Journal of Chromatography, 533 (1990) 224–228
Biomedical Applications
Elsevier Science Publishers B.V , Amsterdam

CHROMBIO 5461

Note

Determination of the calcium antagonist nimodipine in plasma by capillary gas chromatography and nitrogen detection

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(First received April 9th, 1990, revised manuscript received July 4th, 1990)

Nimodipine, Bay e 9736 or isopropyl-2-methoxyethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate, is a dihydropyridine calcium antagonist with a preferential cerebrovascular action [1].

Krol *et al.* [2] have described a gas chromatographic (GC) method for the determination of the drug in plasma. It involves packed-column GC with electron-capture detection (ECD), with oxidation of nimodipine to its pyridine analogue. As this analogue is a possible metabolite of nimodipine, the method is not suitable for the quantitative analysis of nimodipine. The same authors also described a high-performance liquid chromatographic (HPLC) method with UV detection, but 2.5 ml of plasma were needed to achieve a sensitivity of 1 ng/ml of plasma [2]. Jakobsen *et al.* [3] used packed-column GC with ECD for direct determination of nimodipine; the metabolites of nimodipine were not mentioned.

We have developed a specific and sensitive GC assay for the determination of nimodipine in plasma using fused-silica capillary GC with nitrogen–phosphorus detection (NDP) and on-column injection, allowing quantitation of nimodipine in the presence of its metabolites.

EXPERIMENTAL

Materials

Nimodipine (Bay e 9736), metabolite I (Bay m 9822), metabolite II (Bay m 5397) and metabolite III (Bay o 1762), three lipophilic metabolites found in plas-

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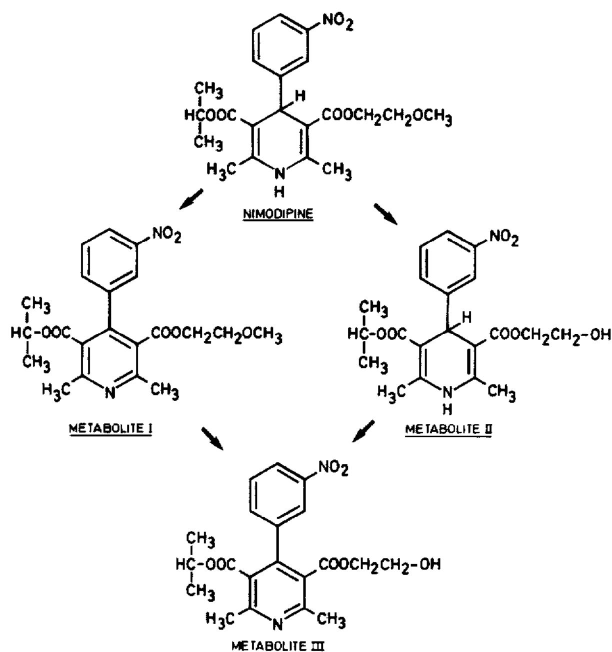

Fig 1 Structures of nimodipine and three of its lipophilic metabolites

ma after oral administration of nimodipine to humans [2], were supplied by Bayer (Wuppertal, F.R.G.) The molecular structures are shown in Fig. 1.

Bay a 4815 (Fig. 2) and nitrendipine (Bay e 5009) were also obtained from Bayer. A preliminary attempt to use nitrendipine as internal standard failed as it has, under the circumstances used, the same retention time as metabolite III, and therefore Bay a 4815 was used as internal standard.

Stock solutions (1 mg/ml, equivalent to base) were prepared in methanol and were stored in amber glass tubes at −20°C. Appropriate dilutions were made every week. Toluene was "Baker analyzed" from J. T. Baker (Deventer, The Netherlands).

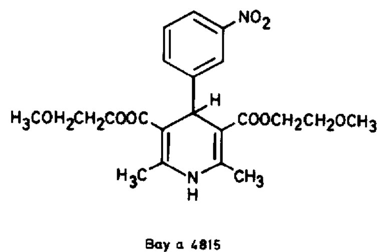

Fig 2 Structure of the internal standard (Bay a 4815)

Gas chromatography

A Hewlett-Packard Model 5880 gas chromatograph, equipped with a nitrogen–phosphorus ionization detector, was used. GC was performed on a 10 m × 0.31 mm I.D. cross-linked 5% phenylmethyl silicone fused-silica capillary column, with a film thickness of 1.03 μm and a phase ratio of 75 (Hewlett-Packard, Avondale, PA, U.S.A.). A Hewlett-Packard capillary on-column injection port system (19, 430H) was used. Samples were injected with a Hamilton 10-μl syringe and a fused-silica needle (0.18 mm O.D.).

The oven temperature profile was: initial value, 90°C; initial time, 1.00 min; programming rate, 25°C/min; final value, 255°C. The detector temperature was 310°C. Helium was used as carrier gas at a flow-rate of 6.0 ml/min and as make-up gas at 20 ml/min. The detector was operated with hydrogen at 3.5 ml/min and with air at 75 ml/min. The peak heights were recorded on a Hewlett-Packard 3390 A recording integrator.

Extraction procedure

The internal standard (70 ng in 7 μl methanol) was added to 1 ml of plasma in a brown, glass-stoppered centrifuge tube. After alkalinization with 0.2 ml of 2.0 *M* sodium hydroxide, the mixture was extracted with 1 ml of toluene by shaking horizontally for 20 min. The phases were separated by centrifugation (10 min, 2200 *g*, 4°C).

The toluene phase was transferred with a Pasteur pipette into a 6-ml brown, glass-stoppered conical tube and evaporated under a stream of nitrogen at 40°C. Another 1 ml of toluene was added to the aqueous phase, which was shaken and centrifuged as before; this 1 ml was then transferred to the same conical tube and again evaporated under nitrogen. Before evaporation was complete, the wall of the conical tube was washed down with 0 2 ml of toluene and the toluene was evaporated to dryness. The residue was reconstituted in 5 μl of toluene and a 0.2-μl aliquot was used for capillary GC–NPD analysis. The whole procedure was carried out under subdued lighting as nimodipine shows some degradation on exposure to daylight and UV light [3] The samples were stored at below −20°C until analysis (within 24 h).

RESULTS AND DISCUSSION

Fig. 3 demonstrates that under the conditions described, nimodipine is fully separated from its metabolites and the internal standard. Using a high-capacity column, symmetrical peaks are obtained for all the products. Calibration curves prepared in the concentration range 2–50 ng/ml were linear The curves ($n=4$) gave a mean (± S.D.) linear regression line of $y = 0.02521\ (\pm\ 0.00948) + 0.03674\ (\pm\ 0.00191)\ x$, with $r = 0.99972\ (\pm\ 0.00096)$, where y is the nimodipine peak height divided by the internal standard peak height and x is the concentration of nimodipine (nanograms of free base per millilitre). Toluene was found to

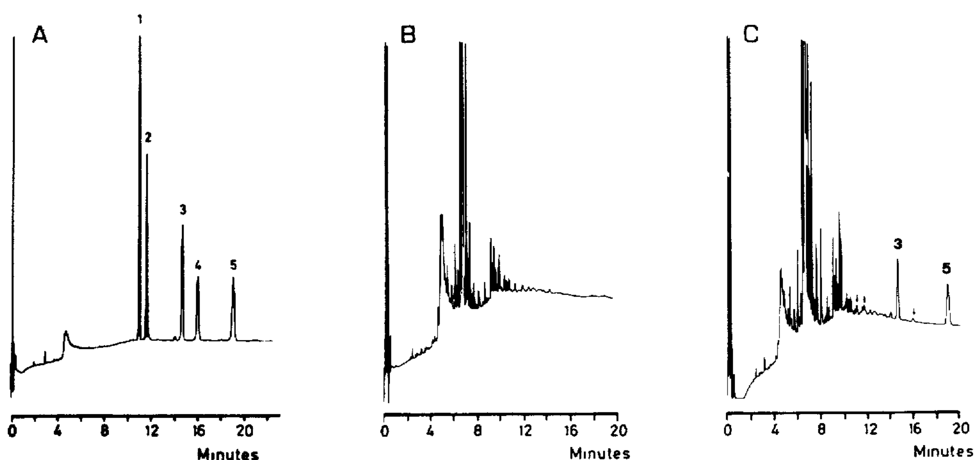

Fig 3 Gas chromatograms of (A) a standard solution, containing 30 ng/μl metabolite I (1), metabolite III (2), nimodipine (3), metabolite II (4) and the internal standard (5), in an injection volume of 0 3 μl, (B) an extract of 1 ml of blank plasma from a dog and (C) an extract of 1 ml of plasma from the same dog sampled 15 min after i.v injection of 90 μg/kg nimodipine (3) and spiked with 70 ng/ml internal standard (5) The arrows indicate the retention times of the metabolites

be an excellent extraction solvent, with a mean (± S.D.) extraction recovery for nimodipine of 94.8 ± 2.2% for 5–50 ng/ml.

The between-day reproducibility and accuracy of the assay method are demonstrated by a coefficient of variation (C.V.) of 9.4% ($n = 9$) and a mean result of 21.4 ng/ml, using pooled dog plasma spiked with nimodipine (20.0 ng/ml) and analysed over three weeks.

The within-day precision and accuracy are given in Table I. With the described instrumentation and procedure, the detection limit of the assay is 0.5 ng/ml of plasma.

Fig. 3 also shows an example of a chromatogram from dog plasma obtained before and 15 min after intravenous (i.v.) administration of nimodipine (90 μg/kg). The extracts of blank dog plasma do not contain components with retention

TABLE I

WITHIN-DAY ACCURACY AND PRECISION FOR NIMODIPINE

Concentration (ng/ml)	n	Mean result (%)	C.V. (%)
5	10	100 0	5 1
10	5	99 5	4 9
50	5	97 0	6 6

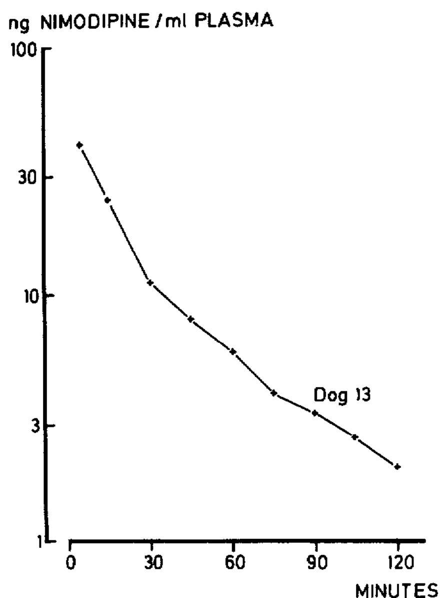

Fig 4 Plasma concentration–time curve of nimodipine after i v. injection of 90 μg/kg in a dog

times equal to those for nimodipine and its metabolites. The plasma concentration–time curve of nimodipine after administration of a 90 μg/kg i.v. dose to a dog is shown in Fig. 4. Only trace amounts of the metabolites are present in plasma after i.v. administration of nimodipine; these concentrations are too low for accurate measurement.

By using a high-load, inert fused-silica capillary column, a specific, sensitive and reproducible analysis of nimodipine is possible.

ACKNOWLEDGEMENTS

The authors thank Dr. K. D. Raemsch, Dr. K. Brandau and Dr. E. Moeller from Bayer (Wuppertal, F.R.G.) for providing standards.

REFERENCES

1 S Kazda and R Towart, *Acta Neurochir*, 63 (1982) 259
2 G J Krol, A J Noe, S C Yeh and K D. Raemsch, *J Chromatogr*, 305 (1984) 105
3 P Jakobsen, E O Mikkelsen, J Laursen and F. Jensen, *J Chromatogr*, 374 (1986) 383