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Determination of Enalapril and its Active Metabolite Enalaprilat in Plasma and Urine by Gas Chromatography/Mass Spectrometry

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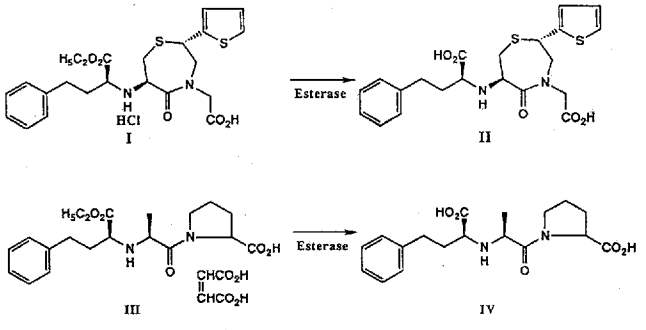
The method for the simultaneous determination of angiotensin-converting enzyme (ACE) inhibitor enalapril and its active metabolite enalaprilat in plasma and urine was developed by gas chromatography/mass spectrometry. Enalapril and enalaprilat in plasma and urine were extracted and cleaned up by using Sep-Pak C18 and silica cartridges. Derivatization was carried out using diazomethane and trifluoroacetic anhydride. Detection by selected ion monitoring was selected to *m/z* 288 (enalaprilat) and 302 (enalapril). The detection limit of enalapril and enalaprilat was 200 pg/mL in plasma and 2 ng/mL in urine. This method was applied to the pharmacokinetic analysis of enalapril and enalaprilat in body fluids.

INTRODUCTION

Inhibitors of angiotensin-converting enzyme (ACE; E.C. 3.4.15.1), such as enalapril (Patchett *et al.*, 1980), ramipril (Witte et al., 1983) etc. have offered a new approach to the treatment of hypertension and congestive heart failure.

It is very important to determine the concentration of ACE inhibitors in plasma and urine with respect to pharmacokinetics and pharmacodynamics. The analytical methods in use for the measurement of ACE inhibitors have been inhibitor binding assay (IBA) (Tocco *et al.*, 1982), radioimmunoassay (RIA) (Hikens *et al.*, 1981), gas chromatography (GC) (Bathala *et al.*, 1984) and gas chromatography/mass spectrometry (GC/MS) (Cohen *et al.*, 1982). It is not impossible for IBA and RIA to determine the prodrug and its active metabolite simultaneously. GC and GC/MS are not sufficiently sensitive to determine the biological fluids.

In the previous paper (Shioya *et al.*, 1989), we reported a GC/MS method using negative ion chemical ionization (NICI) for the determination of an ACE inhibitor (I, CS-622) and its active metabolite (II, RS-5139) in plasma and urine (Scheme 1). We applied this method to the determination of enalapril (III) and its active metabolite enalaprilat (IV, Scheme 1) to compare the bioavailability between I and III in man.



Scheme 1

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Enalapril and enalaprilat were converted to methyl ester and trifluoroacetyl derivatives and determined by GC/MS using electron capture/NICI. Detection by selected ion monitoring (SIM) was selected to *m/z* 288 (enalaprilat) and 302 (enalapril), which were the fragment ions $[M-184]^-$ due to the *N*-trifluoroacetyl(1-ethoxy(or methoxy)carbonyl-3-phenylpropyl)amine moiety. RS-5139 was used as an internal standard.

EXPERIMENTAL

Reagents. Enalapril maleate and enalaprilat were synthesized in the Chemical Research Laboratories of Sankyo Co. Ltd. All reagents were of analytical grade. Sep-Pak C18 and silica cartridges were products of Waters (Milford, MA, USA). Trifluoroacetic anhydride (TFAA) was purchased from Tokyo Kasei (Tokyo, Japan) and used without further purification. *N*-Methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald), for diazomethane generation, was supplied by Aldrich (Milwaukee, USA).

Gas chromatography/mass spectrometry (GC/MS). A Hewlett-Packard 5988A GC/MS system equipped with a Hewlett-Packard 5890 gas chromatograph was used. The gas chromatograph was equipped with a fused silica capillary column (DB-1, 10 m × 0.25 mm i.d., 0.25 μm particle size; J & W, Rancho Cordova, CA, USA), which was directly inserted into the ion source. Helium was used as a carrier gas at a head pressure of 70 kPa. The gas chromatograph was operated in the splitless mode. The injector temperature was maintained at 280°C. The oven temperature was increased from 150°C (1 min) to 280°C at 30°C/min. The mass spectrometer was operated in the NICI mode, with an electron energy of 240 eV. Methane was used as the reagent gas.

Plasma and urine samples. All blood samples were drawn from a peripheral vein into heparinized tubes. The plasma was collected after centrifugation of the samples and stored at −20°C until analysis. Urine samples were stored at −20°C until analysis.

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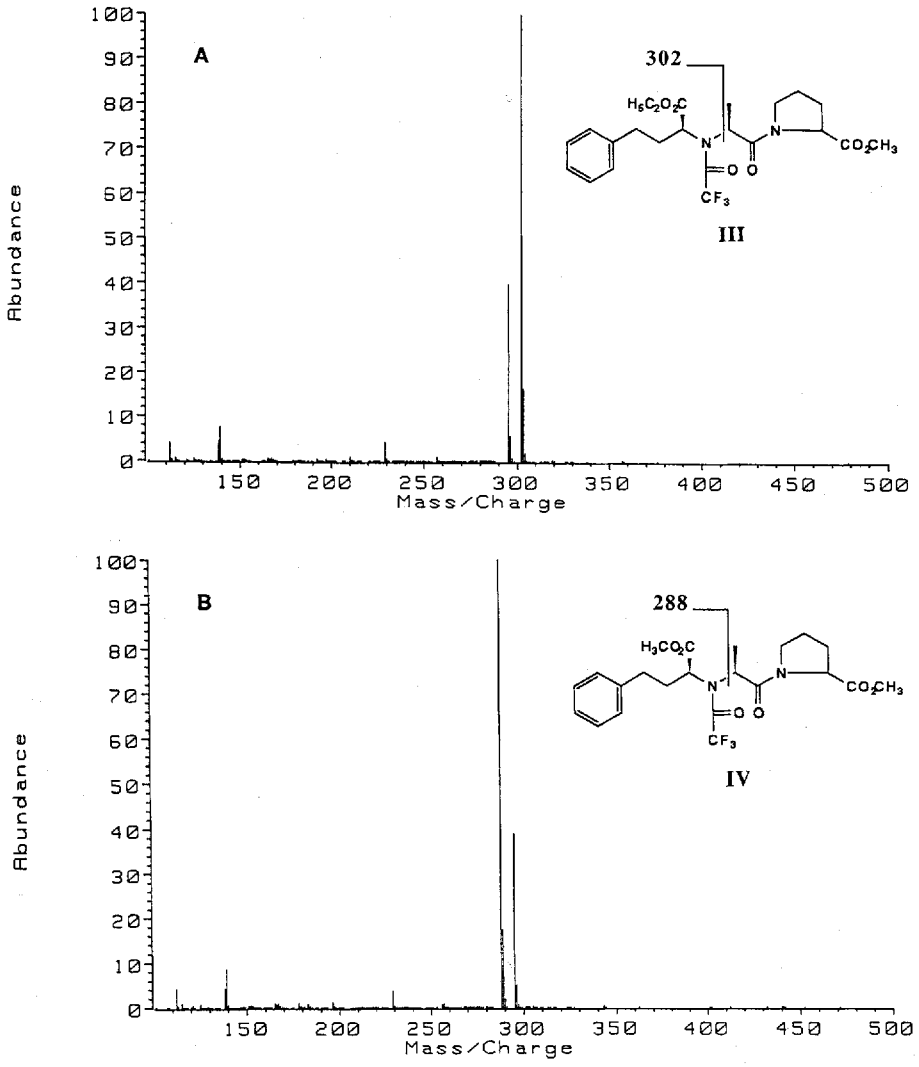

Figure 1. Mass spectra of derivatives of III(A) and IV(B).

Sample preparation. Sample preparation was carried out by the method previously reported (Shioya *et al.*, 1989). The internal standard (II, 100 ng), dissolved in 100 μL of phosphate buffer (pH 7.4), was added to 1 mL of plasma or urine in a centrifuge tube and 5 mL 0.2 M hydrochloric acid was added. The sample was applied to a Sep-Pak C18 cartridge, which had been preconditioned with methanol (5 mL) followed by redistilled water (5 mL). Subsequently the column was washed with 5 mL 0.02 M hydrochloric acid and 6 mL petroleum ether (boiling range 30–70°C). The sample was eluted with 6 mL methanol. The eluant was evaporated by nitrogen gas and methylated by adding diazomethane solution at room temperature for 15 min. It was evaporated to dryness at 40°C under nitrogen gas. The residue was taken up

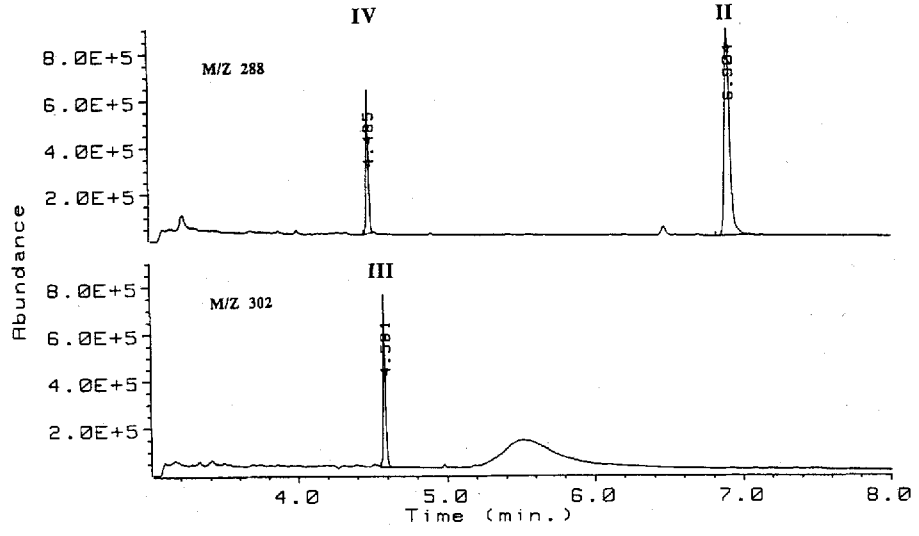

Figure 2. SIM chromatograms of the extract from a 1 mL plasma sample, spiked with 50 ng of III (*m/z* 302), IV (*m/z* 288) and 100 ng of II (*m/z* 288).

Table 1. Accuracy and precision of the proposed method for the determination of III and IV in human plasma and urine

Compound	Concentration added (ng/mL)	Concentration found (mean ± S.D.) (ng/mL)	Coefficient of variation (%)
Plasma			
III	6.25	6.50 ± 0.4	6.8
	25	24.3 ± 1.0	4.3
	100	100.2 ± 8.1	8.1
IV	6.25	6.35 ± 0.48	7.6
	25	24.9 ± 1.6	6.4
	100	98.8 ± 9.1	9.2
Urine			
III	62.5	61.2 ± 3.5	5.7
	250	252.8 ± 13.8	5.4
	1000	998.9 ± 12.0	1.2
IV	62.5	58.5 ± 3.8	6.5
	250	247.9 ± 2.4	1.0
	1000	1001.0 ± 4.0	0.4

in 100 μL methanol, followed by the addition of 1 mL hexane: toluene (1:1). The sample was applied to a Sep-Pak silica cartridge, which had been preconditioned with 5 mL methanol followd by 5 mL chloroform and 5 mL hexane. The sample was eluted with 6 mL chloroform. The eluant was evaporated to dryness at 60°C under nitrogen gas. The residue was taken up in 250 μL ethyl acetate, treated with 5% TFAA in hexane (1 mL) and heated at 60°C for 15 min. Subsequently the sample was evaporated to dryness at 60°C under nitrogen and taken up in 200 μL ethyl acetate, 1 μL of which was injected into the column.

RESULTS AND DISCUSSION

GC/MS determination

The mass spectra of the derivatives of III and IV are shown in Fig. 1. The negative ion mass spectra have base peaks at 302 and 288 $[M-184]^-$, which are the fragment ions due to the *N*-trifluoroacetyl-(1-ethoxy (or methoxy)carbonyl-3-phenylpropyl)amino moiety). The

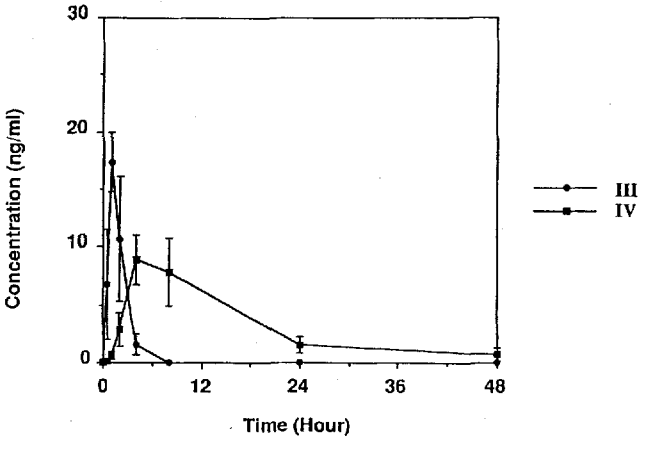

Figure 3. Plasma concentration of III (●) and IV (■) after an oral administration of III (5 mg) to five patients. The mean ± S.D. data are plotted.

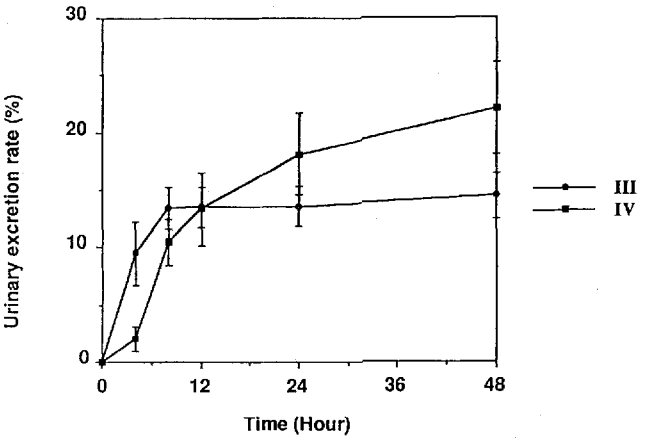

Figure 4. Cumulative urinary excretion of III (●) and IV (■) after an oral administration of III (5 mg) to five patients. The mean ± S.D. data are plotted.

$[M-184]^-$ ion was chosen for monitoring in the SIM mode.

The SIM chromatograms of spiked plasma are shown in Fig. 2; no interfering peaks were observed. The retention times of II, III, and IV were 6.90, 4.58 and 4.49 min, respectively.

The standard curve of III and IV showed good linearity (III: $y = 1.04 \cdot 10^{-2}x + 0.6.10^{-2}$, $r^2 = 0.999$; IV: $y = 1.10 \cdot 10^{-2}x + 0.1 \cdot 10^{-1}$, $r^2 = 0.999$) over the concentration of 1.56–100 ng/mL for a 1 mL plama volume. The detection limit was 200 pg/mL in plasma and 2 ng/mL in urine at a signal-to-noise ratio of 3.

Reproducibility of the method

Accuracy and precision were examined by assaying known concentrations of III and IV in plasma and urine. It is evident from Table 1 that the proposed method is satisfactory in both accuracy and precision. This GC/MS method was used for the quantification of enalapril and enalaprilat in biological fluids with good sensitivity, selectivity and reproducibility.

Quantification of enalapril and enalaprilat in normal human plasma and urine

The method was applied to the determination of III and IV in plasma and urine of healthy volunteers ($N = 5$) orally given 5 mg of III. The plasma concentration of III and IV is shown in Fig. 3. It is clear that III is absorbed and converted to the active metabolite IV, which has a long half-life in plasma. The cumulative

Table 2. Pharmacokinetic parameters of III and IV. The mean ± S.D. data are shown.

Compound	AUC (0–48) (ng · h/mL)	t1/2 (h)	Tmax (h)	Cmax (ng/mL)	Urinary excretion ratio (0–48) (% of dose)
III	37.1 ± 8.5	N.C.[a]	1.1 ± 0.2	22.2 ± 2.4	14.5 ± 2.0
IV	152.1 ± 54.4	15.9 ± 3.0	4.8 ± 0.8	10.0 ± 2.3	22.1 ± 4.0

[a] N.C. = not calculated.

urinary excretion of III and IV is shown in Fig. 4. The Pharmacokinetic parameters of III and IV are shown in Table 2. It is possible to calculate the pharmacokinetic parameters of III and IV accurately and simultaneously by using the GC/MS method.

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