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DETERMINATION OF A NEW ANGIOTENSIN-CONVERTING ENZYME INHIBITOR (CS-622) AND ITS ACTIVE METABOLITE IN PLASMA AND URINE BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY USING NEGATIVE ION CHEMICAL IONIZATION

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SUMMARY

A sensitive and specific gas chromatographic–mass spectrometric method for the simultaneous determination of angiotensin-converting enzyme inhibitor (I, CS-622) and its active desethyl metabolite (II, RS-5139) in plasma and urine was developed Compound D5-RS-5139 was used as an internal standard and measurements were made by electron-capture negative ion chemical ionization Extraction from plasma and urine was carried out using Sep-Pak C_{18} and silica cartridges The extract of plasma or urine was treated with diazomethane followed by trifluoroacetic anhydride to convert I and II into their methyl ester trifluoroacetyl derivatives The detection limit of I and II was 0 5 ng/ml in plasma and 5 ng/ml in urine The proposed method was satisfactory for the determination of I and II in plasma and urine with respect to accuracy and precision Thus it is suitable for measurement of bioavailability and pharmacokinetics of I and II in body fluids

INTRODUCTION

In recent years, inhibitors of angiotensin-converting enzyme (ACE; EC 3.4 15.1) have offered a new approach to the treatment of hypertension and congestive heart failure. α-{(2*S*,6*R*)-6-[(1*S*)-1-Ethoxycarbonyl-3-phenylpropyl]amino-5-oxo-2-(2-thienyl)perhydro-1,4-thiazepin-4-yl} acetic acid hydrochloride (I, CS-622) is a new ACE inhibitor, and it is a prodrug that is deesterified in vivo by esterase to release an active metabolite RS-5139 (II, Fig 1). Compound I has a very high potency, so it is therapeutically active at

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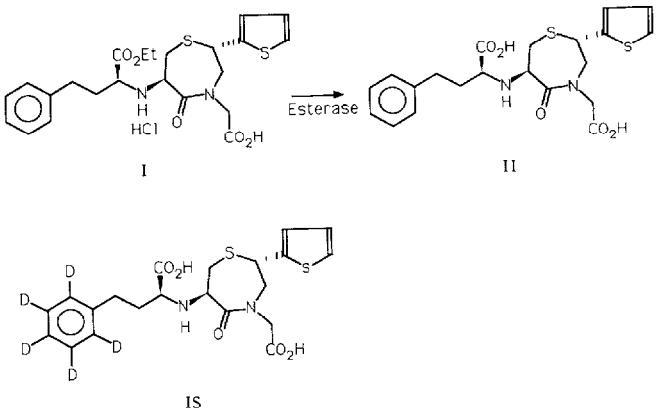

Fig 1 Structures of I, II and the internal standard (IS)

low concentration in plasma. Thus it is very important to determine its concentration in plasma and urine in order to study the pharmacokinetics and pharmacodynamics

Analytical methods in use at present for the measurement of I and II are inhibitor binding assay (IBA) [1], radioimmunoassay [2] and liquid or gas chromatography [3,4]. IBA and radioimmunoassay are very sensitive, but it is difficult to distinguish the prodrug and its active metabolite at the same time. Column liquid and gas chromatography are specific but less sensitive than other methods.

This paper describes a gas chromatographic–mass spectrometric (GC–MS) method that uses negative ion chemical ionization (NICI). The fused-silica capillary GC column and the selected-ion monitoring (SIM) technique make the method very specific and highly sensitive

EXPERIMENTAL

Materials and reagents

Compounds I and II and the internal standard (IS, Fig. 1) were synthesized by Yanagisawa et al. [5]. All reagents were of analytical grade Sep-Pak C_{18} and silica cartridges were products of Waters Assoc. (Milford, MA, U.S A.) Trifluoroacetic anhydride (TFAA) was purchased from Tokyo Kasei (Tokyo, Japan) and used without further purification. N-Methyl-N-nitroso-*p*-toluenesulphonamide (Diazald), for diazomethane generation, was supplied by Aldrich (Milwaukee, WI, U.S.A.)

Gas chromatography–mass spectrometry

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, U.S.A), 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 250°C. The oven temperature was increased from 200°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 on centrifugation of the samples and stored at −20°C until analysis.

Urine samples were stored at −20°C until analysis

Sample preparation

Sample preparation was done by the modified method of Hajdu et al. [6]. The internal standard (200 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 of 0.2 *M* hydrochloric acid was added. The sample was applied to a Sep-Pak C_{18} cartridge, which had been preconditioned with methanol (5 ml) followed by redistilled water (5 ml). Subsequently, the column was washed with 5 ml of 0.02 *M* hydrochloric acid and 6 ml of petroleum ether (boiling range 30–70°C). The sample was eluted with 6 ml of methanol The elute 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. The residue was taken up in 100 μl of methanol, followed by the addition of 1 ml of hexane–toluene (1 1). The sample was applied to a Sep-Pak silica cartridge, which had been preconditioned with 5 ml of methanol followed by 5 ml of chloroform and 5 ml of hexane. The sample was eluted with 6 ml of chloroform. The elute was evaporated to dryness at 60°C under nitrogen. The residue was taken up in 250 μl of 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 of ethyl acetate, 2 μl of which were injected into the column.

RESULTS AND DISCUSSION

The mass spectra of the derivatives of I, II and IS are shown in Fig. 2. The negative ion mass spectra have base peaks at 288, 293 and 302 $[M-284]^-$, which are the fragment ions due to the N-trifluoroacetyl-(1-ethoxy(or methoxy)carbonyl-3-phenylpropyl)amine moiety and are used as SIM ions. These fragment ions are common to the derivatives of non-sulphydryl-containing

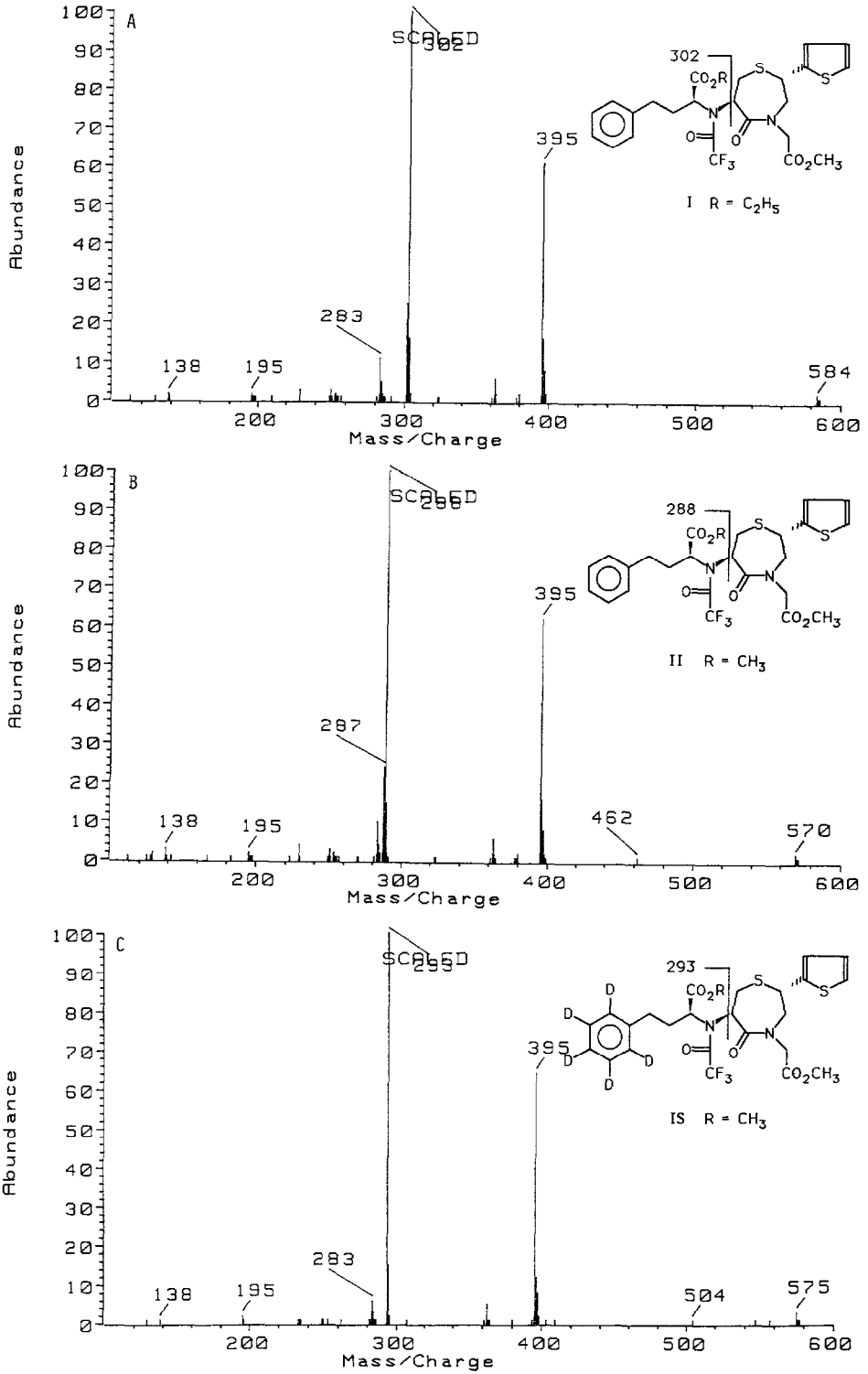

Fig 2 Mass spectra of derivatives of I (A), II (B) and IS (C)

ACE inhibitors, such as enalapril and ramiprıl. So this method ıs applicable to the measurement of the other non-sulphydryl-containing ACE inhibitors in the body fluids.

The SIM chromatograms of spiked plasma are shown in Fig. 3 and no interferıng peaks were observed The retention times of I, II and IS were 5.2, 5.06 and 5.04 min, respectively.

The standard curves of I and II showed good linearity (I:

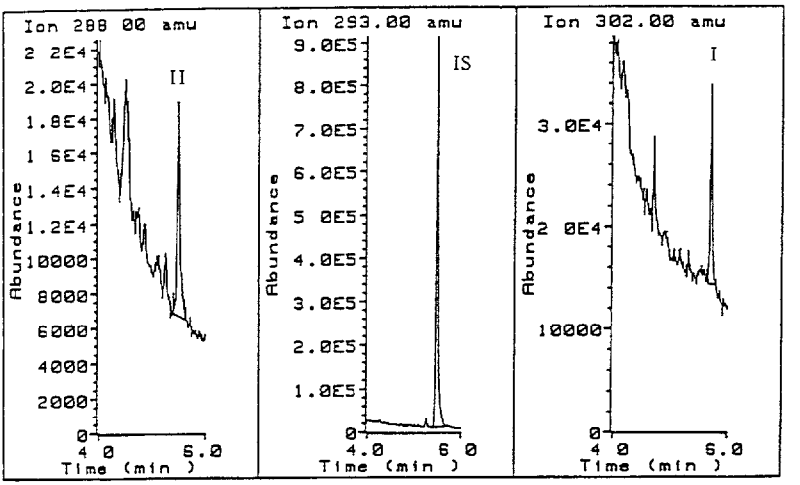

Fıg 3 SIM chromatograms of the extract from a 1-ml plasma sample, spıked wıth 1 56 ng of I (m/z 302) and II (m/z 288), and 200 ng of IS (m/z 293)

TABLE I

ACCURACY AND PRECISION OF THE PROPOSED METHOD FOR THE DETERMINATION OF I AND II IN HUMAN PLASMA AND URINE

Compound	Concentratıon added (ng/ml)	Concentratıon found (mean ± S D) (ng/ml)	Coefficıent of varıatıon (%)
Plasma			
I	5	6 5±0 2	4 9
	15	16 3±0 7	4 6
II	20	19 3±0 7	3 8
	60	58 6±1 3	2 5
Urıne			
I	50	50 6±2 2	3 0
	150	151 5±4 2	2 8
II	200	200 1±3 8	1 9
	600	578 8±7 6	1 3

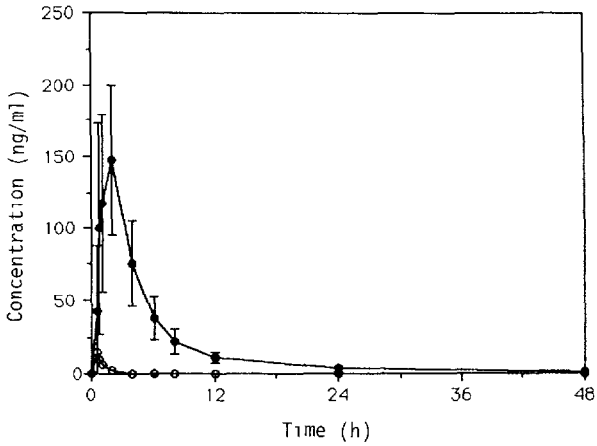

Fig 4 Plasma concentrations (mean ± S D $n=6$) of I (○) and II (●) after oral administration of 5 mg of I

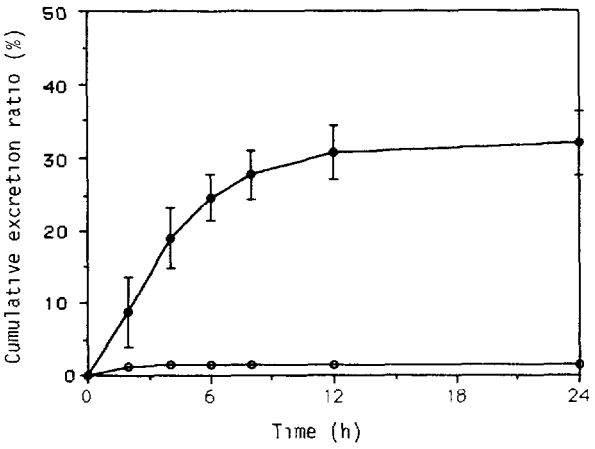

Fig 5 Cumulative urinary excretion ratios (mean ± S D , $n=6$) of I (○) and II (●) after oral administration of 5 mg of I

$y=7.6\cdot10^{-3}x+1.0\cdot10^{-2}, r^2=0.999$, II $y=5.2\cdot10^{-3}x+0.8\cdot10^{-2}, r^2=0.999$) over the concentration range 1.56–100 ng/ml for a 1-ml sample volume. It is evident from the data in Table I that the proposed method is satisfactory in both accuracy and precision. The detection limit is 0.5 ng/ml in plasma and 5 ng/ml in urine at a signal-to-noise ratio of 3.

The method was applied to the determination of I and II in the plasma and urine of healthy volunteers orally dosed with 5 mg of I. Plasma concentrations of I and II are shown in Fig. 4. The cumulative urinary excretions of I and II are shown in Fig. 5. It is apparent that I is rapidly absorbed and metabolized to the pharmacologically active II.

In conclusion, this GC–MS method can be applied to the determination of I and II in plasma and urine at the same time and is suitable for studies of the pharmacokinetics and pharmacodynamics of I and II

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