Journal of Chromatography, 164 (1979) 534—540
Biomedical Applications
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 414

Note

Determination of naftidrofuryl in the plasma of humans by high-performance liquid chromatography

R.R. BRODIE, L.F. CHASSEAUD and T. TAYLOR

Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre, Huntingdon (Great Britain)

and

J.O. HUNTER and P.J. CICLITIRA*

Addenbrookes Hospital, Cambridge (Great Britain)

(Received May 16th, 1979)

Naftidrofuryl is a new drug [1—4] recently introduced for the treatment of cerebral and peripheral vascular disorders [5—7]. The metabolism of the drug has been studied in animals using radioisotopic techniques [8] and a fluorimetric assay for measurement of the parent drug has also been used [9] with a limit of detection in plasma of about 0.1 μg/ml set by background interference. The sensitivity and specificity of the assay for the measurement of the drug in plasma can be improved by using a procedure based on reversed-phase high-performance liquid chromatography. The results obtained are described in this paper.

EXPERIMENTAL

Materials

All reagents were of analytical grade and all inorganic reagents were prepared in freshly glass-distilled water. Diethyl ether was freshly redistilled prior to use and acetonitrile was HPLC, far U.V. grade (Fisons Scientific Apparatus, Loughborough, Great Britain).

Standard solutions of naftidrofuryl [N-diethylaminoethyl-2-tetrahydrofurfuryl-3-(1′-naphthylpropionate) as the oxalate salt, Fig. 1] and LS 140 [the internal standard, oxalate salt of N-dimethylaminoethyl-2-tetrahydrofurfuryl-3-(1′-naphthylpropionate)] were prepared at a concentration of 10 μg/ml in acetonitrile and stored at 4°. Samples of naftidrofuryl and LS 140 were supplied by Lipha (Lyon, France).

*Drummond Research Fellow, 1977—1979.

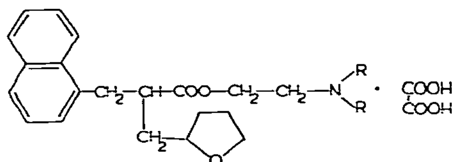

Fig. 1. Chemical structure of naftidrofuryl (R = C_2H_5) and LS 140 (internal standard, R = CH_3) oxalate salt.

Extraction procedure

Plasma samples (1 ml) were transferred into conical centrifuge tubes (10 ml), spiked with internal standard (10 μl, containing 100 ng LS 140) and made alkaline by the addition of ammonia solution (0.5 ml), 0.88 ammonia—water (1:10). The mixture was extracted with diethyl ether (5 ml) by vortexing it for 2 min on a Whirlymixer (Fisons Scientific Apparatus). The extracts were centrifuged at 2000 *g* for 10 min and the separated ether layer carefully transferred to another conical centrifuge tube. The ether extract was evaporated to dryness under nitrogen at 37° and the walls of the tube rinsed with more ether to ensure that all the residue was at the bottom of the tube. The ether was again evaporated and the residue dissolved in mobile phase (20 μl). After centrifugation at 2000 *g* for 10 min, as much as possible of the clear solution was injected into the chromatograph.

Calibration procedure

Samples of control (drug-free) plasma (1 ml) were spiked with naftidrofuryl oxalate at concentrations of 20, 50, 100, 200 and 300 ng/ml and with internal standard at a fixed concentration of 100 ng/ml. The samples are taken through the extraction procedure described previously.

Instrumentation

The liquid chromatograph consisted of a Waters Model 6000A pump (Waters Assoc., Cheshire, Great Britain) fitted to an LC3 variable wavelength UV detector (Pye Unicam, Cambridge, Great Britain) operated at 222 nm at λ_{max} for naftidrofuryl dissolved in the mobile phase. Injection was by syringe (25 μl, Precision Sampling, Baton Rouge, La., U.S.A.) via a U6K universal injector (Waters Assoc.). Peak area ratio measurements were quantified using a 3380A computing integrator (Hewlett-Packard, Slough, Great Britain). Mass spectra were obtained using a Micromass 16F mass spectrometer (V.G. Organic, Cheshire, Great Britain) operated in the electron impact mode of ionisation using an electron beam energy of 70 eV and a trap current of 100 μA. The ion source was operated at a temperature of 210° and samples were introduced by direct insertion probe.

Chromatography

Chromatography was performed in a reversed-phase mode. The column was constructed of stainless steel (30 cm × 0.4 cm I.D.) and prepacked with μBondapak C_{18} (mean particle diameter 10 μm) (Waters Assoc.). A precolumn constructed of stainless steel (7 cm × 0.2 cm I.D.) and dry-packed with

pellicular Co:Pell®ODS (particle diameter 25—37 μm) (Whatman, Maidstone, Great Britain) was installed in series in front of the main analytical column to protect it from contamination and was changed routinely if the back pressure in the system increased. The precolumn appeared to have no effect on the chromatographic separation or resolution and was used merely to remove endogenous material from the extracted samples. The mobile phase consisted of 50% acetonitrile in aqueous potassium dihydrogen orthophosphate (0.5%, w/v) with the final pH adjusted to pH 4 with phosphoric acid. A flow-rate of 2 ml/min was maintained.

Fig. 2 illustrates the separation of naftidrofuryl from the internal standard with retention times of 3.5 min and 2.8 min, respectively, and a total analysis time of 10 min.

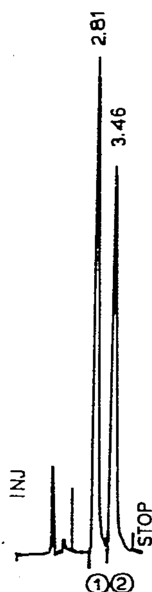

Fig. 2. Chromatogram of reference standards. Peaks: 1 = internal standard (LS 140); 2 = naftidrofuryl. Conditions: column, μBondapak C_{18} (30 cm × 4 mm I.D.); mobile phase, 50% acetonitrile in aqueous potassium dihydrogen orthophosphate (0.5%, w/v) pH 4; flow-rate, 2 ml/min; UV detection at 222 nm with 1 V output to 3380A integrator.

Plasma samples

The method of analysis was applied to plasma samples obtained from two male volunteer subjects after each had received an oral dose of 100 mg of naftidrofuryl oxalate contained in a capsule (batch No. 22.267; Lipha). The conditions of the volunteer studies were similar to those described by Brodie et al. [10].

RESULTS AND DISCUSSION

Concentrations of naftidrofuryl (as oxalate salt) were calculated from calibration lines constructed by plotting peak area ratios of drug to internal standard over the concentration range 20—300 ng/ml naftidrofuryl in plasma. Extraction and measurement at each concentration were repeated on five occasions and peak area ratios showed a coefficient of variation of ± 5% at 20 ng/ml and ± 3% at 300 ng/ml, indicating a good precision for the measurement of naftidrofuryl in plasma.

The calibration line was linear ($y = -0.0187 + 0.0095\,x$, correlation coefficient $r = 0.9987$) and where the value of the intercept was not significantly different from zero ($P > 0.05$). The equation of the line forced through the origin was $y = 0.0094\ (\pm\ 0.0001\ \text{S.D.}) \cdot x$, where y is the peak area ratio and x is the concentration of naftidrofuryl (ng/ml) present. The accuracy of the method defined by 95% confidence limits of the least squares regression line forced through the origin, i.e. taking the calibration line as an estimate of the concentration of naftidrofuryl in plasma, was ± 57.5% at 20 ng/ml, ± 8.7% at 134 ng/ml and ± 4.1% at 300 ng/ml. The recovery of internal standard from plasma was 92% ± 4 S.D. ($n = 5$). The mean overall recovery of naftidrofuryl from plasma (95% ± 5 S.D., $n = 25$) over the concentration range 20—300 ng/ml was calculated by comparing peak area ratio measurements of non-extracted standards to those of extracted standards corrected for recovery of internal standard (Table I).

No interfering peaks were present in the predose (control) plasma (Fig. 3). The limit of detection based on the instrumental parameters used was 5 ng naftidrofuryl per ml with an integrator slope sensitivity setting of 3 mV/min. Since naftidrofuryl is fluorescent (excitation λ_{max} 286 nm and emission λ_{max} 326 nm), it should be possible to improve the sensitivity of the assay using the appropriate fluorescence detector.

Concentrations of naftidrofuryl are reported as the oxalate salt, concentrations of the free base can be determined by allowing for molecular weight differences (conc. of free base = 0.81 × conc. of oxalate salt). When applied

TABLE I

PRECISION OF THE METHOD AND RECOVERIES OF NAFTIDROFURYL FROM PLASMA

Concentration of naftidrofuryl added to plasma (ng/ml)	Coefficient of variation (%) ($n = 5$)	Recovery (%)
20	5	96
50	5	94
100	3	92
200	3	94
300	3	97
mean		95 ± 5 S.D.

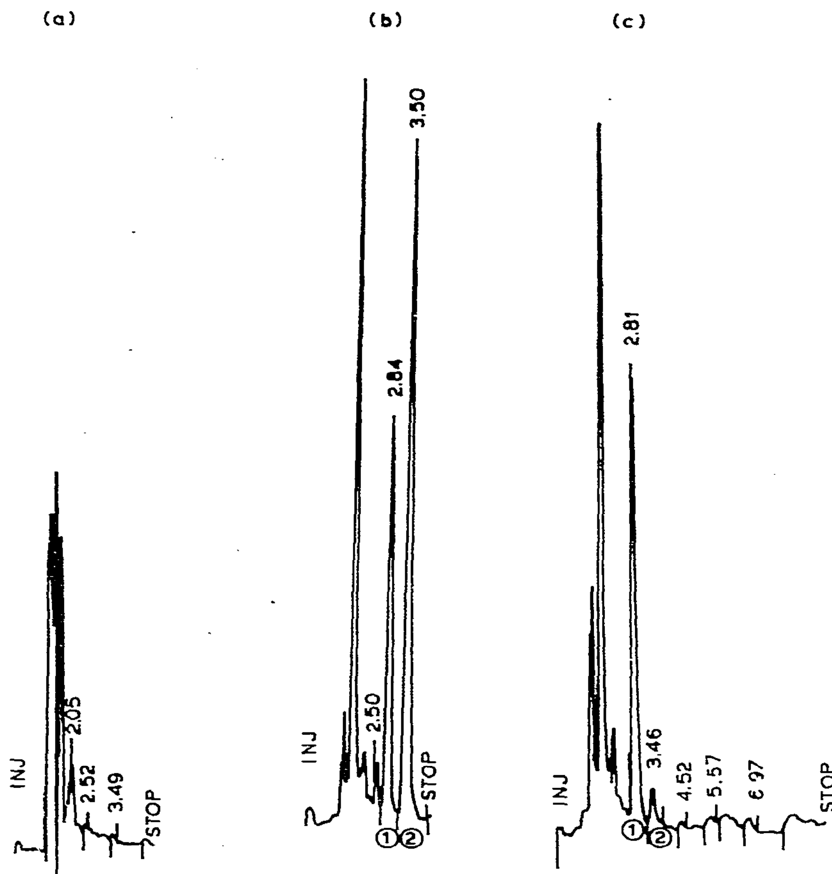

Fig. 3. (a) Predose (control) plasma extract; (b) 0.5 h postdose plasma extract containing 194 ng/ml naftidrofuryl; (c) 5 h postdose plasma extract containing 11 ng/ml naftidrofuryl. Conditions as for Fig. 2; peaks: 1 = internal standard; 2 = naftidrofuryl.

to the collected samples, the method showed that peak concentrations of naftidrofuryl occurred at 0.75 and 0.5 h after dosing (215 ng/ml and 212 ng/ml in Subjects 1 and 2, respectively) and were below the limit of detection (5 ng/ml) at 7 h after dosing (Table II). The descending portion of the concentration—time curves appeared to be composed of at least two linear sections, presumably associated with distribution and elimination phases, respectively. The half-life of the terminal linear section was approximately 1.2 h (Fig. 4).

The specificity of the method was tested by collection of the effluent corresponding to the naftidrofuryl peak which was then extracted and subjected to mass spectrometry. The mass spectrum obtained by this procedure was identical to that obtained with authentic reference material showing a molecular ion at *m/e* 383 and fragments at *m/e* values of 368, 267, 141, 99 and 86 present in similar proportions in both spectra.

TABLE II

CONCENTRATIONS OF NAFTIDROFURYL (AS THE OXALATE SALT) IN THE PLASMA OF HUMAN SUBJECTS AFTER SINGLE ORAL DOSES OF 100 mg NAFTIDROFURYL

Time after dosing (h)	Concentrations of naftidrofuryl (ng/ml)		
	Subject 1	Subject 2	Mean
0.5	194	212	203
0.75	215	135	175
1.0	190	79	135
1.5	94	56	75
2.0	68	33	51
2.5	40	25	33
3.0	35	16	26
4.0	19	10	15
5.0	11	5	8
7.0	<5	<5	<5

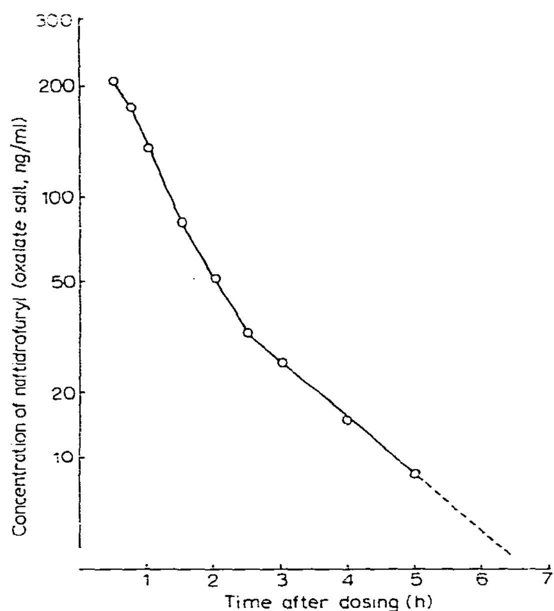

Fig. 4. Semi-logarithmic plot of mean plasma concentrations of naftidrofuryl with time after an oral dose of 100 mg to two human subjects.

ACKNOWLEDGEMENTS

We are grateful to Dr. A. Meynaud, Lipha, Lyon, France, for his help, advice and encouragement. We also thank Miss A. Roberts for her technical assistance and Mr. S.R. Biggs for performing the mass spectrometry.

REFERENCES

1 L. Fontaine, M. Grand, J. Chabert, E. Szarvasi and M. Bayssat, Chim. Ther., No. 6 (1968) 463.
2 L. Fontaine, M. Grand, E. Szarvasi and M. Bayssat, Chim. Ther., No. 1 (1969) 39.
3 A. Meynaud, M. Grand and L. Fontaine, Arzneim.-Forsch., 23 (1973) 1431.
4 A. Meynaud, M. Grand, M. Belleville and L. Fontaine, Therapie, 30 (1975) 777.
5 T.G. Judge, Scot. Med. J., 23 (1978) 43.
6 H. Forrest, Scot. Med. J., 23 (1978) 45.
7 J.R. Cox, in La Malattia Arteriosclerotica: Moderni Aspetti Etiopatogenetici e Terapeutici, Centro Minerva Medica, Italy, 1976, p. 41.
8 A.G. Rico, J.C. Godfrain, B. Sacaze, P. Benard, J.P. Braun, L. Fontaine and L. Belleville, Therapie, 29 (1974) 281.
9 L. Fontaine, M. Belleville, J.C. Léchevin, M. Silie, J. Delahaye and M. Boucherat, Chim. Ther., No. 1 (1969) 44.
10 R.R. Brodie, L.F. Chasseaud, T. Taylor, D.A. O'Kelly and A. Darragh, J. Chromatogr., 146 (1978) 152.