Journal of Chromatography, 434 (1988) 123–133
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4437

GAS CHROMATOGRAPHIC–NEGATIVE-ION CHEMICAL IONIZATION MASS SPECTROMETRIC DETERMINATION OF A NEW DIHYDROPYRIDINE CALCIUM ANTAGONIST (MPC-1304) AND ITS METABOLITES IN HUMAN PLASMA AND URINE

YUKIHIKO UMENO, EIJI MATSUSHIMA, MADOKA MANIWA and TERUYOSHI MARUNAKA*

Biological Research Laboratory, Taiho Pharmaceutical Co., Ltd., Ebisuno, Hiraishi, Kawauchi-cho, Tokushima 771-01 (Japan)

(First received June 24th, 1988; revised manuscript received August 17th, 1988)

SUMMARY

A gas chromatographic–negative-ion chemical ionization mass spectrometric method was developed for the determination of a new calcium antagonist, (±)-methyl 2-oxopropyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate, and its metabolites in plasma and urine. The sample was extracted with *n*-hexane–diethyl ether. The dried organic layer was subjected to acetylation: the aqueous layer was acidified and extracted with ethyl acetate, and after the ethyl acetate extract was dried the resulting residue was subjected to methylation. Aliquots of each reactant solution were injected into the gas chromatograph–mass spectrometer, equipped with a chemical ionization source and negative-ion monitoring mode, and analysed by the selected-ion monitoring method using deuterium-labelled internal standards. Detection was limited to 0.02–0.05 ng/ml of plasma and urine for each metabolite. A precise and sensitive assay for the determination of a new dihydropyridine calcium antagonist and its metabolites in plasma and urine was thus established.

INTRODUCTION

(±)-Methyl 2-oxopropyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (I, MPC-1304) is a 1,4-dihydropyridine derivative, developed as a calcium antagonist [1]. Compound I is metabolized in the body to (±)-methyl 2-hydroxypropyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (I-keto-H_2) and further to (±)-methyl 5-carboxy-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3-pyridinecarboxylate (NF-COOH, desmonomethyl ester-nifedipine), with I and these metabolites then proceeding to their respective dehydro forms (pyridine forms) (DH-I, DH-I-keto-H_2 and DH-

0378-4347/88/$03.50

NF-COOH). Metabolite DH-NF-COOH is further converted into methyl 5-carboxy-2-methyl-6-hydroxymethyl-4-(2-nitrophenyl)-3-pyridinecarboxylate (DH-NF-COOH-OH) and its lactone form (DH-NF-COOH-OH(L)). The metabolic pathways of compound I are illustrated in Fig. 1.

For the determination of other 1,4-dihydropyridine calcium antagonists and their metabolites in biological fluids, various methods were reported, e.g. gas chromatography (GC) with an electron capture detection [2–6] or nitrogen–phosphorus ionization detection [7,8], high-performance liquid chromatography (HPLC) [9–12], combined HPLC and GC [13–15] or GC with electron-impact mass spectrometry (EIMS) [16–18]. Most authors reported that the dihydropyridine compounds were oxidized to their respective pyridine forms and then analysed. However, these methods were not suitable for determining these compounds, since the metabolite in the pyridine form compounds will be detected. An assay method using GC with negative-ion chemical ionization MS (NICIMS) for the individual determination of both dihydropyridine and pyridine compounds has been reported [19].

On investigating the metabolic fate of compound I, both dihydropyridine and pyridine form compounds were determined as metabolites. The GC–NICIMS method was investigated using selective monitoring and resulted in the development of a specific, precise and sensitive method for measuring compound I and its metabolites.

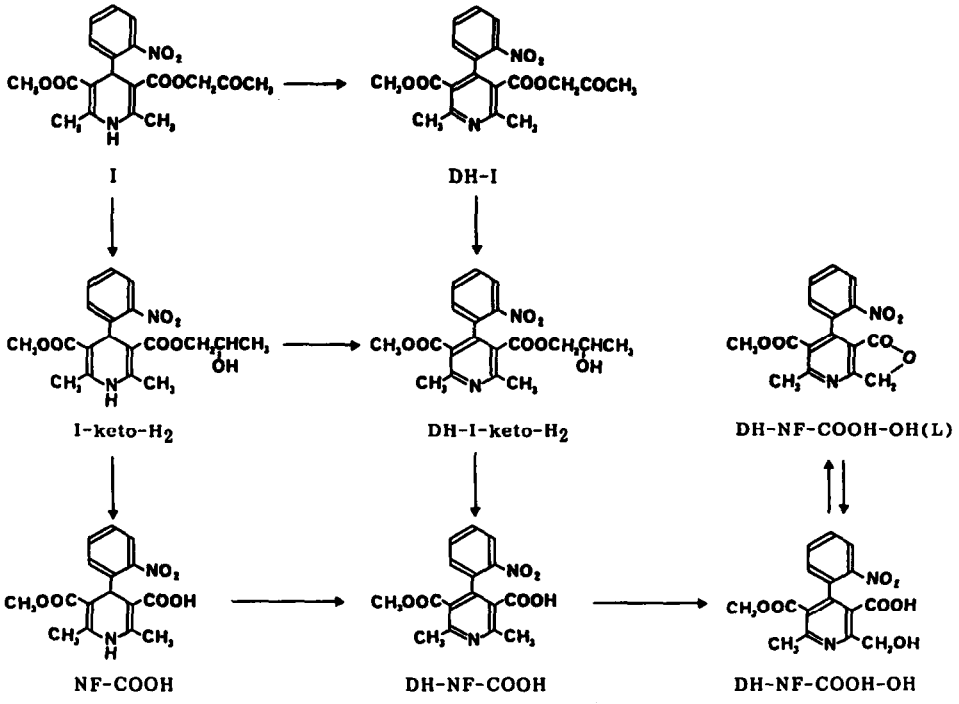

Fig. 1. Metabolic pathway of dihydropyridine calcium antagonist I.

EXPERIMENTAL

Materials

Compound I, its metabolites and deuterium-labelled internal standards, [$^2H_3$]I, [$^2H_3$]DH-I, [$^2H_3$]NF-COOH methyl ester and [$^2H_3$]DH-NF-COOH methyl ester, were supplied by Maruko Pharm. (Nagoya, Japan) and our laboratory. Diazomethane was freshly prepared with N-nitrosomethylurea (ICN, Biomedicals, Plainview, NY, U.S.A.) and 40% potassium hydroxide in diethyl ether. Other chemicals used were all purchased from Wako Pure Chemicals (Osaka, Japan). *n*-Hexane, ethyl acetate and benzene were of liquid chromatographic reagent grade.

Extraction procedure and derivatization

Blood samples were collected in heparinized containers and immediately centrifuged for 15 min at 2000 *g* in a refrigerated centrifuge under a yellow fluorescent lamp (FLR40S·Y-E/M, National, Osaka, Japan) in order to separate the plasma. The resulting plasma and urine were then frozen in a dark room until analysis.

The internal standards, [$^2H_3$]I (1 ng for plasma and urine), [$^2H_3$]DH-I (0.5 ng for plasma and urine), [$^2H_3$]NF-COOH methyl ester (1 ng for plasma and 2 ng for urine) and [$^2H_3$]DH-NF-COOH methyl ester (1 ng for plasma and 5 ng for urine), and 0.1 ml of 1 *M* sodium carbonate were added to 1.0 ml of plasma or urine. The samples were extracted with 4 ml of *n*-hexane–diethyl ether (1:1) at room temperature for 5 min. This extraction was repeated, and the combined organic solvent extracts containing compound I, I-keto-H_2, DH-I, DH-I-keto-H_2 and DH-NF-COOH-OH(L) were dried under nitrogen gas at water temperature ($<15°C$). The resulting residue was subjected to acetylation at room temperature for more than 2 h by addition of 0.2 ml of a mixed solution of acetic anhydride and pyridine (1:1). The reactant solution was evaporated to dryness under nitrogen gas at water temperature and redissolved in 20 μl of benzene. Then 1 μl of this final solution was subjected to GC–NICIMS analysis.

The aqueous layer, containing NF-COOH, DH-NF-COOH and DH-NF-COOH-OH, was acidified to pH 3.0 with 0.3 ml of 1 *M* hydrochloric acid and then extracted with 6 ml of ethyl acetate at room temperature for 5 min. The ethyl acetate layer was evaporated to dryness under nitrogen gas at water temperature. The residue obtained was then methylated at room temperature for more than 2 h by addition of 0.2 ml of diethyl ether solution containing saturated diazomethane. The reactant solution was dried under nitrogen gas at water temperature and redissolved in 20 μl of benzene, and 1 μl of this solution was injected into the gas chromatograph–mass spectrometer.

All the above procedures were carried out under a yellow fluorescent lamp.

Gas chromatography–mass fragmentography

A JEOL JMS DX-303 gas chromatograph–mass spectrometer, with a CI ion source and NI mode equipped with a JMA-DA5100 data system (Tokyo, Japan), was used.

The chemical bonded-type fused-silica capillary column of the gas chromato-

graph was coated with methylsilicon (12.5 m×0.33 mm I.D., Shimadzu, Kyoto, Japan) and was conditioned at 280°C for 24 h. The injector and separator temperatures were 280°C. The ion source temperature was set at 210°C for the determination of compound I, I-keto-H_2, DH-I, DH-I-keto-H_2 and DH-NF-COOH-OH(L) and at 150°C for NF-COOH, DH-NF-COOH and DH-NF-COOH-OH. Analyses were carried out with an initial column temperature of 200°C and a temperature rise of 16°C/min to 300°C. Helium was used as the carrier gas at a flow-rate of 20 ml/min. The split ratio was 20:1. This splitless injection was carried out using a moving needle.

The mass spectrometer was operated under the following conditions: ionization energy, 200 eV; ionization current, 300 μA; accelerating voltage, 3.0 kV; ion multiplier voltage, 1.6–2.0 kV; reactant gas, isobutane. The mass fragment ions selected were the *m/z* 281 ion $[M-107]^-$ of I, the *m/z* 386 ion $[M]^-$ of DH-I, the *m/z* 297 ion $[M-135]^-$ and an additional *m/z* 281 ion $[M-151]^-$ of acetylated I-keto-H_2, the *m/z* 430 ion $[M]^-$ of acetylated DH-I-keto-H_2, the *m/z* 346 ion $[M]^-$ of methylated NF-COOH, the *m/z* 344 ion $[M]^-$ of methylated DH-NF-COOH, the *m/z* 328 ion $[M]^-$ of DH-NF-COOH-OH(L), the *m/z* 328 ion $[M-H_2O]^-$ of DH-NF-COOH-OH, the *m/z* 284 ion $[M-107]^-$ of $[^2H_3]$I, the *m/z* 389 ion $[M]^-$ of $[^2H_3]$DH-I, the *m/z* 349 ion $[M]^-$ of methylated $[^2H_3]$NF-COOH and the *m/z* 347 ion $[M]^-$ of methylated $[^2H_3]$DH-NF-COOH.

Calibration curves

Calibration curves were prepared by adding known amounts of compound I and its metabolites (0.02, 0.05, 0.1, 0.5, 1.0, 5.0, 25.0 and 100 ng/ml for plasma and 0.1, 0.5, 2.0, 10, 50, 200 and 1000 ng/ml for urine) to 1.0-ml aliquots of human plasma and urine, and then analysing the mixture using the same extraction procedure and derivatization.

Calibration curves for the determination of I and its metabolites by GC–NICIMS were obtained by plotting the ratio of the peak areas of the respective metabolites to that of the internal standard against the concentrations of these compounds. All of the calibration curves gave good results.

RESULTS AND DISCUSSION

Extraction procedure and derivatization

The extraction procedure had to be carried out under a yellow fluorescent lamp, since the dihydropyridine form compounds are otherwise converted into their respective pyridine forms.

Compound I, I-keto-H_2, DH-I, DH-I-keto-H_2 and DH-NF-COOH-OH(L) could be recovered from aqueous solution with an organic solvent, while NF-COOH, DH-NF-COOH and DH-NF-COOH-OH remained in the aqueous layer. During the investigation of several extraction procedures for the plasma and urine samples, the following procedure was found to be the most reliable, with the highest recovery for all compounds; no decomposition was observed. The extraction with *n*-hexane–diethyl ether from an aqueous solution, adjusted to pH 8.0 with sodium carbonate solution, was employed for quantitative separation of I, I-keto-H_2, DH-

I, DH-I-keto-H_2 and DH-NF-COOH-OH(L) from NF-COOH, DH-NF-COOH and DH-NF-COOH-OH. The addition of 1 *M* hydrochloric acid to the aqueous layer containing the remaining NF-COOH and DH-NF-COOH and DH-NF-COOH-OH, adjustment to pH 3.0, and subsequent extraction with ethyl acetate resulted in good, constant recoveries.

Under the above procedure, some of DH-NF-COOH-OH(L) and DH-NF-COOH-OH at low concentrations were cleaved to DH-NF-COOH-OH and DH-NF-COOH-OH(L), respectively. However, this problem was overcome by calculation as total concentrations, since these compounds are tautomers and are in equilibrium in the body [13].

In determining the concentrations of compound I and its metabolites by GC–NICIMS, it was possible to analyse I, DH-I and DH-NF-COOH-OH(L), whereas other metabolites, I-keto-H_2, DH-I-keto-H_2, NF-COOH, DH-NF-COOH and DH-NF-COOH-OH, had to be derivatized because of their non-volatility or adsorption on the column packing. Acetylation with a mixed solution of acetic anhydride and pyridine for I-keto-H_2 and DH-I-keto-H_2 gave a higher detection sensitivity and a better separation by GC–NICIMS. On the other hand, the methylated derivatives of the metabolites with a carboxylic acid moiety, NF-COOH, DH-NF-COOH and DH-NF-COOH-OH, were found to produce satisfactory results. The DH-NF-COOH-OH methyl ester was cleaved by the heat of the injection port of the gas chromatograph, but it could be monitored quantitatively in the form of DH-NF-COOH-OH(L). All these derivatizations gave quantitative results.

The acetyl derivatives were found to be stable at 5 °C for one to two weeks and methyl derivatives were stable at 5 °C for several weeks. In addition, no changes of the dihydropyridine form compounds to the pyridine form compounds were observed during the above extraction procedure and derivatization.

Gas chromatography–negative-ion chemical ionization mass spectrometry

The GC–EIMS method for the pyridine form compounds gave a good separation and sensitivity. However, the sensitivities of the dihydropyridine form compounds were poor and not suitable for the assay of these compounds after administration of compound I. Accordingly, the method for assay of compound I and its metabolites by GC–NICIMS was investigated.

In the NICI mass spectra of compound I and its metabolites using isobutane as a reactant gas, all compounds gave their respective molecular ions $[M]^-$. However, the peak intensities of I and I-keto-H_2 were too weak for them to be selected as monitoring ions. As shown in Fig. 2, it was found that the sensitivities of the $[M]^-$ and m/z 281 ions of I and I-keto-H_2 were influenced by the ion source temperature, and at 210 °C the m/z 281 ions of these compounds showed higher peak intensities than the $[M]^-$ ions at 150 °C. No apparent influences on the peak intensity of $[M]^-$ ions of their pyridine form compounds were observed when the ion-source temperature was increased from 150 to 210 °C. The ion source temperature was set at 201 °C for monitoring the derivatized *n*-hexane–diethyl ether extract containing I, I-keto-H_2, DH-I, DH-I-keto-H_2 and DH-NF-COOH-OH(L). The NF-COOH, DH-NF-COOH and DH-NF-COOH-OH metabolites were analysed at 150 °C, which is a usual ion source temperature for CI analysis.

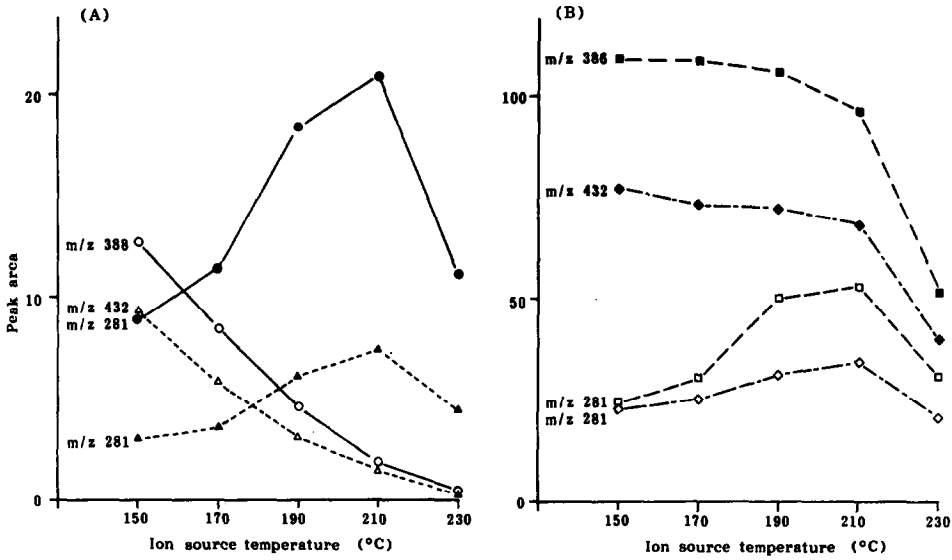

Fig. 2. Influence of ion source temperature on peak intensity of the detected fragment ions by GC–NICIMS for compound I and its metabolites. (● and ○) I; (▲ and △) I-keto-H_2; (■ and □) DH-I; (♦ and ◇) DH-I-keto-H_2.

The fragment ions of m/z 281 $[M-107]^-$ of I, m/z 281 $[M-135]^-$ of acetylated I-keto-H_2 and m/z 328 $[M-H_2O]^-$ of DH-NF-COOH-OH, and the molecular ions $[M]^-$ of other metabolites, m/z 386 of DH-I, m/z 430 of acetylated DH-I-keto-H_2, m/z 346 of methylated NF-COOH, m/z 344 of methylated DH-NF-COOH and m/z 328 of DH-NF-COOH-OH(L), were selected for multiple-ion detection by GC–NICIMS analysis, since no influences on biological constituents could be observed.

The NICI mass spectra of compound I and its metabolites measured with the above ion source temperatures using isobutane as a reactant gas are shown in Fig. 3.

The deuterium-labelled internal standards, $[^2H_3]$I, $[^2H_3]$DH-I and the methylated $[^2H_3]$NF-COOH and $[^2H_3]$DH-NF-COOH, were used for multiple-ion detection. The mass fragment ions detected for GC–NICIMS were the m/z 284 ion $[M-107]^-$ of $[^2H_3]$I, the m/z 389 ion $[M]^-$ of $[^2H_3]$DH-I, the m/z 349 ion $[M]^-$ of methylated $[^2H_3]$NF-COOH and the m/z 347 ion $[M]^-$ of methylated $[^2H_3]$DH-NF-COOH, respectively.

The acetylated I-keto-H_2 could also be monitored with the m/z 281 ion as well as compound I. None of the fragment ions detected had any appreciable influence on the biological constituents and gave an excellent separation on measurement by GC–NICIMS.

Very small amounts of I and I-keto-H_2 (each ca. 0.5%) and NF-COOH (ca. 2.0%) were cleaved to their respective pyridine form compounds on the injection port, column or ion source of the GC–MS apparatus. Therefore, the respective concentrations had to be corrected.

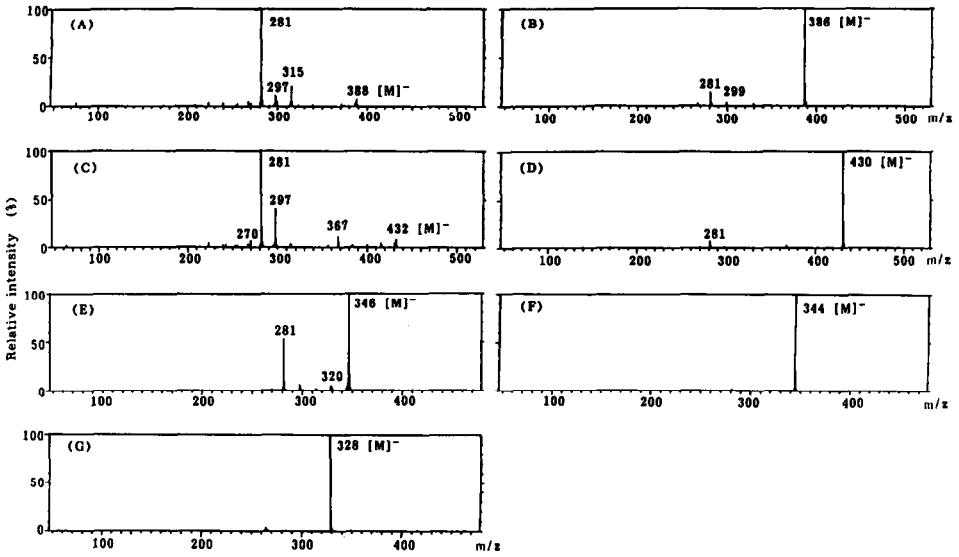

Fig. 3. NICI mass spectra of compound I and its metabolites: (A) I; (B) DH-I; (C) acetylated I-keto-H_2; (D) acetylated DH-I-keto-H_2; (E) methylated NF-COOH; (F) methylated DH-NF-COOH; (G) DH-NF-COOH(L).

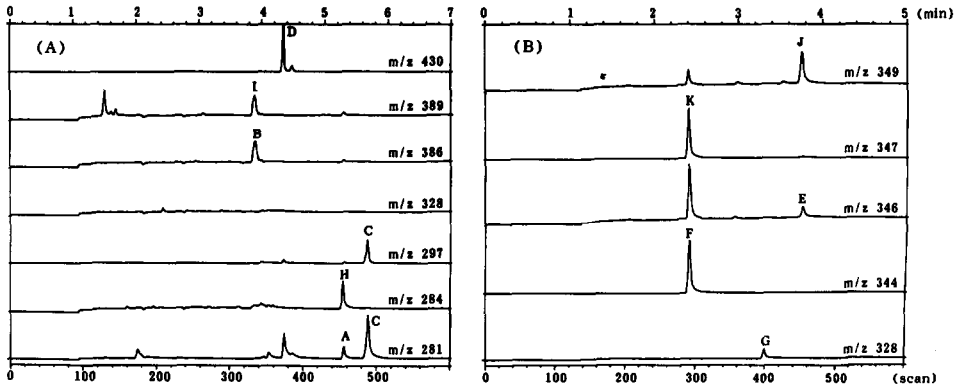

Fig. 4. Typical chromatograms by GC–NICIMS showing the separation of compound I, its metabolites and internal standards in human plasma prepared from (A) the *n*-hexane–diethyl ether extract and (B) the ethyl acetate extract. Peaks: A = I; B = DH-I; C = acetylated I-keto-H_2; D = acetylated DH-I-keto-H_2; E = methylated NF-COOH; F = methylated DH-NF-COOH; G = DH-NF-COOH-OH and DH-NF-COOH-OH(L); H = [$^2H_3$]I; I = [$^2H_3$]DH-I; J = methylated [$^2H_3$]NF-COOH; K = methylated [$^2H_3$]DH-NF-COOH.

Typical GC–NICIMS profiles of compound I, its metabolites and internal standards prepared from human plasma and urine following administration of I are illustrated in Figs. 4 and 5. The retention times of I, acetylated I-keto-H_2, DH-I, acetylated DH-I-keto-H_2 and DH-NF-COOH-OH(L) prepared from the organic layer were ca. 5.0–5.3, 5.4–5.7, 3.7–3.9, 4.1–4.3 and 3.3–3.5 min, and those of meth-

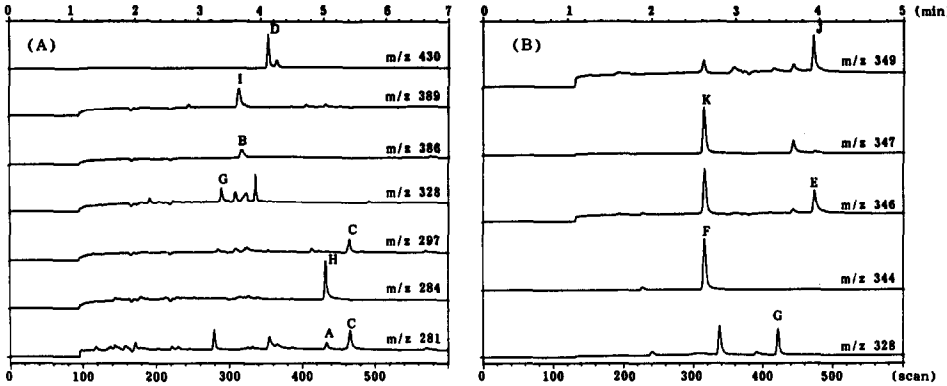

Fig. 5. Typical chromatograms by GC–NICIMS showing the separation of compound I, its metabolites and internal standards in human urine prepared from (A) the *n*-hexane–diethyl ether extract and (B) the ethyl acetate extract. For peak identification, see Fig. 4.

TABLE I

RECOVERIES OF COMPOUND I AND ITS METABOLITES FROM HUMAN PLASMA AND URINE

Metabolite	Added (ng/ml)	Recovery from plasma (%)	Added (ng/ml)	Recovery from urine (%)
I	1.0	98.3±3.1	10.0	98.9±3.2
	0.5	102.7±1.2	2.0	97.3±1.4
	0.1	100.0±0.0	0.5	91.3±8.3
	0.05	106.7±11.5	0.1	100.0±10.0
DH-I	1.0	98.7±5.1	10.0	97.3±4.7
	0.5	100.7±1.2	2.0	100.0±4.8
	0.1	100.0±0.0	0.5	95.3±3.1
	0.05	106.7±11.5	0.1	96.7±5.8
	0.02	100.0±0.0		
I-keto-H_2	1.0	100.7±0.6	10.0	97.4±0.6
	0.5	97.3±3.1	2.0	99.7±2.9
	0.1	93.3±5.8	0.5	93.3±5.0
	0.05	120.0±0.0	0.1	96.7±5.8
DH-I-keto-H_2	1.0	95.0±1.7	10.0	94.5±0.7
	0.5	104.0±7.2	2.0	97.8±0.8
	0.1	100.0±0.0	0.5	93.3±4.2
	0.05	106.7±11.5	0.1	103.3±5.8
	0.02	100.0±0.09		
NF-COOH	1.0	103.7±2.1	100	98.4±1.1
	0.5	94.0±3.5	20	97.1±6.4
	0.1	96.7±5.8	5	98.5±3.7
	0.05	86.7±30.6	1	113.0±13.2
DH-NF-COOH	1.0	96.7±4.5	1000	95.4±1.9
	0.5	96.0±5.3	200	100.5±1.6
	0.1	110.0±10.0	50	97.6±3.2
	0.05	106.7±11.5	10	103.7±6.6
DH-NF-COOH-OH	100.0	83.6±1.3	1000	99.3±6.7
and	25.0	112.2±5.5	200	101.2±6.5
DH-NF-COOH-OH(L)	5.0	88.5±3.9	50	92.4±8.4
	1.0	95.0±10.3	10	85.1±1.8

ylated NF-COOH, methylated DH-NF-COOH and DH-NF-COOH-OH prepared from the aqueous layer were ca. 3.8–3.9, 2.4–2.6 and 3.3–3.5 min, respectively.

Recovery, sensitivity and accuracy

Known amounts of compound I and its metabolites were added to control samples of human plasma and urine at concentrations of 0.02, 0.05, 0.1, 0.5, 1.0, 5.0, 25.0 and 100 ng/ml for plasma and 0.1, 0.5, 2.0, 10, 50, 200 and 1000 ng/ml for urine, and the samples were analysed. As summarized in Table I, satisfactory recoveries of each compound prepared from plasma and urine were obtained.

The detection limits for I and its metabolites using the present method were 0.02–0.05 ng/ml in plasma and urine for each compound. The reproducibility of this method was ±2.8–4.3% at a concentration of 0.02–1000 ng/ml for each metabolite.

Stability

Compound I and its metabolites were found to be stable in the residue of the extract at −20°C for up to seven to ten days following extraction.

The stability of compound I in human plasma and urine at −20°C and 5°C and room temperature under a yellow fluorescent lamp was investigated. As shown

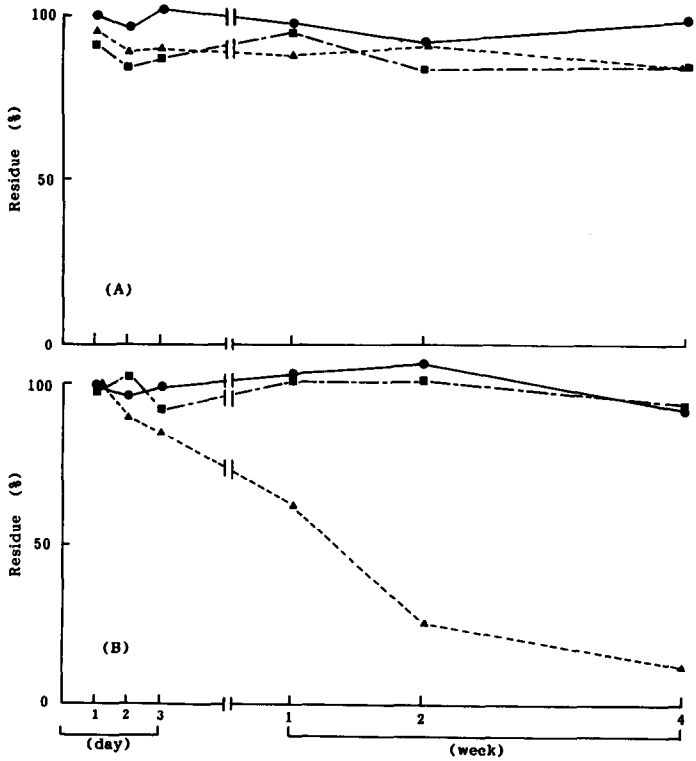

Fig. 6. Stability of compound I in (A) dog plasma and (B) dog urine at −20°C (●), 5°C (■) and room temperature (▲).

in Fig. 6, no significant degradation of I was observed over a four-week period or longer in all conditions, except in urine at room temperature.

Application

The time course of changes in the concentrations of compound I and its metabolites after oral administration of I was measured by the described method. Fig. 7 shows the mean ± S.D. plasma concentrations of I and its metabolites obtained from five healthy volunteers. The terminal half-lives of I and the active metabolite I-keto-H_2 in this experiment were ca. 1.1 and 3.8 h, respectively. The detailed results for clinical pharmacological studies on compound I using the present GC–NICIMS method will be reported elsewhere.

The present GC–NICIMS method was also applied to animal plasma and urine. The results obtained for the chromatographic separation, recoveries, sensitivity and precision were in good agreement with those obtained with human plasma and urine. Furthermore, this method can be applied for the determination of nifedipine (NF) and its metabolites, dehydro-NF (DH-NF), NF-COOH, DH-NF-COOH, DH-NF-COOH-OH(L) and DH-NF-COOH-OH, except that the *n*-hexane–diethyl ether extract containing NF, DH-NF and DH-NF-COOH-OH(L)

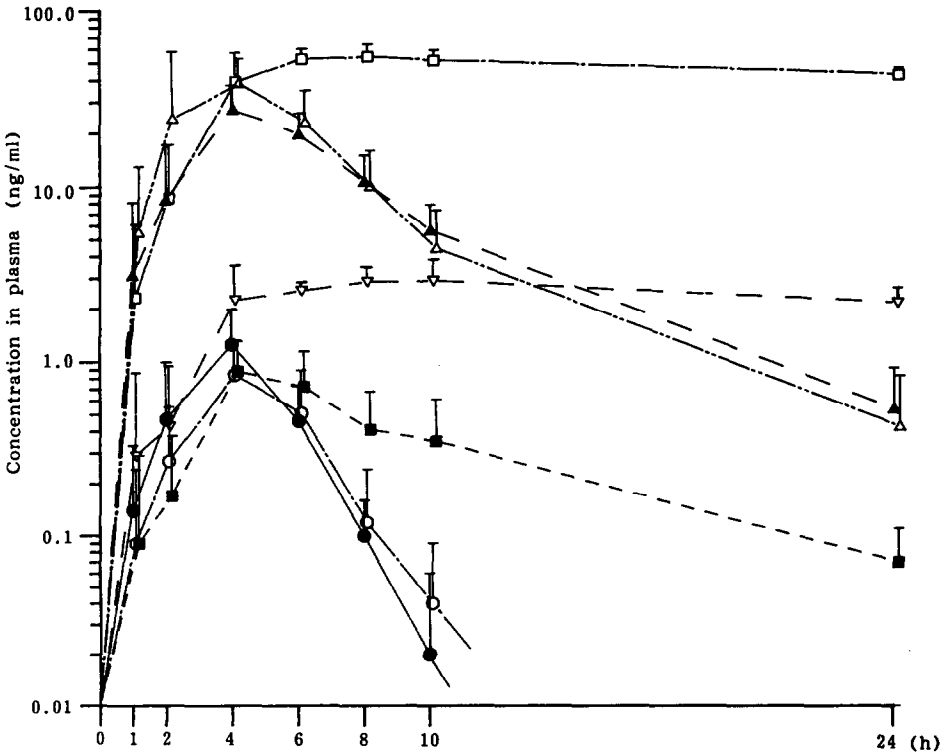

Fig. 7. Plasma levels of compound I and its metabolites after oral administration of I at a dose of 5 mg to healthy volunteers. Each point is the mean ± S.D. of five men: (●)I; (○) DH-I; (▲) I-keto-H_2; (△) DH-I-keto-H_2; (■) NF-COOH; (□) DH-NF-COOH; (▽) DH-NF-COOH-OL(L) and DH-NF-COOH-OH.

was not subjected to acetylation and the ethyl acetate extract containing NF-COOH, DH-NF-COOH and DH-NF-COOH-OH was ethylated at room temperature by addition of diethyl ether containing saturated diazoethane. The recoveries of NF and DH-NF were as follows: NF, 98.6 ± 2.0% from human plasma and 97.6 ± 1.7% from human urine; DH-NF, 98.3 ± 2.9% from human plasma and 98.3 ± 1.7% from human urine.

The specification, precision and sensitivity of this assay appear to be satisfactory for basic and clinical pharmacological investigations on compound I.

ACKNOWLEDGEMENTS

The authors thank the members of the Synthesis Department of Maruko Pharm. Co. for preparing authentic samples of compound I, its metabolites and deuterium-labelled internal standards.

REFERENCES

1 S. Ohno, K. Mizukoshi, O. Komatsu, K. Ichihara and T. Morishima, Jpn. Pat., 58-67668 (1983).
2 P. Jakobsen, O.L. Pedersen and E. Mikkelsen, J. Chromatogr., 162 (1979) 81.
3 S. Kondo, A. Kuchiki, K. Yamamoto, K. Akimoto, K. Takahashi, N. Awata and I. Sugimoto, Chem. Pharm. Bull., 28 (1980) 1.
4 S.R. Hamann and R.G. McAllister, Jr., Clin. Chem., 29 (1983) 158.
5 J. Dokladalova, J.A. Tykal, S.J. Coco, P.E. Durkee, G.T. Quercia and J.J. Korst, J. Chromatogr., 231 (1982) 451.
6 B.J. Schmid, H.E. Perry and J.R. Idle, J. Chromatogr., 425 (1988) 107.
7 M.T. Rosseel and M.G. Bogaert, J. Chromatogr., 279 (1983) 675.
8 N. Kurosawa, S. Morishima, E. Owada, K. Ito, K. Ueda, A. Takahashi and T. Kikuiri, Yakugaku Zasshi, 104 (1984) 775.
9 T. Sadanaga, K. Hikida, K. Tameto, Y. Matsushima and Y. Ohkura, Chem. Pharm. Bull., 30 (1982) 3807.
10 P.R. Bach and the Clinical Investigation of Duchenne Dystrophy Group, Clin. Chem., 29 (1983) 1344.
11 R.A. Janis, G.J. Krol, A.J. Noe and M. Pan, J. Clin. Pharmacol., 23 (1983) 266.
12 S. Kobayashi, J. Chromatogr., 420 (1987) 439.
13 K.D. Raemsch and J. Sommer, Supp. II Hypertension, 5 (1983) II-18.
14 J. Kann, G.J. Krol, K.D. Raemsch, D.E. Burkholder and M.J. Levitt, J. Cardiovascular Pharmacol., 6 (1984) S968.
15 G.J. Krol, A.J. Noe, S.C. Yeh and K.D. Raemsch, J. Chromatogr., 305 (1984) 105.
16 S. Higuchi and Y. Shiobara, Biomed. Mass Spectrom., 5 (1978) 220.
17 S. Higuchi and S. Kawamura, J. Chromatogr., 223 (1981) 341.
18 O. Beck and T. Ryman, J. Chromatogr., 337 (1985) 402.
19 Y. Tokuma, T. Fujiwara and H. Noguchi, Mass Spectrosc. (Japan), 33 (1985) 211.