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Note

Determination of naftidrofuryl in human plasma by high-performance liquid chromatography with fluorescence detection

L.M. WALMSLEY*, P.A. WILKINSON, R.R. BRODIE and L.F. CHASSEAUD

Department of Metabolism and Pharmacokinetics, Huntingdon Research Centre, Huntingdon PE18 6ES (U.K.)

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Naftidrofuryl (nafronyl) is a vasodilator used for the treatment of cerebral and peripheral vascular disorders [1—3]. Plasma concentrations of this drug have been determined both by fluorimetry [4, 5] and by high-performance liquid chromatography (HPLC) [6]. By their nature, fluorimetric assays tend to be less specific than those involving chromatography; they are also more likely to be affected by interference from metabolites and other substances.

The HPLC method described in this paper has been modified from that reported previously [6]. In the previous procedure [6], the internal standard may be subject to metabolite interference. Accordingly, the conditions for chromatography have been modified and fluorescence detection has been used in preference to ultraviolet absorption detection, in order to improve the specificity of the assay procedure whilst maintaining its sensitivity.

This present method differs in several respects from the HPLC method recently employed by Garrett [7] to investigate the pharmacokinetics of naftidrofuryl in the dog. Although both methods require fluorescence detection, they differ in the wavelengths selected, columns used and the internal standards utilised; furthermore, sub-sampling the plasma into ammonia solution (with the subsequent extraction procedure described in this method) was found to prevent the decomposition of naftidrofuryl in the plasma of those species in which this compound has a shorter half-life due to plasma esterase activity. The method here described has been successfully applied to the analysis of naftidrofuryl in both human and animal plasma.

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EXPERIMENTAL

Materials

Acetonitrile was HPLC grade (Fisons, Loughborough, U.K.). All other reagents were of analytical grade and all inorganic reagents were prepared in freshly glass-distilled water.

Standard solutions of naftidrofuryl [3-(1-naphthyl)-2-tetrahydrofurfuryl-propionic acid 2-(diethylamino)ethyl ester as the oxalate salt, Fig. 1], and the internal standard [3-(1-naphthyl)-2-tetrahydrofurfurylpropionic acid 2-(dimethylamino)ethyl ester, also as the oxalate salt, Fig. 1], were prepared in acetonitrile and stored in the dark at 4°C throughout the study. Analytical standards of these compounds were supplied by Lipha (Lyon, France).

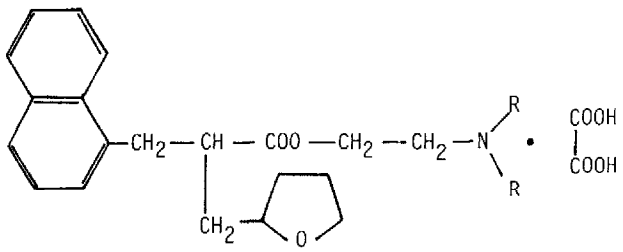

Fig. 1. Structure of naftidrofuryl (R = C_2H_5) and internal standard (R = CH_3) as their oxalate salts.

Extraction procedure

Plasma samples (1 ml), taken into balanced oxalate (12 mg ammonium oxalate and 8 mg potassium oxalate) tubes, were transferred into screw-capped centrifuge tubes (10 ml capacity) containing ammonia solution (10%, 1 ml), and spiked with internal standard (500 ng in 50 μl acetonitrile). Where plasma concentrations of naftidrofuryl exceeded the calibration range, aliquots (0.5 ml) of plasma were made up to volume (1 ml) with control plasma and analysed in the normal manner. The samples were extracted into freshly redistilled diethyl ether (5 ml) by rotary mixing for 5 min, then centrifuged for 5 min. The organic layers were removed into conical tubes and evaporated to dryness at room temperature, under a stream of nitrogen. Residues were reconstituted in mobile phase (60 μl), transferred to autosampling vials and aliquots (20 μl) were chromatographed.

Calibration procedure

Aliquots of blank human plasma (1 ml), taken into balanced oxalate tubes, were transferred into tubes containing ammonia solution (10%, 1 ml) and spiked with naftidrofuryl at concentrations equivalent to 20, 200, 400, 600 and 800 ng free base per ml and with internal standard at a fixed concentration of 500 ng/ml. Samples were then submitted to the extraction procedure described previously.

Instrumentation

The liquid chromatograph consisted of an M6000A pump (Waters Assoc., Northwich, U.K.) coupled to an LS-3 fluorescence detector (Perkin-Elmer,

Beaconsfield, U.K.) operated at an excitation wavelength of 286 nm and an emission wavelength of 335 nm. Injection was via an automatic injector (Waters' Intelligent Sample Processor, WISP 710A). Chromatograms were recorded using an HP 3380A computing integrator (Hewlett-Packard, Slough, U.K.). Peak heights and peak height ratio measurements were made manually since this gave greater precision of measurement than the computing facility of the integrator.

Chromatography

The column used for the analysis of the plasma samples was constructed of stainless steel (12.5 cm × 0.5 cm I.D.) pre-packed with Spherisorb ODS (mean particle diameter 5 μm, Hichrom, Reading, U.K.). A pre-column (5 cm × 0.2 cm I.D.) constructed of stainless steel and dry-packed with pellicular Co:Pell® ODS (particle diameter 25—37 μm, Whatman, Maidstone, U.K.) was installed in front of the analytical column to protect it from contamination.

Chromatography was performed in the reversed-phase mode, using a mobile phase consisting of acetonitrile (70%, v/v) in sodium dihydrogen orthophosphate buffer (0.2%, w/v, containing 3 ml/l of 1 *M* orthophosphoric acid). The mobile phase was passed through the column at a flow-rate of 3 ml/min.

Fig. 2 illustrates the separation of the internal standard and naftidrofuryl, the retention times of which were 6.5 and 8.6 min, respectively. Retention times were sensitive to both the pH and phosphate concentration in the mobile phase which were, therefore, carefully controlled.

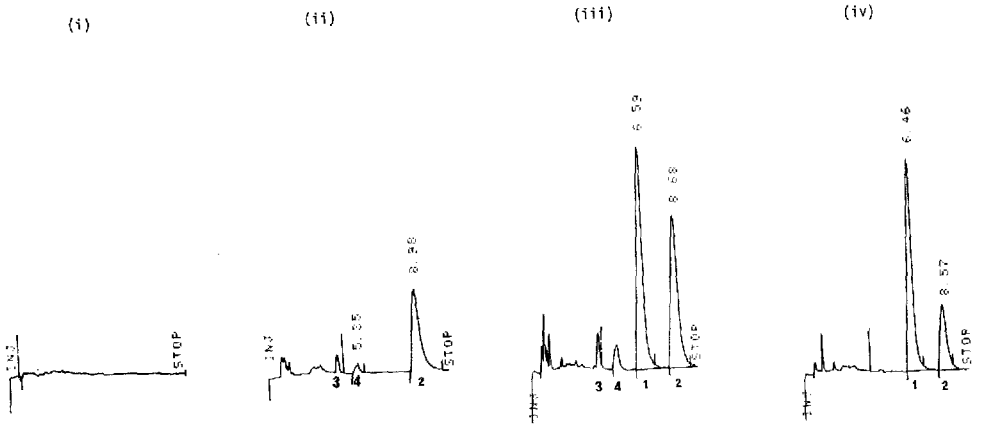

Fig. 2. Chromatograms of (i) pre-dose (control) plasma; (ii) post-dose plasma (no internal standard added); (iii) post-dose plasma plus internal standard; (iv) spiked calibration standard, 200 ng/ml naftidrofuryl plus internal standard. Conditions: column, Spherisorb ODS (12.5 cm × 0.5 cm I.D.); mobile phase, acetonitrile (70%, v/v) in aqueous sodium dihydrogen orthophosphate (0.2%, w/v, containing 3 ml/l of 1 *M* orthophosphoric acid); flow-rate 3 ml/min; fluorescence excitation 286 nm, emission 335 nm.

Studies in human subjects

The method of analysis was applied to plasma samples obtained during a study of naftidrofuryl administered as 300 mg of the oxalate salt (Praxilene®, Lipha) to five human subjects. The conditions of the study were similar to those described previously [8].

RESULTS AND DISCUSSION

Precision and accuracy

Extraction and measurement at each of three concentrations in plasma over the calibration range, was repeated on five separate occasions. The precision of measurement of the assay, as indicated by the coefficient of variation of peak height ratio measurements of drug to internal standard, was ± 10% at 20 ng/ml, ± 1% at 300 ng/ml and ± 1% at 500 ng/ml.

Daily calibration lines were constructed from duplicate measurements at each of six concentrations over the calibration range 0 to 800 ng/ml. Plots of peak height ratio against concentration were linear ($y = a + bx$), where y is the peak height ratio and x is the concentration of naftidrofuryl free base. The mean standard error of the fitted lines, an index of the accuracy of the measurement, was ± 28 ng/ml.

Recovery

The recovery of internal standard (500 ng/ml) from plasma (1 ml) was determined by comparison of peak height ratio measurements of internal standard to naftidrofuryl, of standards taken through the extraction procedure, to those injected into the chromatograph without extraction. The mean recovery of internal standard was 92 ± 3% S.D. ($n = 11$). The mean recovery of naftidrofuryl from plasma was determined by comparison of peak height ratios of extracted standards corrected for 100% recovery of internal standard, with those of non-extracted standards; the mean recovery was 88 ± 2% S.D. (Table I).

TABLE I

RECOVERY OF NAFTIDROFURYL ADDED TO PLASMA OVER THE CONCENTRATION RANGE 100—500 ng/ml

Concentration of naftidrofuryl (free base) (ng/ml)	Mean peak height ratios of non-extracted standards	Mean peak height ratios of standards extracted from plasma	Mean relative recovery (%)	Mean overall recovery of naftidrofuryl*
100	0.17	0.16	94	86
200	0.33	0.32	97	89
300	0.49	0.46	94	86
400	0.64	0.61	95	87
500	0.79	0.78	99	91

*Corrected for 100% recovery of the internal standard.

Specificity and limits of detection

No peaks with retention times similar to those of naftidrofuryl or internal standard were present in extracts of pre-dose (control) plasma. No peak with the same retention time as the internal standard was present in extracts of post-dose plasma analysed without the addition of internal standard (Fig. 2), indicating the absence of metabolite interference with the internal standard used.

The limit of detection of naftidrofuryl free base was taken as the lowest point on the calibration line (20 ng/ml). This was the minimum concentration detected by the assay based on the analysis of 1 ml of plasma using 500 ng/ml internal standard and maintaining all peaks on scale. Where a narrower calibration range was desired, the method was successfully applied to achieve a lower limit of detection of naftidrofuryl of 4 ng/ml.

Concentrations of naftidrofuryl in human plasma

The mean concentrations of naftidrofuryl in the plasma of five human subjects after single oral doses of 300 mg of naftidrofuryl oxalate, analysed by the HPLC method described are presented in Table II. Plasma concentrations declined monoexponentially with a half-life of 2.0 ± 0.4 h S.D., similar to that reported previously [6].

TABLE II

MEAN CONCENTRATIONS (± S.D.) OF NAFTIDROFURYL FREE BASE (ng/ml) IN THE PLASMA OF FIVE HUMAN SUBJECTS AFTER ADMINISTRATION OF SINGLE ORAL DOSES OF 300 mg OF NAFTIDROFURYL OXALATE

Time (h)	Concentration (ng/ml, ± S.D.)
Pre-dose	<20
0.25	156 ± 54
0.5	548 ± 227
0.75	600 ± 293
1	559 ± 115
1.5	446 ± 98
2	313 ± 89
3	162 ± 48
4	104 ± 31
5	63 ± 19
6	49 ± 12
8	<20

ACKNOWLEDGEMENT

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REFERENCES

1 A. Meynaud, M. Grand and L. Fontaine, Arzneim.-Forsch., 23 (1973) 1431.
2 A. Meynaud, M. Grand, M. Belleville and L. Fontaine, Thérapie, 30 (1975) 777.
3 T.G. Judge, Scot. Med. J., 23 (1978) 43.
4 L. Fontaine, M. Grand, E. Szarvasi and M. Bayssat, Chim. Ther., 4 (1969) 39.
5 M. Belleville and J.C. Lechevin, Ann. Pharm. Fr., 26 (1968) 641.
6 R.R. Brodie, L.F. Chasseaud, T. Taylor, J.D. Hunter and P.J. Ciclitira, J. Chromatogr., 164 (1979) 534.
7 E.R. Garrett, J. Pharm. Sci., 72 (1984) 635.
8 R.R. Brodie, L.F. Chasseaud, T. Taylor, D.A. O'Kelly and A. Darragh, J. Chromatogr., 146 (1978) 152.