Journal of Chromatography, 528 (1990) 191–198
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5229

Note

Determination of mepirodipine in plasma by capillary column gas chromatography–negative-ion chemical ionization mass spectrometry

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(First received October 24th, 1989; revised manuscript received January 15th, 1990)

Mepirodipine hydrochloride, (+)-(3′*S*,4*S*)-3-(1′-benzyl-3′-pyrrolidinyl)-methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride, is a new potent dihydropyridine calcium antagonist and is currently undergoing clinical trials for the treatment of hypertension [1,2]. The concentrations of mepirodipine in human plasma are relatively low owing to its low effective dose [3,4] and extensive metabolism [5], so a sensitive and specific method is required for its determination.

Many methods have been reported for the determination of dihydropyridine calcium antagonists in biological fluids, such as high-performance liquid chromatography (HPLC) or the combination of HPLC with gas chromatography (GC) [6–11], GC with electron-capture detection (ECD) [12–14] or nitrogen–phosphorus ionization detection (NPD) [15], GC with electron-impact mass spectrometry (EI-MS) [16–18], radioreceptor assay [19,20] and radioimmunoassay [21]. However, some of them were not sensitive enough (HPLC, GC–ECD, GC–NPD) and others lacked specificity (GC–EI-MS, radioreceptor assay, radioimmunoassay). Recently, more specific and sensitive capillary column gas chromatography–negative-ion chemical ionization mass spectrometry (GC–NICI-MS) has been reported for some dihydropyridine drugs [22–24].

0378-4347/90/$03.50

This paper describes the use of a similar technique in a highly sensitive and specific method for the determination of mepirodipine in human plasma.

EXPERIMENTAL

Materials

Mepirodipine hydrochloride and its deuterium-labelled analogue, [$^2H_4$]mepirodipine hydrochloride (internal standard, I.S.), were supplied by our Central Research Laboratories (Tokyo, Japan). Their molecular structures are shown in Fig. 1. All other chemicals used in the study were of analytical grade and commercially available.

Gas chromatography–mass spectrometry

A JMS DX-303 gas chromatograph–mass spectrometer combined with a JMA DA-5000 data system (JEOL, Tokyo, Japan) was used. The gas chromatograph was equipped with a solventless injection system (JEOL). A 5% phenylmethylsilicone-coated fused-silica capillary column (4 m×0.31 mm I.D., coating thickness 0.17 μm, Hewlett-Packard, Tokyo, Japan) was used, and the column outlet was directly coupled to the ion source of the mass spectrometer. A sample was introduced into a solventless injector maintained at 280°C. The column oven, separator and ion source temperatures were set at 280, 300 and 300°C, respectively. Helium was used as a carrier gas at a flow-rate of 2 ml/min.

The mass spectrometer was equipped with an EICI ion source and an electron multiplier, which was combined with a post-accelerated detector (JEOL) for positive- and negative-ion detection. Ionization energy (CI mode) and ionization current were 200 eV and 300 μA, respectively. Isobutane was used as a reagent gas. The electron multiplier and post-accelerated detector voltages were 1.2–1.5 and 10 kV, respectively. Mass fragment ions were selected at *m/z* 474 for $[M-17]^-$ of mepirodipine and at *m/z* 478 for $[M-17]^-$ of I.S.

Extraction procedures

To 1 ml of plasma sample in a 10-ml centrifuge tube, 0.5 ml of I.S. aqueous solution, 0.5 ml of saturated sodium bicarbonate solution and 4 ml of diethyl

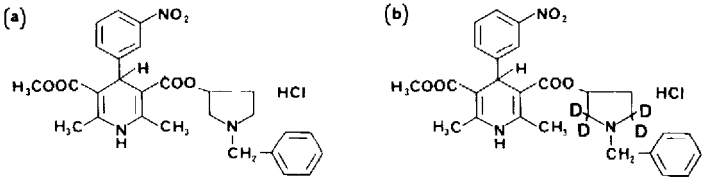

Fig. 1. Chemical structures of mepirodipine hydrochloride (a) and deuterium-labelled mepirodipine hydrochloride (b).

ether were added. The tube was vortex-mixed for 1 min and centrifuged at ca. 1000 *g* for 5 min. The organic layer was transferred to another centrifuge tube containing 3 ml of 0.1 *M* hydrochloric acid. The tube was vortex-mixed for 1 min and centrifuged at ca. 1000 *g* for 5 min. The organic layer was discarded, then the remaining aqueous layer was adjusted to pH 9 with the saturated sodium bicarbonate solution and vortex-mixed with 4 ml of diethyl ether. The tube was centrifuged at ca. 1000 *g* for 5 min. The organic layer was transferred to another centrifuge tube and evaporated to dryness on a water-bath at 45 °C. Ethyl acetate (25 μl) was added to the residue. The ethyl acetate solution (2–3 μl) was injected into the gas chromatograph–mass spectrometer.

Extraction recoveries

A 1-ml volume of control plasma sample containing mepirodipine hydrochloride (1 or 10 ng) was carried through the above procedure without addition of I.S. The I.S. (1 or 10 ng), dissolved in ethyl acetate, was added to the extraction residue and the ethyl acetate solution was evaporated to dryness under reduced pressure. Recoveries were calculated by comparing the peak-height ratios with those obtained when the drug and I.S., dissolved in ethyl acetate, were processed without the extraction procedure.

Preparation of calibration curve

An aliquot (100 μl) of each mepirodipine hydrochloride standard solution was added to 1 ml of control plasma in a 10-ml centrifuge tube. The spiked plasma samples were processed as described above. A calibration curve was prepared by plotting the peak-height ratios of mepirodipine to I.S. against mepirodipine hydrochloride concentrations in plasma.

Stability in plasma

To 1 ml of control plasma sample, 0.5 ml of mepirodipine hydrochloride aqueous solution (0.20 and 2.00 ng) was added and incubated at room temperature (10–20 °C) for 24 h without protection from light. The concentrations of mepirodipine hydrochloride in the samples before and after the incubation were determined.

Human studies

Mepirodipine hydrochloride (20 mg) was administered orally to three healthy male volunteers (56–74 kg, age 23–37 years) after an overnight fast in clinical studies, and the plasma concentrations of unchanged drug were determined [25].

RESULTS AND DISCUSSION

When a packed column was used for the determination of 1,4-dihydropyridine compounds in GC–MS, they were partially oxidized to their pyridine an-

alogues in the column [16,17]. This difficulty has been overcome by using a capillary column in which the compounds are stable, and some determination methods have been successfully established [18,22–24]. We used a similar technique in the present study.

The NICI mass spectra of mepirodipine and I.S. are shown in Fig. 2. The base peaks of mepirodipine and I.S. were observed at *m/z* 474 ($[M-17]^-$) and at *m/z* 478 ($[M-17]^-$), respectively, and these were used to monitor the drug and I.S. The oxidation of mepirodipine to its pyridine analogue in the capillary column was checked by measuring the molecular ion peak of the pyridine analogue of mepirodipine at *m/z* 489. About 7% of the drug was converted into the pyridine analogue in the capillary column. However, it was considered that the concentrations of the unchanged drug showed the correct values because the deuterium-labelled compound was used as I.S., and it has the same physicochemical properties as the non-labelled compound.

Typical chromatograms are shown in Fig. 3. A chromatogram for control

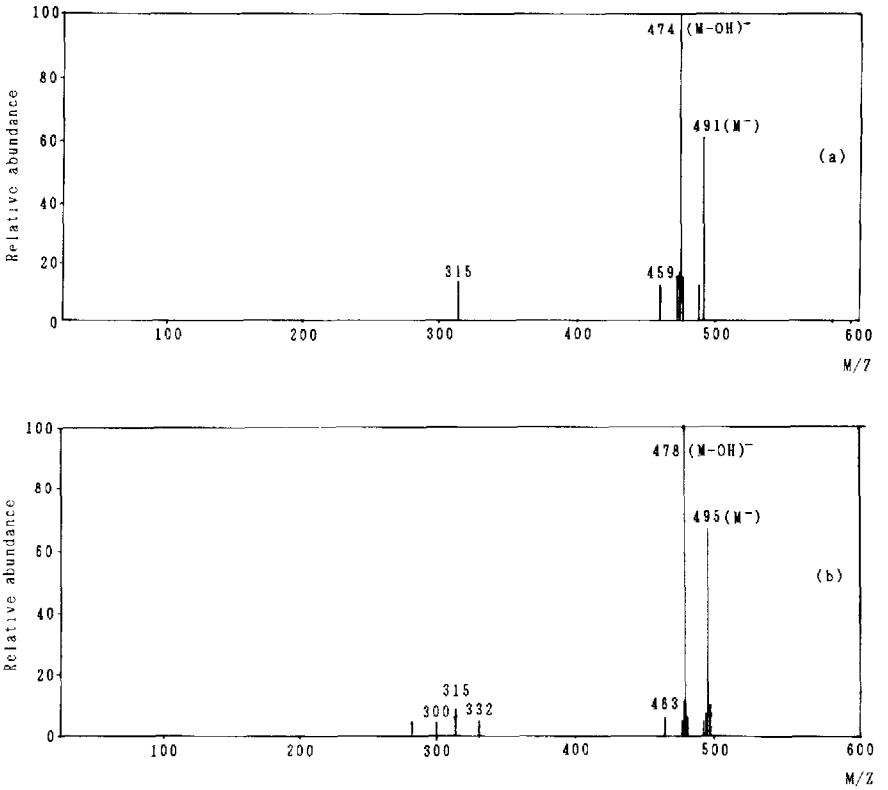

Fig. 2. NICI mass spectra of mepirodipine hydrochloride (a) and [$^2H_4$]mepirodipine hydrochloride (b).

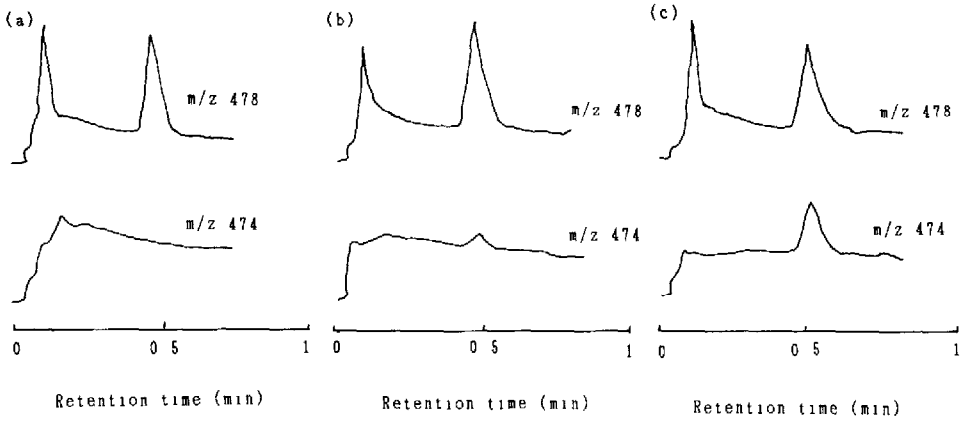

Fig. 3. Chromatograms of (a) control plasma containing 1 ng of I.S., (b) control plasma spiked with mepirodipine hydrochloride (0.03 ng/ml) containing 1 ng of I.S and (c) plasma obtained from a human subject 4 h after administration containing 1 ng of I.S. (0.72 ng/ml).

TABLE I

CALIBRATION CURVES FOR HUMAN PLASMA

Each value represents the mean ± S D. of four curves.

Concentration range (ng/ml)	Slope	Intercept	Correlation coefficient
0.03–0.54	2.13 ± 0 09	0.00 ± 0.01	0.999 ± 0.00
0.54–5.40	2.16 ± 0.07	0.04 ± 0.10	0.999 ± 0.00

human plasma gave a clean baseline at *m/z* 474. No interference peak was observed near the retention time of mepirodipine and I.S. in a chromatogram for the plasma sample from a healthy subject at 4 h after oral administration of 20 mg of mepirodipine hydrochloride.

Each calibration curve prepared on different days showed good linearity in the concentration ranges 0.03–0.54 and 0.54–5.40 ng/ml (Table I). The limit of determination was 30 pg/ml. At this concentration, the signal-to-noise ratio for the peak of mepirodipine was ca. 3. This high sensitivity was due to the use of the post-accelerated detector and the solventless injection system. The detector increased the sensitivity three to four times, and the injection system allowed larger sample volumes to be injected and gave sharper peaks.

The precision and accuracy were determined by analysing five replicate samples at each of three concentrations (Table II). The precision, expressed as the coefficient of variation, ranged from 1.7 to 2.5%. The accuracy, expressed as the percentage nominal concentration, ranged from 96 to 110%.

Mepirodipine in plasma samples could be extracted with diethyl ether, ethyl

TABLE II

PRECISION AND ACCURACY OF THE METHOD IN HUMAN PLASMA

Actual concentration (ng/ml)	Concentration found (mean ± S D., $n=5$) (ng/ml)	Coefficient of variation (%)	Nominal concentration (%)
0.05	0.048 ± 0.001	2 1	96
0.33	0.367 ± 0.009	2 5	110
1.00	1.061 ± 0.018	1.7	106

TABLE III

STABILITY OF MEPIRODIPINE IN HUMAN PLASMA AT ROOM TEMPERATURE (10–20°C)

Each value represents the mean ± S.D. of five samples.

Concentration added (ng/ml)	Concentration found (ng/ml)	
	Initial	After 24 h
0.20	0.20 ± 0.00	0.19 ± 0.01
2 00	2.00 ± 0.01	1.99 ± 0.04

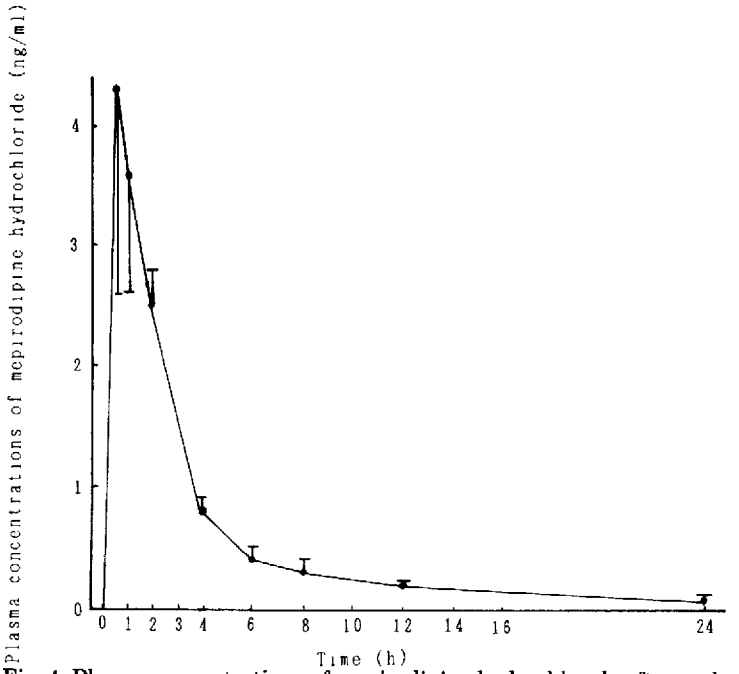

Fig. 4. Plasma concentrations of mepirodipine hydrochloride after oral administration at a dose of 20 mg to healthy volunteers. Each point is the mean ± S.E. of three males [25].

acetate, chloroform and benzene. In this study, diethyl ether was used as the extraction solvent, because the extraction recovery was the highest among these solvents. Mepirodipine was highly extracted with diethyl ether when the pH of the aqueous layer was in the range 5–11. We selected a pH of ca. 9 for the extraction because of the minimum coextraction of endogenous substances. The absolute overall extraction recoveries (mean ± S.E., $n=6$) were 60.4 ± 2.9% for 10 ng/ml and 57.3 ± 3.5% for 1 ng/ml.

The stability of mepirodipine in human plasma was evaluated by incubating five replicate samples at each of two concentrations for 24 h at room temperature without protecting from light. Table III shows that the drug was very stable in human plasma.

Fig. 4 shows plasma concentrations of mepirodipine hydrochloride determined by the described method. The plasma concentration reached its maximum (4.33 ng/ml) 1 h after dosing and then decreased to 0.04 ng/ml at 24 h. The mean $AUC_{0-24\,h}$ was 13.1 ng·h/ml and the terminal elimination half-life was estimated as 7.5 h.

The method was specific and sensitive enough to allow pharmacokinetic analysis of the drug in clinical studies. Further, a large number of samples (more than 200) could be measured in a day, since the retention time of mepirodipine and I.S. was ca. 30 s.

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