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DETERMINATION OF MANIDIPINE AND ITS PYRIDINE METABOLITE IN HUMAN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION AND COLUMN SWITCHING

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SUMMARY

A highly sensitive and selective high-performance liquid chromatographic method using column switching is described for the determination of the dihydropyridine calcium antagonist manidipine (I), 2-[4-(diphenylmethyl)-1-piperazinyl]ethyl methyl (±)-1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate, and its pyridine metabolite (II), 2-[4-(diphenylmethyl)-1-piperazinyl]ethyl methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate, in human serum The method is based on the combination of the column-switching technique and ion-pair chromatography In the first ODS column, I and II are preseparated from endogenous substances in serum with a mobile phase containing sodium nonane sulphonate as an ion-pair reagent After column switching, in the second ODS column, the heart-cut fraction containing I and II is further separated from the co-eluted substances through the first column with a mobile phase containing no ion-pair reagent By using microbore columns with a diameter of 2 1 mm, the sensitivity is almost double that given by conventional bore columns with a diameter of 4 6 mm The method offers high sensitivity and selectivity with short-wavelength ultraviolet detection at 230 nm The detection limits of both I and II are 0 1 ng/ml using 1 ml of serum The method is suitable for the pharmacokinetic study of I 2HCl after oral administration to man

INTRODUCTION

Manidipine (I), 2-[4-(diphenylmethyl)-1-piperazinyl]ethyl methyl (±)-1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate (Fig

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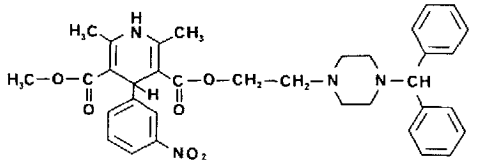
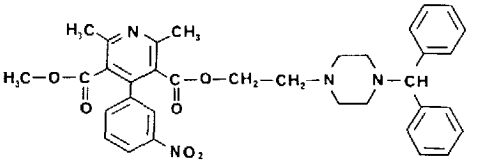


I



II

Fig 1 Structures of manidipine (I) and its pyridine metabolite (II)

1), is a newly developed potent 1,4-dihydropyridine calcium antagonist having long-lasting activity [1] After oral administration of I·2HCl to man at a dose which causes hypotension, very low concentrations (0 1–10 ng/ml) of the unchanged drug and its pyridine metabolite (II), 2-[4-(diphenylmethyl)-1-piperazinyl]ethyl methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate, were found in serum Therefore, a sensitive analytical method for I and II is required for pharmacokinetic studies

I has UV absorption maxima at 230 and 352 nm The UV absorption [molar absorptivity at 230 nm (32 000)] is about five times larger than that at 352 nm A preliminary high-performance liquid chromatographic (HPLC) study using a standard solution of I showed that UV detection at 230 nm offered sufficient sensitivity provided that there were no interferents present at the same retention time as I in the HPLC system However, UV detection at a short wavelength such as 230 nm is generally not suitable for the sensitive determination of a drug in biological fluids because of poor selectivity

In a previous paper [2], we described a column-switching HPLC system utilizing ion-pair formation, which permitted the sensitive determination of basic compounds in serum by UV detection at a short wavelength The detection limit for I was 0 2 ng/ml using 1 ml of serum In this paper, the development of a more sensitive HPLC method for the simultaneous determination of I and II is described, involving the use of microbore HPLC and column switching to optimize the conditions for measurements in serum after oral administration of I·2HCl to man

EXPERIMENTAL

Reagents and materials

I and its pyridine metabolite (II) were synthesized in the Central Research Division, Takeda Chemical Industries (Osaka, Japan) Acetonitrile and *n*-

hexane were of HPLC grade (Wako, Osaka, Japan) All other reagents were of analytical-reagent grade and used without further purification

Extraction from serum

To 1 0 ml of serum in a 10-ml brown centrifuge tube was added 1 0 ml of 0 02 *M* disodium hydrogenphosphate (pH 10), as I was light-sensitive The mixture was extracted twice with 5 ml of *n*-hexane–diethyl ether (2 1, v/v) The organic layer was evaporated to dryness under nitrogen and the residue was reconstituted with 200 μl of the mobile phase for column 1 (MP1) and an aliquot of 150 μl was injected on to the HPLC column

Chromatography

The HPLC system consisted of two LC-6A pumps, two SPD-6A UV detectors, a CTO-6A column oven, an FCV-2AH six-port switching valve and an SIL-6A autosampler, all of which were controlled automatically by an SCL-6A controller (all from Shimadzu, Kyoto, Japan) Two U-228 dual-pen recorders (Nippon Denshi Kagaku, Kyoto, Japan) were used to record the chromatograms before and after column switching Two ODS columns, Develosil ODS-3K (3 μm particle size, 100 mm$\times$2 1 mm I D) for column 1 and Develosil ODS-5K (5 μm particle size, 100 mm$\times$2 1 mm I D) for column 2 (both from Nomura Chemical, Aichi, Japan) were used The mobile phase for column 1 (MP1) was 0 02 *M* potassium dihydrogenphosphate–acetonitrile (54 46, v/v) containing 5 m*M* sodium nonane sulphonate (SNS) adjusted to pH 3 0 with 85% (w/v) orthophosphoric acid The mobile phase for column 2 (MP2) was 0 02 *M* potassium dihydrogenphosphate–acetonitrile (54 46, v/v) adjusted to pH 3 0 with 85% (w/v) orthophosphoric acid The temperature and the flow-rate for both columns were 40°C and 0 3 ml/min, respectively

HPLC system and procedure

The HPLC system and the procedure were similar to those reported previously [2] A schematic diagram of the HPLC system with the column switching valve is shown in Fig 2 The retention times of I and II in column 1 were checked each day before analysis to determine the precise time programme for the column switching At time zero, the sample was injected on to column 1, which was eluted with MP1, and column 2 was eluted with MP2 (valve position A) Just before the elution of II from column 1, the valve was switched to position B and the eluate fraction containing I and II was introduced on to column 2 After the elution of I, the valve was switched back to position A Then the introduced eluate was further separated by column 2 followed by detection of II and I by UV adsorption at 230 nm, while column 1 was conditioned with MP1 for the next injection The analysis of each sample was completed within 40 min The valve operation was carried out automatically by the SCL-6A controller according to the predetermined time programme

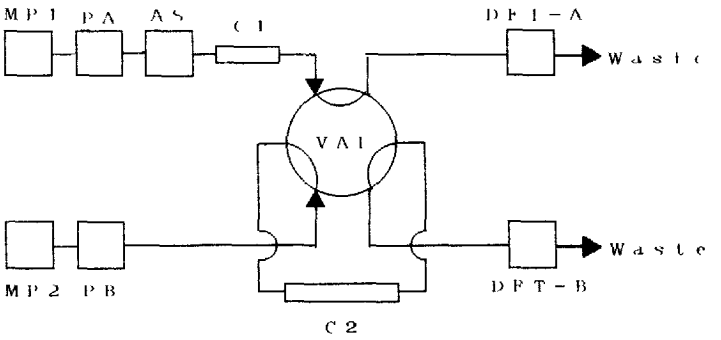


Fig 2 Schematic diagram of the HPLC system PA, PB = pumps A and B, AS = autosampler, VAL = six-port valve, C1, C2 = columns 1 and 2, DET-A, DET-B = UV detectors A and B The solid and dotted lines in the switching valve indicate valve positions A and B, respectively

Calibration graph

Drug-free serum spiked with known amounts of I and II was analysed according to the analytical procedure described above. Peak heights of I and II were plotted against the serum concentration to give the calibration graphs The percentage recoveries of I and II in serum were calculated from the peak-height ratios of spiked standard samples relative to directly injected standard solutions

RESULTS

Chromatography

Fig 3 shows typical chromatograms of the standard and drug-free serum The sensitive determination of I and II was impossible owing to interferences from endogenous substances

To utilize ion-pair formation for the first separation step in column switching, various ion-pair reagents, including alkane sulphonates ($C_nH_{2n+1}SO_3$, $n=5$–10) and sodium dodecyl sulphate (SDS, $n=12$), were investigated Heptane sulphonate ($n=7$) did not retard the elution of I and decane sulphonate ($n=10$) and SDS retarded it too much (Fig 4) In previous work [2], sodium octane sulphonate (SOS, $n=8$) was used, but SNS ($n=9$) was found to be the most suitable for the present purpose

The relationship between the retention times of I and II and the pH of the mobile phase is shown in Fig 5 Curves 1 and 2 represent the retention times of II and I respectively, with 5 m*M* SNS, which correspond to the separation of II and I in column 1, and curves 3 and 4 represent the retention times of II and I, respectively, without 5 m*M* SNS, which correspond to the separation of II and I in column 2

In each column, the separation of II and I was improved as the pH increased,

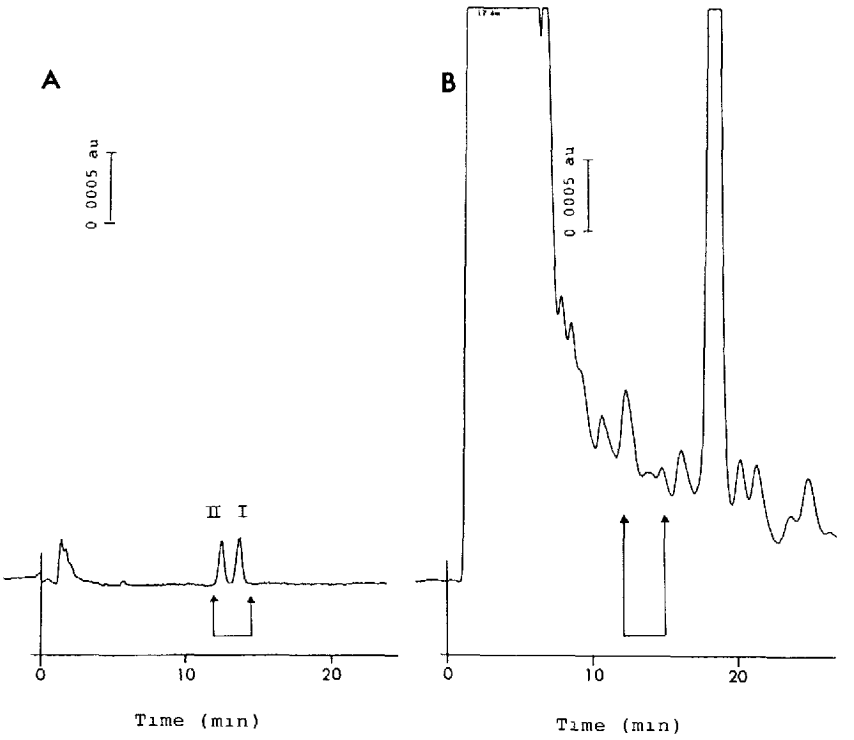


Fig 3 Chromatograms of (A) standard solution and (B) drug-free serum obtained with column 1 monitored by detector A The marked zone indicates the heart-cut fraction For HPLC conditions, see text

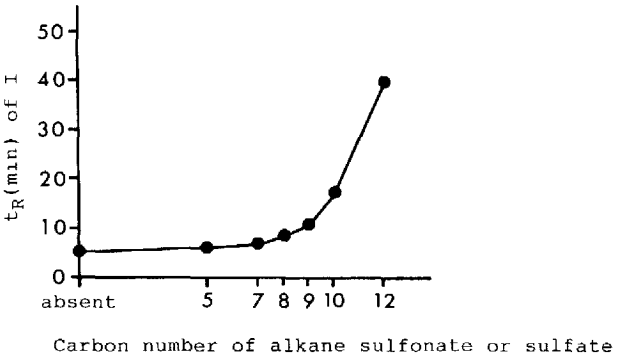


Fig 4 Effect of the alkyl chain length of alkane sulphonate or sulphate in the mobile phase on the retention time of I with column 1 HPLC conditions column, Develosil ODS-5K (5 μm particle size, 100 mm×2 1 mm I D), mobile phase, 0 02 *M* potassium dihydrogenphosphate–acetonitrile (54 46, v/v) containing 5 m*M* alkane sulphonate or sulphate adjusted to pH 3 0 with 85% (w/v) orthophosphoric acid, temperature, 40 °C, flow-rate, 0 3 ml/min, detection, UV 230 nm

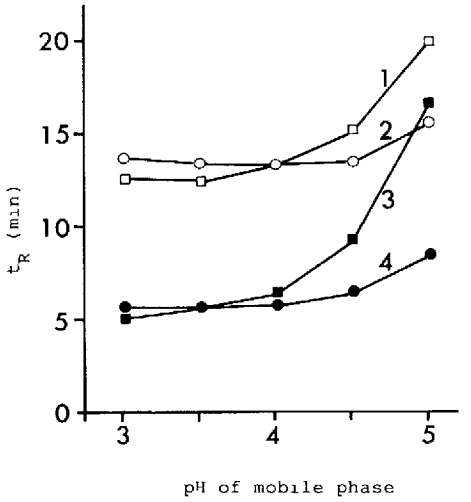


Fig 5 Relationship between retention times of I and II and pH of the mobile phase 1, II (containing 5 m*M* sodium nonane sulphonate), 2, I (containing 5 m*M* sodium nonane sulphonate), 3, II, 4, I HPLC conditions column, Develosil ODS-5K (5 μm particle size, 100 mm × 2 1 mm I D), mobile phase, 0 02 *M* potassium dihydrogenphosphate–acetonitrile (54 46, v/v) containing 5 m*M* sodium nonane sulphonate (or no additive) adjusted to pH 3–5 with 85% (w/v) orthophosphoric acid, temperature, 40 °C, flow-rate, 0 3 ml/min, detection, 230 nm

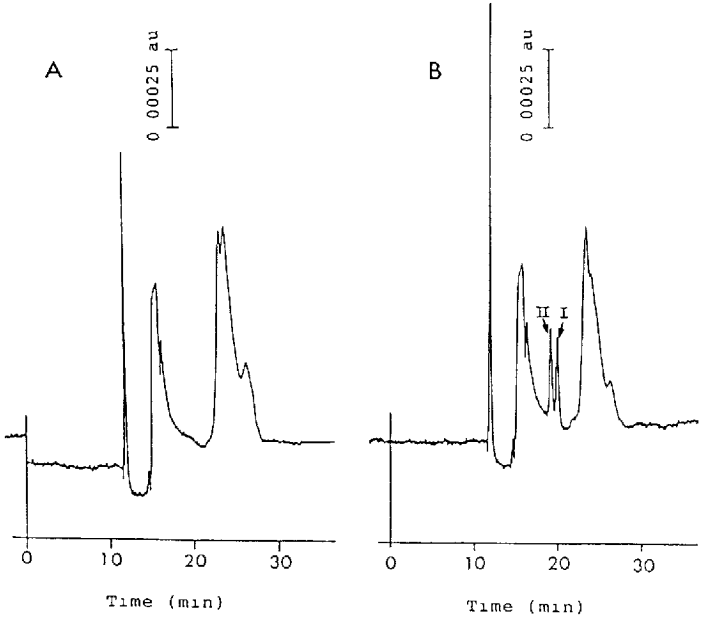


Fig 6 Chromatograms of (A) drug-free serum and (B) serum spiked with I and II (both 1 ng/ml) obtained by column switching monitored with detector B For HPLC conditions, see text

but as the separation was improved in column 1, the 'heart-cut' volume increased, which reduced the sensitivity of the response in column 2, i e , at pH 4 (heart-cut volume 0 5 ml) the separation of II and I proved insufficient and at pH 4 5 (heart-cut volume 1 0 ml) the sensitivity of II and I in the column 2 was low, in spite of the good separation

We therefore selected pH 3 (heart-cut volume 0 78 ml) as the optimum pH from the standpoints of good separation and sensitivity Under these conditions, I and II were well separated with high sensitivity and no interferences were observed at their retention times, as shown in Fig 6

Linearity and recovery

The calibration graph was obtained by analysing spiked drug-free serum samples of I and II in the concentration range 0.2–10 ng/ml The equations of the regression lines were $y=27\ 4x-0\ 38$ ($r=1\ 0000$) for I and $y=21\ 26x+1\ 49$ ($r=0\ 9996$) for II The intra-assay recoveries of I and II from low spiked serum samples (both 1 ng/ml, $n=4$) were 97 4% with a coefficient of variation (C.V) of 2 8% for I and 92.7% with a C V of 4 7% for II The inter-assay recoveries of I and II from low spiked serum samples (2 ng/ml for I and 1 ng/ml for II, $n=8$) were 96 0% with a C V of 1.6% for I and 94 4% with a C V of 4.6% for II The recovery and reproducibility for I and II in serum are also shown in Table I The limits of detection of both compounds were 0 1 ng/ml in human serum at a signal-to-noise ratio of 3, using 1 ml of serum

Application of the method

Fig. 7 shows the time course of the mean serum levels of I and II after postprandial oral administration of a 20-mg dose of I·2HCl to healthy volunteers

TABLE I

RECOVERY AND REPRODUCIBILITY IN THE DETERMINATION OF I AND II ADDED TO DRUG-FREE SERUM

For extraction from serum and HPLC conditions, see text

Analyte added to serum (ng/ml)	Recovery (mean ± C V) (%)			
	Intra-assay		Inter-assay	
	I	II	I	II
0 2	91 9	90 9	–	–
1 0 ($n=4$)	97 4±2 8	92 7±4 7	–	–
1 0 ($n=8$)	–	–	–	94 4±4 6
2 0 ($n=8$)	–	–	96 0±1 6	–
5 0	103 7	100 4	–	–
10 0	99 1	99 3	–	–

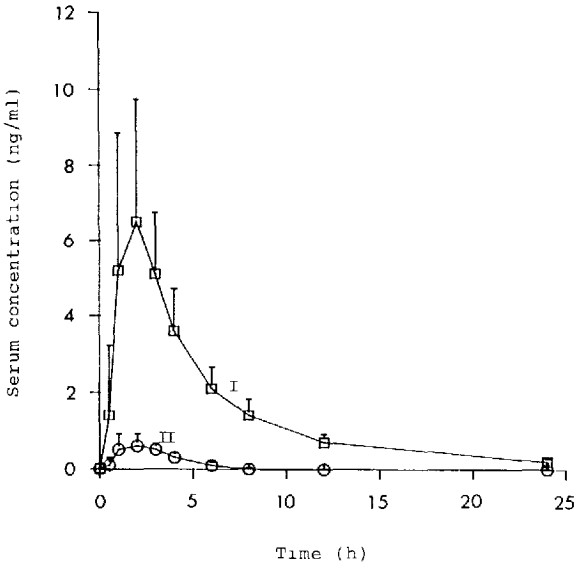


Fig 7 Mean serum levels of I and II after postprandial oral administration of a 20-mg dose of I 2HCl in man Each point and bar represent the mean ($n=6$) and the standard deviation, respectively

as an example The concentrations of I and II peaked at 2 h with mean values of 6 5 and 0 6 ng/ml, respectively, and decreased with mean half-lifes of 6 9 and 1 3 h, respectively The area under the serum concentration–time curve (AUC) values of I were 9 0–12 0 times higher than those of II The present method was sensitive enough for application to the pharmacokinetic study of I in man

DISCUSSION

HPLC has been widely utilized for the determination of 1,4-dihydropyridine compounds. Nifedipine in plasma was determined by HPLC with electrochemical detection (ED) [3], and nicardipine [4] and nimodipine [5] in plasma were determined by HPLC with UV detection at 254 and 238 nm, respectively The molar absorptivity of nicardipine at 238 nm was higher than that at 254 nm but, owing to the interferences by endogenous substances at the shorter wavelength, the drug was determined at 254 nm [4] The reported sensitivity of the determination of 1–2 ng/ml was not sufficient for the present purpose

As oral administration of I·2HCl in man at a dose that reduces blood pressure yields very low serum concentrations of I and II, it is necessary to develop a sensitive analytical method In a previous study [2] we successfully developed a sensitive method for the determination of I in serum by applying a column-switching technique combined with ion-pair chromatography In that study, conventional-bore columns (4 6 mm I D) were used for both columns 1 and 2, resulting in a detection limit of 0 2 ng/ml for I using 1 ml of serum A

more sensitive method is required for pharmacokinetic studies after oral administration of, e g , 20 mg of I·2HCl to man

Furthermore, dihydropyridine compounds are known to be metabolized to pyridine-type metabolites having less potent pharmacological activities Therefore, both the unchanged drug and the pyridine metabolite should be measured simultaneously

Simpson and Brown [6,7], and Schlabach and Wilson [8] reported that use of a microbore column instead of a conventional-bore column resulted in increased sensitivity Accordingly, we introduced microbore columns (2 1 mm I D) into our column-switching HPLC system The sensitivity of the determination of I was almost doubled compared with that obtained with conventional-bore columns

For the selective separation of I and II in a chromatogram, the pH of the mobile phase containing SNS was adjusted to 3 0, instead of the pH of 3.5 used previously At pH 3 0, I and II were separated well in the first column and determined selectively and sensitively in the second column

In conclusion, a highly sensitive and selective method for the determination of I and II in serum was established using microbore columns in a column-switching HPLC system utilizing ion-pair chromatography and two mobile phases at pH 3 The method has permitted pharmacokinetic studies of I and II in human serum after oral administration of I·2HCl at doses between 5 and 40 mg

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