Journal of Chromatography, 310 (1984) 219—222
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2168

Note

High-performance liquid chromatographic determination of nifedipine in plasma

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(First received January 12th, 1984; revised manuscript received March 23rd, 1984)

Nifedipine [dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylate] is one of the most useful drugs known as a calcium antagonist, and has been widely used clinically. Thus the measurement of nifedipine in plasma is required for pharmacokinetic studies and to examine relationship between plasma levels and clinical effects.

Several methods for the assay of plasma levels of nifedipine have been described, including a fluorescence method [1], gas chromatography [2—4], gas chromatography—mass spectrometry [5], and high-performance liquid chromatography (HPLC) [6, 7], but many of these methods are inappropriate for clinical use because they have a low sensitivity, are time-consuming, need a large amount of plasma, and require expensive equipment not usually available in a clinical laboratory. A recent paper [8] describes a rapid assay method using HPLC, but the reproducibility of this method at low concentrations (10 ng/ml in plasma) is relatively inferior. Here we describe a simple and reproducible HPLC method using a reversed-phase column and an ultraviolet (UV) detector.

0378-4347/84/$03.00

EXPERIMENTAL

Materials

Nifedipine was kindly supplied by Kanebo (Tokyo, Japan). Butamben (*n*-butyl-*p*-aminobenzoate) was purchased from Kishida Chemicals (Osaka, Japan). All chemicals were of reagent grade and used without further purification.

Procedures

Methanol (100 μl) and acetonitrile (2 ml) were added to 0.5 ml of plasma in a brown test tube, and agitated with a Vortex mixer. After centrifugation at 1500 *g* for 5 min, 2 ml of the supernatant were transferred into a brown test tube containing 1 ml of distilled water, and then 4.5 ml of acetone—chloroform mixture (1:1, v/v) were added. The mixture was shaken for 10 min and then centrifuged at 1500 *g* for 5 min. After aspirating the aqueous layer, 5 ml of the organic layer were transferred into a brown test tube, and reduced to dryness in a centrifugal evaporator (Model RD-21, Yamato Scientific, Tokyo, Japan) at 45°C for 30 min. The residue was dissolved in 100 μl of the mobile phase containing butamben as internal standard (2 μg/ml), and 20 or 30 μl of the solution were injected into the HPLC system.

Chromatographic conditions

A liquid chromatograph (Hitachi 635 A) equipped with a high-pressure sampling valve (638-0801, 1—150 μl) and multiwavelength UV detector (Hitachi 638-41) was used. For the stationary phase, a reversed-phase column (Zorbax ODS, 4—6 μm 25 cm × 4.6 mm I.D.; Du Pont de Nemour, Wilmington, DE, U.S.A.) was used; the column was warmed at 55°C using a constant-temperature water bath circulator. The mobile phase consisted of 0.01 *M* disodium hydrogen phosphate buffer—methanol (45:55). Before mixing, the buffer was brought to pH 6.1 with 50% phosphoric acid. The flow-rate was 0.8 ml/min and the pressure was approximately 70 kg/cm^2. The wavelength was 280 nm at 0.0025 a.u.f.s.

Calibration graph

Standard solutions containing 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 μg/ml nifedipine in methanol were prepared under very subdued light. Instead of 100 μl of methanol, 100 μl of each standard solution were added to 0.5 ml of drug-free plasma and the samples were processed as described above. The ratios of the peak height of nifedipine to that of butamben (internal standard) were used to construct a calibration graph. Stock solutions of both nifedipine and internal standard (1 mg/ml) in methanol were stored in complete darkness; these solutions were freshly prepared every month.

Monitoring of plasma concentrations

An experiment was performed with two healthy subjects aged 27 and 29 years, weighing 60 and 53 kg, respectively. First, basal blood samples were taken after overnight fasting. Then 10 mg of nifedipine (Adalat; Bayer Yakuhin, Osaka, Japan) were administered orally with 100 ml of tap water. Blood samples were drawn through an indwelling venous catheter at 0.33,

0.5, 0.75, 1, 1.5, 2, 3, and 4 h. Food and beverages were restricted for 4 h after administration.

RESULTS AND DISCUSSION

Sample preparation

Nifedipine can be extracted at neutral and alkaline pH by several organic solvents such as heptane, diethyl ether, chloroform, and ethyl acetate. In preliminary experiments, however, endogenous biological interfering substances were also extracted by these organic solvents, and showed a peak immediately after the peak of the internal standard. However, it was found that these interfering peaks became negligible when a mixture of acetone–chloroform (1:1, v/v) was used for the extraction.

Selectivity

Fig. 1 shows chromatograms of blank plasma, plasma sample spiked with 40 ng/ml nifedipine, and plasma sample at 3.0 h after the administration of nifedipine to the patient (Adalat, 10 mg). Nifedipine and internal standard were well separated from endogenous substances. In this procedure a peak due to metabolites of nifedipine was not observed near the peak of nifedipine.

The calibration curve of peak height ratio was linear with a correlation coefficient of 0.9998. The coefficient of variation at 10 ng per 0.5 ml of plasma was 3.02% (n = 7).

The relative recovery of nifedipine from plasma containing 40 ng/ml was estimated by comparing it with the recovery from an aqueous sample (distilled water) and was found to be 99.1 ± 3.2% (mean ± S.D., n = 7). Plasma was spiked with nifedipine by the same procedure as described for the calibration graph. The limit of sensitivity for quantitation was 5 ng/ml plasma.

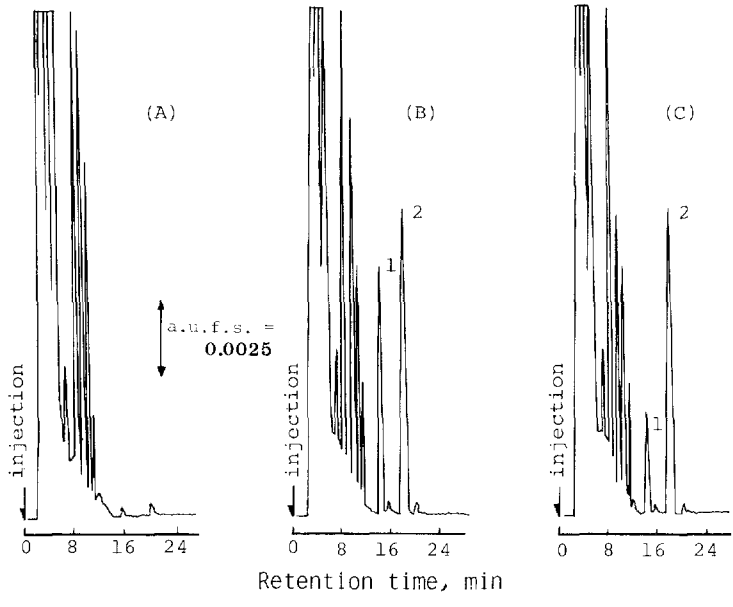

Fig. 1. High-performance liquid chromatograms of blank plasma (A), plasma sample spiked with 40 ng/ml nifedipine (B), and plasma sample after the administration of nifedipine to a patient (C). Peaks: 1 = nifedipine, 2 = internal standard.

No interfering peaks were detected for plasma spiked with propranolol, trichloromethiazide, or L-α-methyldopa in accepted therapeutic concentrations.

Plasma concentration profile

Plasma concentrations of nifedipine were monitored using the newly developed assay method. The plasma concentration profiles are shown in Fig. 2. The absorption rates and the elimination profiles were significantly different between the two subjects. In our recent studies investigating the relationship between the plasma levels of nifedipine and the lowering of the blood pressure in hypertensive patients, substantial variations in the rate of appearance in plasma were seen (unpublished data). Also, substantial variations in plasma levels of nifedipine [8] and its analogue [9] among the subjects were reported.

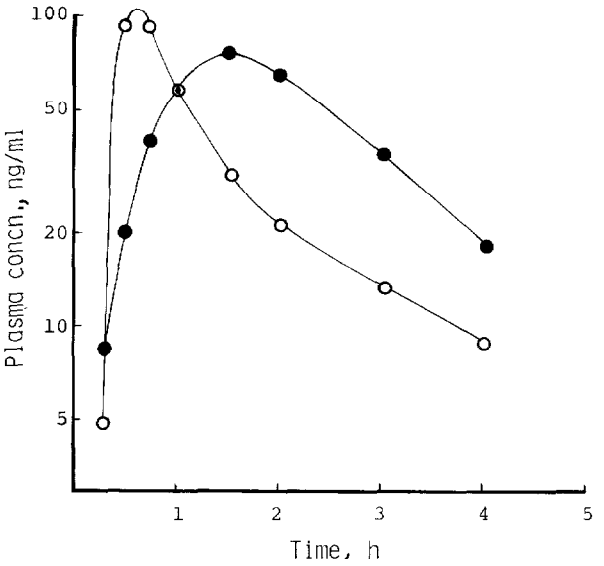

Fig. 2. Plasma concentration profiles of nifedipine after oral administration of 10 mg of nifedipine to each of two volunteers.

It is possible to determine low plasma concentrations of nifedipine rapidly, reproducibly, and sensitively by the method described in this report. Our results suggest that the method is useful for both therapeutic drug monitoring and pharmacokinetic studies.

REFERENCES

1 K. Schlossmann, Arzneim.-Forsch., 22 (1972) 60.
2 S. Kondo, A. Kuchiki, K. Yamamoto, K. Akimoto, K. Takahashi, N. Awata and I. Sugimoto, Chem. Pharm. Bull., 28 (1980) 1.
3 P. Jakobsen, O.L. Pedersen and E. Mikkelsen, J. Chromatogr., 162 (1979) 81.
4 S.R. Hamann and R.G. McAllister, Jr., Clin. Chem., 29 (1983) 158.
5 S. Higuchi and Y. Shiobara, Biomed. Mass Spectrom., 5 (1978) 220.
6 P. Pietta, A. Rava and P. Biondi, J. Chromatogr., 210 (1981) 516.
7 T. Sadanaga, K. Hikida, K. Tameto, Y. Matsushima and Y. Ohkura, Chem. Pharm. Bull., 30 (1982) 3807.
8 P.R. Bach and the Clinical Investigation of Duchenne Dystrophy Group, Clin. Chem., 29 (1983) 1344.
9 S. Higuchi and S. Kawamura, J. Chromatogr., 223 (1981) 341.