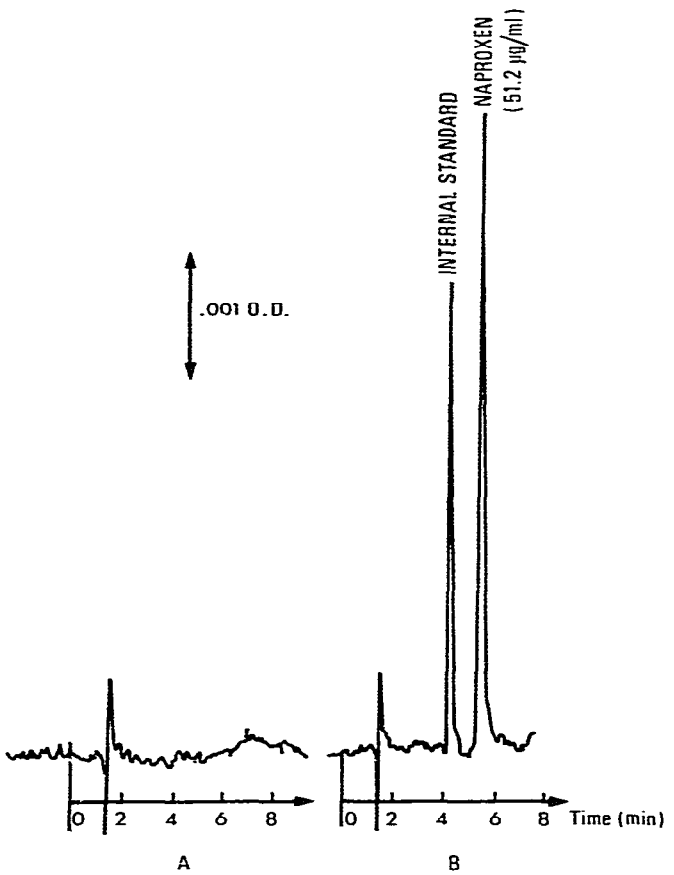

Fig. 2. Typical chromatograms of human plasma extracts for naproxen determination (a) before administration of the dose, (b) 8 h after oral administration of a 500-mg dose (1 μl injected). For chromatographic conditions, see the text.

TABLE I

REPRODUCIBILITY OF THE HPLC METHOD FOR THE MEASUREMENT OF NAPROXEN AT DIFFERENT CONCENTRATIONS IN PLASMA

Spiked concentrations (μg/ml)	Number of observations	Found concentrations (mean values) (μg/ml)	Standard deviation (μg/ml)	Coefficient of variation (%)
1	5	1.3	0.28	21.6
5	5	5.1	0.55	10.9
10	4	10.0	0.99	9.9
20	4	18.8	1.51	8.1
40	3	40.9	0.74	1.9
60	3	60.3	2.50	4.2
80	4	80.1	2.57	3.2
100	5	100.1	2.64	2.7
120	4	119.7	3.27	2.8

with these peaks. The retention times were 4.18 and 5.35 min, respectively, for internal standard and naproxen; with a respective reduced height equivalent to a theoretical plate of 5.8 and 4.7 (mean diameter of the stationary phase was 5.7 μm as determined with a Coulter Counter TA_2 from Coultronics, Margency, France), the interval between each injection was 7 min.

The procedure was quantified by the internal standard method using the peak height ratio method. The response was linear between 1 and 120 μg/ml under the conditions described above, and the day-to-day variation in the slope of the calibration curve was < 3%. The accuracy of the method was determined by analysing spiked plasma samples of naproxen and the results are shown in Table I. The coefficients of variation ranged from 22% for 1 μg/ml to < 3% for the 120 μg/ml plasma drug concentrations (Table I).

DISCUSSION

To extract naproxen from plasma, four different extraction solvents were tried: hexane, toluene, chloroform and diethyl ether. The same conditions described above for chloroform were used. The recoveries of the two compounds (naproxen and its internal standard) were in the following increasing order: hexane < toluene < chloroform = diethyl ether. Chloroform and diethyl ether extracts had almost similar chromatograms but the diethyl ether extracts showed a high and strongly retained peak with a retention time of 40 min. Although obtained at 230 nm, the chromatogram of blank plasma (Fig. 2) extracted with chloroform is considerably cleaner than that presented by Westerlund et al. [8] or Upton et al. [9], and is comparable to that obtained by Burgoyne et al. [10] who used a more specific fluorescence detector and a two-stage extraction.

Different mobile phases were investigated using the same column before making the final selection of the chromatographic conditions: acetonitrile and sodium acetate (0.1 *M*) in different ratios; acetonitrile 30%, KH_2PO_4 (0.01 *M*, pH 6) 70%; acetonitrile 40%, KH_2PO_4 (0.01 *M*, pH 4.6) 60%; acetonitrile and KH_2PO_4 (0.005 *M*, pH 3) in different ratios. The separation and efficiencies were poor with large band tailing peaks when sodium acetate mobile phases were used. Replacing 0.1 *M* sodium acetate with 0.01 *M* KH_2PO_4 at the same pH partly improved the chromatograms. Decreasing the pH or the phosphate concentration of the mobile phase containing KH_2PO_4 increased both the efficiency and selectivity, as well as reducing peak tailing. The same effects were observed by decreasing the concentration of KH_2PO_4 from 0.01 *M* to 0.005 *M* at the same pH. Finally, the chromatograms obtained using pure orthophosphoric acid at a given pH were better than those obtained using a phosphate salt. This suggests that the chromatographic characteristics of naproxen and its internal standard are markedly dependent on the composition of the mobile phase.

The selected mobile phase, acetonitrile—H_3PO_4 (pH 3) (45:55, v/v) (which allowed the system to show more than 37,000 theoretical plates/meter for naproxen on a 15-cm column packed with Spherisorb ODS 5 μm) was similar to that used by Burgoyne et al. [10], namely methanol—acetic acid (0.1 *M*)

(70:30, v/v) at 1.00 ml/min on a 30-cm column (apparently 48,000* theoretical plates/meter for naproxen on μBondapak C_{18}). These two acidic mobile phases were completely different from that used by: (1) Westerlund et al. [8], methanol—phosphate (pH 7) (40:60, v/v) at 1.00 ml/min on a 10-cm column (5500* theoretical plates/meter for naproxen on LiChrosorb RP-8, 5 or 10 μm); (2) Upton et al. [9], acetonitrile—phosphate (pH 7) (8:92, v/v) at 2.00 ml/min on a 40-cm column (3850* theoretical plates/meter for naproxen on Spherisorb ODS 5 μm). The pH, which was the main difference between the four mobile phases, appeared to be one of the most important parameters for obtaining good efficiency and selectivity.

Using the present chromatographic system, it was possible to resolve most of the commonly prescribed anti-inflammatory agents from naproxen (Table II).

TABLE II

CAPACITY FACTORS OF SOME NON-STEROIDAL ANTI-INFLAMMATORY AGENTS

For chromatographic conditions, see the text.

Drug	k'
Paracetamol	0.8
Salicylic acid	1.5
Internal standard	2.5
Alclofenac	3.2
Ketoprofen	3.4
Oxyphenobutazone	3.9
Naproxen	4.1
Sulindac	4.3
Flurbiprofen	5.9
Ibuprofen	7.0
Indomethacin	7.3
Phenylbutazone	8.5
Flufenamic acid	12.1

Only oxyphenbutazone and sulindac were likely to interfere with the measurement of naproxen in plasma. Possible interferences by the metabolites of naproxen in plasma after naproxen administration was considered to be negligible [12]. Because of the simple extraction procedure and the short time of the chromatographic analysis, at least 30 samples can be analysed daily by only one person. If a faster rate of analysis is needed this procedure can be automated. Since the final extract is dissolved in 1 ml of solvent, of which 10 μl are injected, an automatic injector can be used without any modification of the method.

The mean plasma naproxen levels obtained after the administration of a 500-mg single dose (Naprosyn®) clearly demonstrate that the method has sufficient sensitivity for pharmacokinetic studies (Fig. 3). The sensitivity of the method may be greatly increased by reducing the quantity of solvent used in the final pre-injection stage.

*Calculated from the chromatograms shown in the respective papers.

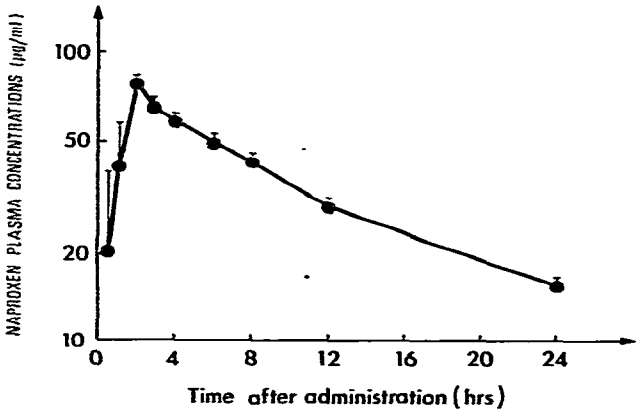

Fig. 3. Mean (± S.E.M.) plasma concentrations of naproxen after administration of a 500-mg dose as Naprosyn® to three adults.

In conclusion, the HPLC method for naproxen in plasma that has been developed, has been demonstrated to be both simpler and more selective than other methods described previously. It is sufficiently sensitive and can serve as a useful tool for pharmacokinetic and clinical studies involving naproxen.

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