Journal of Chromatography, 420 (1987) 439–444
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3755

Note

Simple method for the determination of nicardipine in plasma using high-performance liquid chromatography

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(First received February 16th, 1987; revised manuscript received April 14th, 1987)

Nicardipine hydrochloride, 2-(N-benzyl-N-methylamino)ethylmethyl 1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate monohydrochloride, is a calcium antagonist with highly potent vasodilating activity [1–3] and is used for the treatment of hypertension and cerebrovascular disease [4,5]. Although nicardipine is well absorbed from the gastro-intestinal tract after oral administration to humans and laboratory animals, its plasma concentrations are relatively low owing to first-pass metabolism [6,7]. Various metabolites generated from nicardipine have been found in humans and laboratory animals [6,8] and manifest little vasodilating activity [9]. Thus, a sensitive and specific method for the determination of plasma nicardipine concentrations is required to examine the pharmacokinetics and the relationship between plasma concentrations and pharmacological effects. Several methods have been reported for the determination of nicardipine in plasma of humans and animals [10–14]. Some of them are available for the specific determination of nicardipine, which include high-performance liquid chromatography (HPLC) [12,13], and the combination of HPLC with gas chromatography (GC) [12] or of thin-layer chromatography with GC with mass spectrometric detection [14]. However, some of these methods require intricate and laborious procedures [12,14], and the others are not sensitive enough for the measurement of clinical samples [13]. The method described in this report is a simple, sensitive and accurate HPLC method using conventional instruments.

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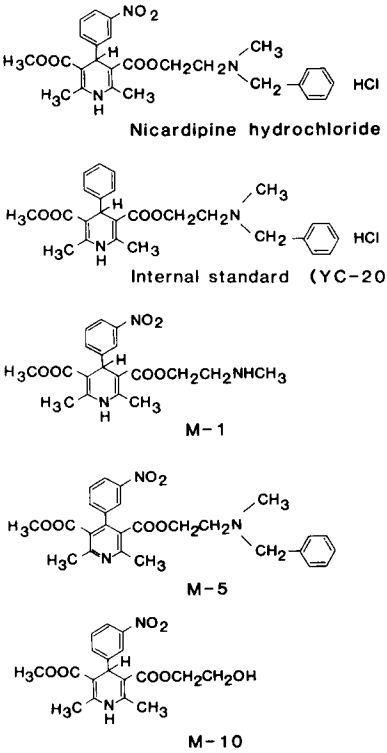

Fig. 1. Chemical structures of nicardipine hydrochloride, the internal standard (YC-204) and metabolites M-1, M-5 and M-10.

EXPERIMENTAL

Materials

Authentic nicardipine hydrochloride, an internal standard (YC-204, I.S.), 2-(N-benzyl-N-methylamino)ethylmethyl 1,4-dihydro-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate monohydrochloride, and metabolites (M-1, M-5 and M-10) were supplied by our Central Research Laboratories. Their chemical structures are shown in Fig. 1. Other chemicals with guaranteed reagent grade were obtained commercially and used without further purification.

Apparatus and chromatographic conditions

The HPLC system consisted of an M-6000 pump, a U6K injector (Nihon Waters, Tokyo, Japan), a variable-wavelength UV detector (SPD-2A, Shimadzu, Kyoto, Japan) and a Shimadzu Chromatopac C-R1A. A stainless-steel column (150 mm × 4 mm I.D.) packed with LiChrosorb Si-60 (mean particle size 5 μm, Merck Japan, Tokyo, Japan) was maintained at 55°C. A mixture of *n*-hexane–chloroform–absolute ethanol (20:5:1) was used as HPLC eluent for analysis of human plasma samples, and *n*-hexane–chloroform–absolute ethanol (20:4:1) for plasma samples from rats, dogs and monkeys. The eluent was passed through the column at a flow-rate of 0.5 ml/min and monitored at a wavelength of 254 nm.

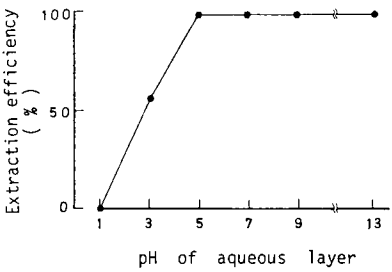

Fig. 2. Effect of pH of the aqueous layer on the extraction of nicardipine (mean of duplicate measurements). In this determination, 80 ng of nicardipine hydrochloride were added to 2 ml each of aqueous solutions with different pH and extracted with diethyl ether–*n*-hexane (1:1). The aqueous solutions were: 0.1 *M* hydrochloric acid (pH 1); 0.1 *M* phosphoric acid–potassium dihydrogen phosphate (pH 3); 0.1 *M* phosphate buffer (pH 5 and 7); 0.1 *M* disodium hydrogenphosphate (pH 9); and 0.1 *M* sodium hydroxide (pH 13).

Standard solutions

Stock solutions of nicardipine and the I.S. were prepared at a concentration of 1 mg/ml in absolute ethanol. Standard solutions containing 0.25, 0.5, 1, 1.5, 2 and 2.5 μg/ml of nicardipine hydrochloride were prepared by diluting the stock solution with absolute ethanol. An I.S. solution (0.25 μg/ml) was prepared in a mixture of 0.1 *M* phosphate buffer (pH 7.4) and absolute ethanol (20:1).

Procedures for sample preparation

I.S. solution (1 ml) and 6 ml of diethyl ether–*n*-hexane (1:1) were added to 1 ml of plasma in a 10-ml brown centrifuge tube equipped with a stopper. The tube was shaken for 15 min on a mechanical shaker and centrifuged for 5 min at 600 *g*. The organic layer was transferred to another 10-ml brown tube and evaporated to dryness in an aspirator. The residue was dissolved in 50 μl of the HPLC eluent, and 20 μl of the solution were injected onto the column.

Calibration curve

A 20-μl aliquot of each nicardipine standard solution was added to 1 ml of blank plasma in a 10-ml brown centrifuge tube equipped with a stopper. The spiked plasma samples were processed as described above. A calibration curve was constructed by plotting the peak-height ratios of nicardipine to I.S. against nicardipine concentrations in plasma.

RESULTS

Extraction procedures for sample preparation

Nicardipine in plasma samples could be extracted with ethyl acetate, diethyl ether, chloroform and benzene. However, various endogenous substances in plasma were coextracted and interfered with the analysis of nicardipine. This interference was reduced greatly when diethyl ether–*n*-hexane (1:1) was used, and nicardipine was completely extracted with this mixture when the pH of the aqueous layer was in the range 5–13 (Fig. 2). A pH value of 7.4 was selected for

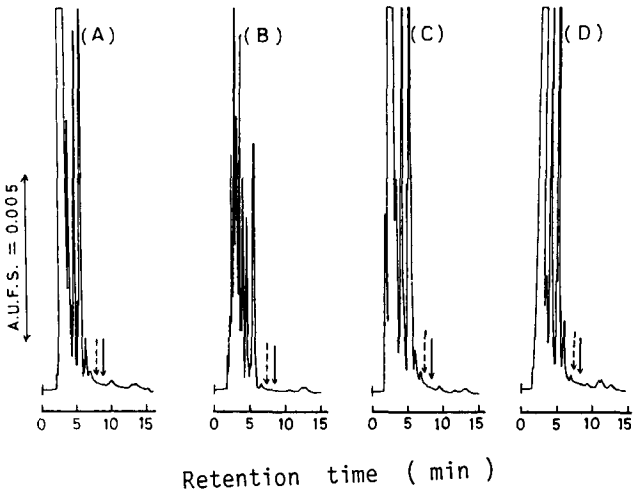

Fig. 3. Chromatograms of blank plasma of (A) humans, (B) monkeys, (C) dogs and (D) rats. The retention times of nicardipine and internal standard are indicated by solid and dashed arrows, respectively.

the extraction because of minimum coextraction of endogenous substances. The extraction efficiencies for 50 ng of nicardipine hydrochloride added to 1 ml of blank plasma of humans and rats were 95.8 and 96.4%, respectively. For the construction of the calibration curve, 20 μl of standard ethanol solution were added to blank plasma. Ethanol caused no effects on the extraction of nicardipine and I.S. when the volume of absolute ethanol was increased to 100 μl. Sample extracts were directly injected onto an analytical column without a precolumn. The column remained useable for longer than 5 months.

Separation of nicardipine, I.S. and endogenous substances

UV absorption maxima of nicardipine in the HPLC eluent were observed at 238 and 355 nm. Although monitoring at 238 nm gave the highest peak for nicardipine, endogenous substances interfered. At 254 nm, however, this interference was much less and no peaks appeared at the retention time of nicardipine, as shown in Fig. 3. Although there were minute peaks caused by endogenous substances near the retention time of I.S., the effects of these peaks on the determination of nicardipine could be neglected by using adequate amounts of I.S. (more than 200 ng). Monitoring at 355 nm was also available in terms of the elimination of interfering peaks, but the peak height for nicardipine was relatively low.

Selectivity

Of various metabolites generated in humans and laboratory animals dosed with nicardipine hydrochloride, the metabolites possessing a primary amine or carboxyl group are expected to be excluded by the extraction process in this method. Other metabolites, such as M-1, M-5 and M-10 (Fig. 1), are possibly coextracted with nicardipine and I.S. with diethyl ether–*n*-hexane. However, when a mixed sample of nicardipine, I.S. and the metabolites was injected, they were completely

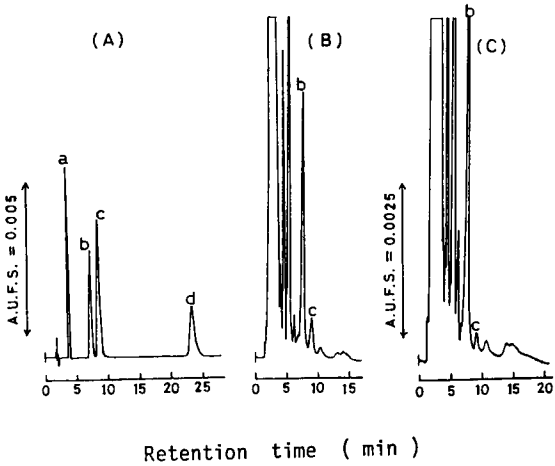

Fig. 4. Chromatograms of (A) mixture of nicardipine, internal standard and metabolites, (B) human plasma sample taken 2 h after oral dosing of 20 mg of nicardipine hydrochloride and (C) human plasma spiked with 5 ng/ml nicardipine hydrochloride. Peaks: a = M-5; b = internal standard; c = nicardipine; d = M-10. The nicardipine concentration of the plasma sample shown in (B) was estimated to be 19.4 ng/ml.

separated from one another, as shown in Fig. 4A. M-1 was not eluted within 60 min. Fig. 4B shows a chromatogram of a plasma sample taken 2 h after administration of a tablet containing 20 mg of nicardipine hydrochloride to a healthy volunteer. No interfering peaks were observed near the peak of nicardipine.

Calibration curve and sensitivity

Calibration curves for spiked plasma of humans and animals showed good linearity in the concentration range 5–1000 ng/ml. A chromatogram for human plasma containing 5 ng/ml of nicardipine hydrochloride is shown in Fig. 4C. The signal-to-noise (S/N) ratio for the peak of nicardipine was ca. 6 at this concentration, so a detection limit at S/N = 2 was calculated to be as low as 1.7 ng of nicardipine hydrochloride per mililitre of plasma.

Accuracy and precision

Table I shows the accuracy and precision of this method, determined from human plasma samples spiked with nicardipine hydrochloride at concentrations of 10, 30 and 100 ng/ml. Five samples were prepared for each concentration. The differences of the mean value measured from the concentration prepared, expressed in percentages, were only 2.0%, 1.0% and 0.1% at 10, 30 and 100 ng/ml, respectively. The coefficients of variation were as low as 2.4% and 0.6% at 30 and 100 ng/ml, respectively. However, slightly greater variation was found at 10 ng/ml.

DISCUSSION

The method described here has been developed in order to determine plasma nicardipine concentrations simply, sensitively and accurately by use of conven-

TABLE I

ACCURACY AND PRECISION OF THE METHOD FOR THE DETERMINATION OF NICARDIPINE IN HUMAN PLASMA

Concentration prepared (ng/ml)	Concentration measured (mean ± S.D., $n=5$) (ng/ml)	Percentage difference (%Δ)★	Coefficient of variation (%)
10	10.2 ± 0.7	2.0	6.9
30	29.7 ± 0.7	−1.0	2.4
100	100.1 ± 0.6	0.1	0.6

★$\%\Delta = \frac{\text{mean of concentration measured} - \text{concentration prepared}}{\text{concentration prepared}} \times 100$

tional instruments available in most laboratories. Nowadays, HPLC is widely used for the analysis of various compounds. Wu et al. [12] reported a specific HPLC–UV method for the determination of nicardipine in human plasma samples, but their method included relatively laborious extraction procedures prior to HPLC analysis. Although a simpler method using HPLC and electrochemical detection was described by Visor et al. [13], their method was used for only rat plasma samples. Even if the method were available for human plasma samples, its sensitivity would not be high enough.

The method in this report has a detection limit of 2 ng/ml and good accuracy and precision, and is expected to allow pharmacokinetic analysis of plasma concentrations of nicardipine administered at a dose of 20 mg, the minimum orally effective dose, to humans. The method consists of a simple extraction procedure and HPLC analysis, and so can be used anywhere that HPLC–UV detection is available. In addition, the method is applicable to routine measurements of plasma concentrations, not only in humans but also in laboratory animals, such as rats, dogs and monkeys.

REFERENCES

1 M. Iwanami, T. Shibanuma, M. Fujimoto, R. Kawai, K. Tamazawa, T. Takenaka, K. Takahashi and M. Murakami, Chem. Pharm. Bull., 27 (1979) 1426.
2 T. Takenaka, S. Usuda, T. Nomura, H. Maeno and T. Sado, Arzneim.-Forsch., 26 (1976) 2172.
3 M. Terai, T. Takenaka and H. Maeno, Biochem. Pharmacol., 30 (1981) 375.
4 T. Takenaka and J. Handa, Int. J. Clin. Pharmacol. Biopharm., 17 (1979) 1.
5 T. Takabatake, H. Ota, Y. Yamamoto, M. Maekawa, S. Arai, N. Hattori and G. Nomura, Int. J. Clin. Pharmacol. Ther. Toxicol., 20 (1981) 346.
6 S. Higuchi, H. Sasaki, Y. Shiobara and T. Sado, Xenobiotica, 7 (1977) 469.
7 S. Higuchi and Y. Shiobara, Xenobiotica, 10 (1980) 447.
8 D.J.M. Graham, R.J. Dow, D.J. Hall, O.F. Alexander, E.J. Mroszczak and D. Freedman, Br. J. Clin. Pharmacol., 20 (1985) 23S.
9 T. Shibanuma, M. Iwanami, M. Fujimoto, T. Takenaka and M. Murakami, Chem. Pharm. Bull., 28 (1980) 2609.
10 S. Higuchi, H. Sasaki and T. Sado, J. Chromatogr., 110 (1975) 301.
11 S. Higuchi and Y. Shiobara, Biomed. Mass Spectrom., 7 (1980) 339.
12 A.T. Wu, I.J. Massey and S. Kushinsky, J. Pharm Sci., 73 (1984) 1444.
13 G.C. Visor, E. Bajka and E. Benjamin, J. Pharm. Sci., 75 (1986) 44.
14 S. Higuchi and S. Kawamura, J. Chromatogr., 223 (1981) 341.