Journal of Chromatography, 275 (1983) 211—216
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1666

Note

Simultaneous determination of nafimidone [1-(2-naphthoylmethyl)imidazole], a new anticonvulsant agent, and a major metabolite in plasma by high-performance liquid chromatography

D.J.M. GRAHAM*, J. EADIE, B.M. LEADBETTER and R. METCALF*

Syntex Research Scotland, Heriot-Watt University, Edinburgh, EH14 4AS (Great Britain)

(First received November 8th, 1982; revised manuscript received January 24th, 1983)

Nafimidone hydrochloride [1-(2-naphthoylmethyl)imidazole] (I) is a new anticonvulsant agent under investigation for use in the treatment of generalised tonic-clonic and partial seizures [1, 2]. Nafimidone has been shown to be rapidly metabolised in rat, dog, monkey and man with the formation of a major non-conjugated metabolite 1-[2-hydroxy-2-(2-naphthyl)ethyl]imidazole (II) [3]. This metabolite has been shown to possess similar anticonvulsant properties to the parent compound [4].

This paper describes two simple high-performance liquid chromatographic (HPLC) methods for the quantitation of nafimidone and its major metabolite in plasma. The methods employ UV and fluorescence detection, the latter involving wavelength switching to optimise sensitivity. The methods have been used to analyse plasma obtained following the administration of nafimidone hydrochloride to dog and monkey.

EXPERIMENTAL

Materials

All reagents were of at least analytical grade except in the case of diethyl ether which was redistilled in glass before use. HPLC grade methanol and acetonitrile were purchased from Rathburn Chemicals (Walkerburn, Great Britain). Heptanesulphonic acid, used as an ion-pair reagent, was of HPLC grade (Fisons Scientific Apparatus, Loughborough, Great Britain).

Nafimidone, [^{14}C]nafimidone, metabolite II and internal standards, 1-[2-(2-naphthoyl)ethyl]imidazole (III) and 1-[(6-methyl-2-naphthoyl)methyl]-

*Present address: Smith, Kline and French Research Ltd., The Frythe, Welwyn, Hertfordshire, Great Britain.

0378-4347/83/$03.00

imidazole (IV) were supplied as hydrochloride salts by Syntex Research (Palo Alto, CA, U.S.A.).

Preparation of standard solutions

Standard solutions of nafimidone, metabolite II and internal standards III and IV were prepared in 0.02 *M* citrate buffer (pH 2.75). These solutions (5—15 $\mu g\ ml^{-1}$) stored at 4°C were stable for at least three months.

Extraction procedure

Duplicate aliquots of plasma (0.05—0.5 ml) were spiked with internal standard [III (UV) or IV (fluorescence), 10—200 ng] buffered by the addition of McIlvaine buffer (0.19 *M* Na_2HPO_4—0.0064 *M* citric acid, pH 7.6, 1 ml) [5] and extracted once with diethyl ether (6 ml). This pH was employed as nafimidone is unstable at high pH. Extraction consisted of mechanised rotation in stoppered tubes followed by centrifugation. The ether fraction was then extracted with 0.1 *M* hydrochloric acid (1 ml). Following removal of the ether fraction the aqueous fraction was again buffered with McIlvaine buffer (pH 7.6, 2 ml) and extracted with diethyl ether (4 ml). The organic layer was then transferred to conical tubes and evaporated to dryness under oxygen-free nitrogen (45°C). The residue was re-dissolved in 40—100 μl of HPLC mobile phase prior to analysis.

A calibration line was prepared by extraction of aliquots of control plasma (0.5 ml) spiked with nafimidone and metabolite II over the expected concentration range.

Instrumentation

Analyses were performed on a Spectra-Physics Model SP8000A liquid chromatograph (Spectra-Physics, St. Albans, Great Britain) equipped with a Waters Assoc. (Cheshire, Great Britain) Model 710A Intelligent Sample Processor (WISP).

A Model CE2012 variable-wavelength UV detector (Cecil Instruments, Cambridge, Great Britain), or a Model PE3000 fluorescence detector (Perkin-Elmer, Beaconsfield, Great Britain) was used as flow monitor.

Chromatography

Two HPLC systems were used for quantitation of nafimidone and metabolite. Both systems utilised stainless-steel columns 150 mm × 4.5 mm I.D. When UV detection was used columns were packed with ODS Hypersil (5 μm) (Shandon Southern Products, Runcorn, Great Britain) and operating conditions were: mobile phase, methanol—acetonitrile—0.2 *M* ammonium carbonate (40:8:52. v/v), flow-rate, 1 ml min^{-1}; detector, 248 nm (for simultaneous detection of nafimidone and metabolite II) or 225 nm (optimum for detection of metabolite II). Use of fluorescence detection required a different system: column, Partisil 10 ODS-3 (silylated) (Whatman, Maidstone, Great Britain), (10 μm); mobile phase, methanol—acetonitrile—water (all containing 0.005 *M* heptanesulphonic acid and 0.087 *M* acetic acid (44:4:41, v/v), flow-rate 1 ml min^{-1}; detector, for nafimidone and internal standard IV λ_{ex} 245 nm, λ_{em} 456 nm, for metabolite II λ_{ex} 255 nm, λ_{em} 333 nm.

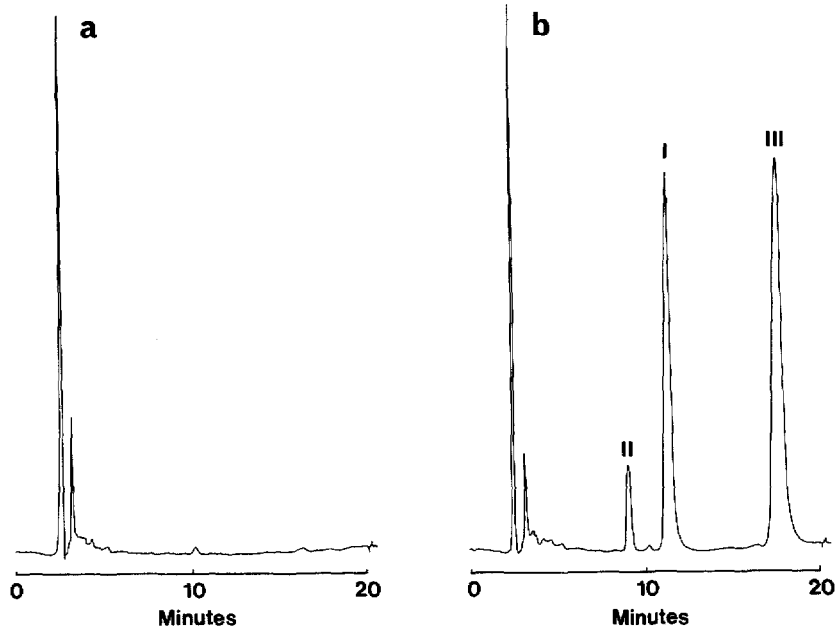

Fig. 1. Chromatograms of extracts of control plasma (a) and of plasma from a male volunteer following administration of nafimidone hydrochloride showing nafimidone (I), metabolite II, and internal standard (III) using UV detection (248 nm) (b).

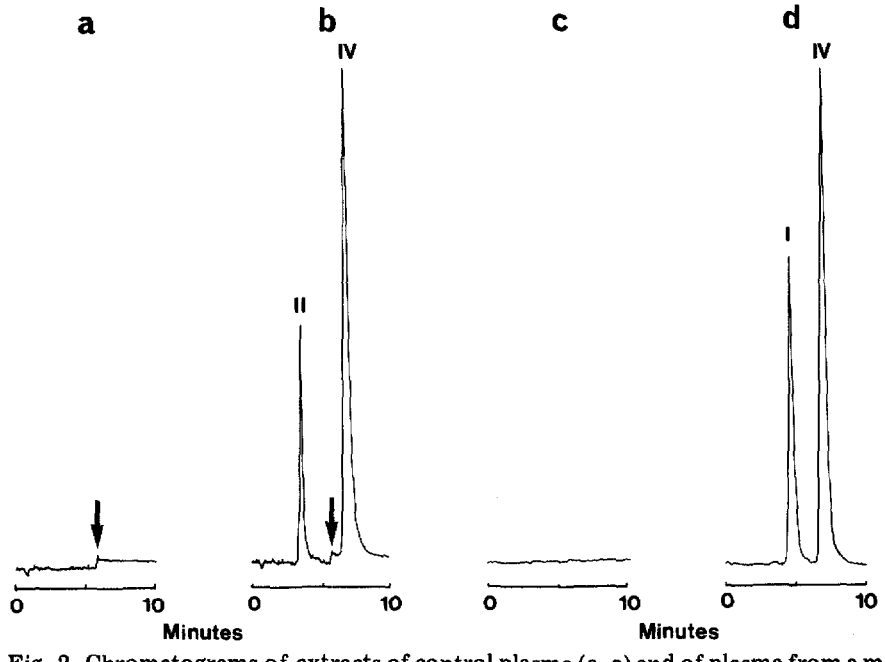

Fig. 2. Chromatograms of extracts of control plasma (a, c) and of plasma from a male volunteer following administration of nafimidone hydrochloride (b, d) showing nafimidone (I), metabolite II and internal standard (IV). Arrows indicate wavelength change from λ_{ex} 255 nm to 245 nm, λ_{em} from 333 nm to 456 nm (a, b). For c and d, λ_{ex} = 245 nm; λ_{em} = 456 nm.

Using the ion-pair HPLC system resolution of nafimidone and metabolite II was incomplete and two injections were made of each extract with the fluorimeter set up for each compound in turn. Nafimidone is not detectable at the optimum excitation/emission wavelengths for metabolite II and the converse is also true. Chromatograms are shown in Figs. 1 and 2.

RESULTS AND DISCUSSION

The precision and accuracy of the method using both detectors was determined by preparing pools of plasma containing nafimidone and metabolite II at several concentrations. Multiple analyses were performed using this spiked plasma and the accuracy and assay variance computed from the results. The results using each detection method are given in Table I.

TABLE I

PRECISION AND ACCURACY FOR THE DETERMINATION OF NAFIMIDONE (I) AND METABOLITE (II) IN PLASMA USING HPLC WITH UV OR FLUORESCENCE DETECTION

UV				Fluorescence			
Actual concn. (ng ml^{-1})	Observed (mean ± S.D., n = 6)	Variance	% of actual	Actual concn. (ng ml^{-1})	Observed (mean ± S.D., n = 6)	Variance	% of actual
Nafimidone							
26	25.5 ± 0.43	1.7	98	43.6	41.7 ± 0.69	1.6	96
127	135 ± 5.1	3.8	106	209	207 ± 7.9	3.8	99
362	378 ± 15.2	4.0	104	396	388 ± 26.0	6.7	98
Metabolite II							
204	209 ± 11.2	5.4	102	511	519 ± 23.7	4.6	102
798	811 ± 38.6	4.8	102	2440	2530 ± 138	5.5	104
1900	1940 ± 108	5.6	102	4640	4710 ± 354	7.5	102

The recovery of nafimidone from plasma was checked by spiking aliquots of control plasma with [^{14}C]nafimidone, extracting as described and determining the amount of radioactivity in the final extract by liquid scintillation counting. Plasma was spiked at 100 ng ml^{-1} and 1 μg ml^{-1} and the percentage recoveries in the final ether extract were 65 ± 7% (S.D.) and 74 ± 5% (S.D.), respectively.

The detection limits for each compound vary with the method used. The limits for each compound for each method are given in Table II. These are based on the extraction of 0.5 ml plasma. The signal-to-noise ratios for the peaks at the limits range from 3 to 6.

Using the extraction methods described no plasma components interfere with the assay. Metabolite II is the only non-conjugated metabolite to have

TABLE II

DETECTION LIMITS FOR THE QUANTITATION OF NAFIMIDONE (I) AND METABOLITE II IN PLASMA USING HPLC WITH UV OR FLUORESCENCE DETECTION

Limits are based on extraction of 0.5 ml plasma. Wavelengths are expressed as nm, detection limits are expressed as ng ml^{-1}.

Detector	Compound	λ_{abs}	λ_{ex}	λ_{em}	Detection limit
UV	I	248	—	—	10
UV	II	248	—	—	100
UV	II	225	—	—	10
Fluorescence	I	—	245	456	3
Fluorescence	II	—	255	333	5

been isolated and identified so it cannot be said with certainty that the assay is specific. However radiochemical studies have shown that II is the main, if not only, non-conjugated metabolite found in plasma of rat, dog and monkey.

Analysis of control plasma spiked with nafimidone or metabolite II has shown that there is no detectable interconversion of the two compounds in vitro.

The above methods of analysis were applied to plasma samples from dog and monkey following single oral doses (10 mg kg^{-1}) of nafimidone hydrochloride administered in solution (0.2% w/w in 0.02 *M* citrate buffer, pH 2.75). Plasma profiles of nafimidone and metabolite II are shown in Fig. 3.

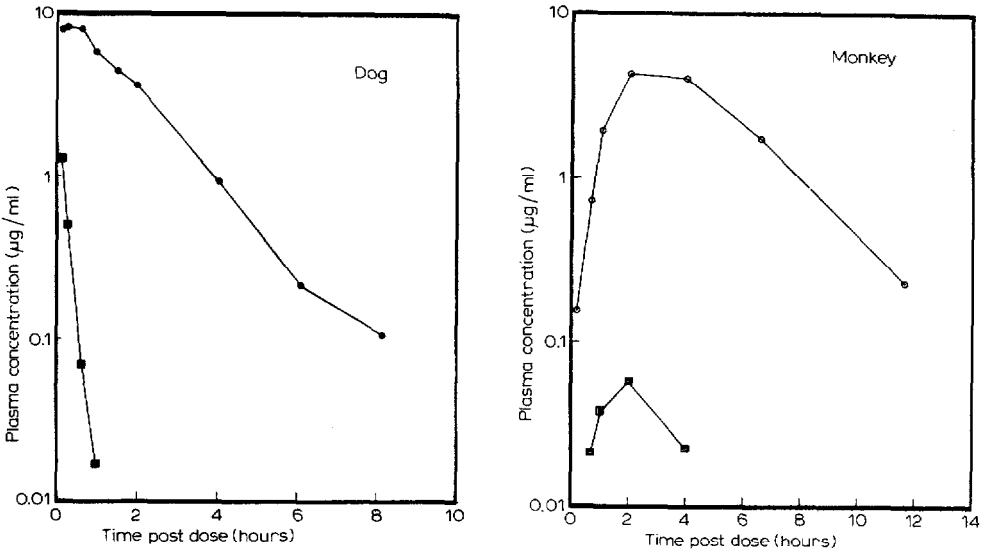

Fig. 3. Plasma profiles of nafimidone (I) (■, □) and metabolite II (●, ○) in dog and monkey following single oral doses of nafimidone hydrochloride at a dose level of 10 mg kg^{-1}.

The interaction of the commonly administered anticonvulsants, phenytoin, carbamazepine, phenobarbitone and sodium valproate with the assay of nafimidone and metabolite II has been studied. None of the anticonvulsants fluoresce at the excitation—emission wavelengths used so do not interfere with the fluorescence assay. However, using the UV assay modification of the mobile phase is necessary to resolve carbamazepine from the compounds of interest. None of the other drugs interfere with the UV assay.

Both of the above methods afford similar limits of detection with regard to the metabolite II. As this is the more significant species biologically either method will be suitable for the pharmacokinetic and clinical evaluation of nafimidone. The methods are also suitable for the quantitation of the metabolite II in saliva and thus will allow the validation of salivary estimation as a means of therapeutic monitoring.

REFERENCES

1 M.B. Wallach, L.R. Hedley, K.E. Paterson and C. Rogers, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 39 (1980).
2 K.A.M. Walker, M.B. Wallach and D.R. Hirschfield, J. Med. Chem., 24 (1980) 67.
3 D.A. Ross, personal communication.
4 M.B. Wallach, personal communication.
5 P.J. Elving, J.M. Markowitz and I. Rosenthal, Anal. Chem., 28 (1956) 1179.