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DETERMINATION OF CAPTOPRIL IN BIOLOGICAL FLUIDS BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

The quantitation of captopril in biological fluids by gas–liquid chromatography using a flame photometric detector is described. Captopril was converted into an adduct with N-ethylmaleimide and then into the hexafluoroisopropyl ester. The latter derivative was separated on a 2% OV-210 column and determined by employing the captopril–N-hexylmaleimide adduct as an internal standard. The blood level and urinary excretion of captopril administered intravenously to dogs were measured by the proposed method.

INTRODUCTION

Captopril [1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline] (SQ-14,225) is a potent, specific and orally active inhibitor of angiotensin-converting enzyme, one of the most important components in the renin–angiotensin system[1–4]. Recently, both *in vitro* and *in vivo* experiments have revealed that captopril is readily converted into the disulphide and unknown sulphur-containing compounds[5]. On the other hand, captopril is a major urinary metabolite in mice[6] and humans[7] when it or its disulphide is administered. The method so far available for the quantitation of captopril in biological fluids involves the measurement of radioactivity on a thin-layer chromatogram[5]. This procedure, however, is not necessarily satisfactory with respect to feasibility and reliability. An urgent need to clarify the metabolic fate of captopril administered intravenously to dogs prompted us to develop a new method for the determination of captopril. This paper describes a gas–liquid chromatographic (GLC) method for the determination of captopril in blood and urine using a flame photometric detector.

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EXPERIMENTAL

Gas–liquid chromatography

A Shimadzu Model 4BM gas chromatograph equipped with a Model FPD 1A flame photometric detector and a coiled glass column (1 m × 3 mm I.D.) was used. The column was packed with 2% OV-210 on Gas-Chrom Q (80–100 mesh). The detector and flash heater were maintained at 250° and the column at 215°. The flow-rate of carrier gas (nitrogen) was 50 ml/min.

Gas chromatography–mass spectrometry

A Shimadzu Model LKB-9000B gas chromatograph–mass spectrometer was used. A coiled glass column (1 m × 2 mm I.D.) was packed with 2% OV-210 on Gas-Chrom Q (80–100 mesh). The flow-rate of carrier gas (helium) was 30 ml/min. The temperature of the column was 195° and those of the injection port and ion source were 270°. The accelerating voltage, ionization voltage and trap current were 3.5 kV, 70 eV and 60 μA, respectively.

Materials

Captopril was supplied by Sankyo (Tokyo, Japan). Hexafluoroisopropanol, trifluoroacetic anhydride, N-ethylmaleimide (NEM) and other chemicals were of analytical-reagent grade.

Synthesis of captopril–N-hexylmaleimide adduct

A solution of maleic anydride (11.8 g) in chloroform (120 ml) was added drop-wise to a stirred solution of *n*-hexylamine (10.1 g) in chloroform (10 ml) at 0° and the resulting solution was evaporated to dryness. A solution of the residue in acetic anhydride (50 ml) was heated with anhydrous sodium acetate (3.3 g) at 100° for 1 h and then at 160° for 10 h. After removal of the precipitate by filtration, the filtrate was poured into ice–water. The solution was stirred to decompose the excess of reagent, adjusted to pH 9 with 5% sodium hydroxide solution and extracted with ethyl acetate (100 ml). After evaporation of the solvent, the oily residue was chromatographed twice on silica gel. The eluate obtained with *n*-hexane was subjected to preparative thin-layer chromatography (TLC) using chloroform–benzene (9:1) as the developing solvent. Elution of the area corresponding to the spot gave N-hexyl-maleimide (NHM) (1.5 g) as a colourless oil. Mass spectrum, *m/e* 181 $[M]^+$. The product was homogeneous as judged by TLC.

The captopril–NHM adduct was prepared by the Michael addition reaction from captopril and NHM in the usual manner. The desired compound was not obtained in the crystalline state but was substantially homogeneous as judged by TLC. Mass spectrum, *m/e* 399 $[M]^+$; TLC with chloroform–methanol–acetic acid (30:8:0.5), $R_F = 0.54$.

Preparation of hexafluoroisopropyl ester derivative

Hexafluoroisopropanol (0.3 ml) and trifluoroacetic anhydride (0.05 ml) were added to a sample containing captopril–NEM (85–4500 ng) and a known amount of captopril–NHM, and the resulting solution was allowed to stand at 50° for 60 min. After removal of the excess of reagent by means of a stream of nitrogen, the residue

was dissolved in ethyl acetate (0.2–0.5 ml) and a 5-μl volume of this solution was injected into the gas chromatograph.

Assay of captopril in blood and urine

A 1-ml volume of blood was added to a 0.2% solution of NEM in 0.1 *M* phosphate buffer and treated twice with 5-ml volumes of 10% metaphosphoric acid. After removal of the precipitate by centrifugation at 1600 *g* for 10 min, the supernatant was extracted three times with 10-ml volumes of ethyl acetate. The organic layers were combined and evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml of acetate buffer (pH 6.0) and extracted twice with 5-ml volumes of ethyl acetate. The aqueous layer was acidified with 0.1 *N* hydrochloric acid (5 ml) and extracted three times with 5-ml volumes of ethyl acetate. The organic layers were combined and evaporated to dryness *in vacuo*. After addition of an internal standard to the residue, the mixture was subjected to derivatization with hexafluoroisopropanol and trifluoroacetic anhydride, followed by gas chromatography.

With urine, 2 ml of the specimen was mixed with a 0.2% solution of NEM in 0.1 *M* phosphate buffer and the solution was processed in the manner described above without the deproteinization step.

Stability test for captopril–NEM in blood and urine

The test sample was prepared by dissolving *ca*. 6.2 μg of captopril in canine blood (1 ml) or urine (2 ml) diluted with a 0.2% solution of NEM in 0.1 *M* phosphate buffer (1 ml), and the solution was allowed to stand in a refrigerator for 5 days. The determination of captopril–NEM was then carried out according to the above procedure.

Recovery test for captopril added to blood and urine

The test sample was prepared by dissolving *ca*. 8.5 μg of captopril in canine blood (1 ml) or urine (2 ml) diluted with a 0.2% solution of NEM in 0.1 *M* phosphate buffer. The determination of captopril was then carried out according to the above procedure.

Determination of captopril in canine blood and urine

Female CSK beagle dogs (body weight 10–12.5 kg) were administered a single intravenous dose of 1 mg/kg of captopril in 0.2 *M* phosphate buffer (pH 7.4). Blood was withdrawn from a superficial vein and urine specimens were collected through a catheter inserted in the bladder. Captopril in these samples was determined by the above procedure described above.

RESULTS AND DISCUSSION

The facile redox reaction between captopril and its disulphide in biological fluids has been reported previously[5]. Our initial effort was therefore directed towards the rapid protection of the sulphydryl group of captopril to prevent its oxidation to the disulphide. Treatment of captopril (I, Fig. 1) with NEM in phosphate buffer readily provided the adduct, whose structure was unequivovally characterized by inspection of the mass and nuclear magnetic resonance spectra.

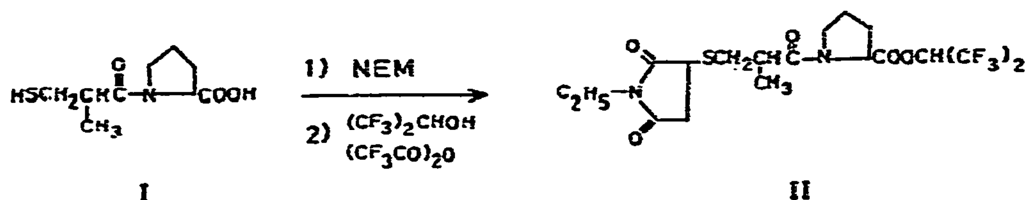

Fig. 1. Derivatization of captopril (I) into the N-ethylmaleimide adduct hexafluoroisopropyl ester (II).

Several attempts to silylate captopril–NEM with N,O-bis(trimethylsilyl)-acetamide or N,O-bis(trimethylsilyl)trifluoroacetamide failed owing to its poor reactivity. Accordingly, derivatization of the proline moiety into the volatile ester was undertaken. When treated with hexafluoroisopropanol in the presence of trifluoroacetic anhydride as a catalyst[8,9], captopril–NEM was converted into the hexafluoroisopropyl ester (II) (Fig. 1). As illustrated in Fig. 2, esterification with these reagents proceeded readily at 50° and was completed in 60 min. The structure of derivatized captopril was characterized by gas chromatography–mass spectrometry (Fig. 3). The molecular ion peak, $[M]^{+}$, and fragment ion peak, $[M-126]^{+}$, formed with the loss of the N-ethylsuccinimidyl residue appeared at *m/e* 492 and 366, respectively. The base peak at *m/e* 264 was assigned to the heptafluoro-isopropyl ester of proline produced by the fission of an amide linkage.

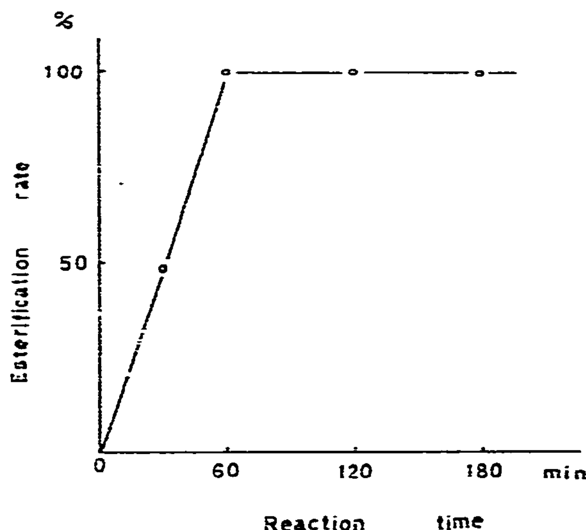

Fig. 2. Time course for esterification of captopril–NEM. The reaction was carried out with hexa-fluoroisopropanol (0.3 ml) and trifluoroacetic anhydride (0.05 ml) at 50°.

This promising derivatization procedure was applied to the determination of captopril by the interval standard method. Of several closely related compounds prepared in these laboratories, captopril–NHM was chosen as the internal standard. OV-210 was found to be most suitable stationary phase for the present purpose. A

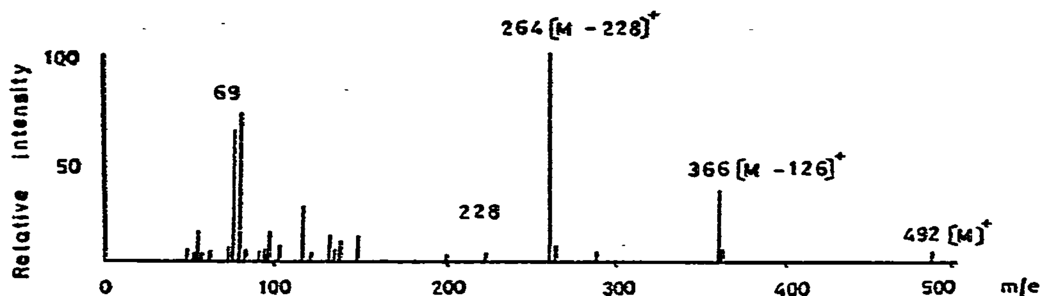

Fig. 3. Mass spectrum of captopril–NEM hexafluoroisopropyl ester.

typical gas chromatogram of captopril and captopril–NHM is shown in Fig. 4. These two compounds gave a single peak of the correct theoretical shape on the gas chromatogram. A calibration graph was constructed by plotting the ratio of the peak height of captopril to that of the internal standard against the amount of the former; satisfactory linearity was observed in the range 1–5 µg of captopril (Fig. 5).

The utility of the present method for the analysis of captopril in blood and urine was tested with dogs. Deproteinization of the blood specimen was accomplished by treatment with metaphosphoric acid in the usual manner. Separation of captopril–NEM in blood and urine was effected by extraction with ethyl acetate. In order to check the validity of the proposed method, a known amount of captopril was added

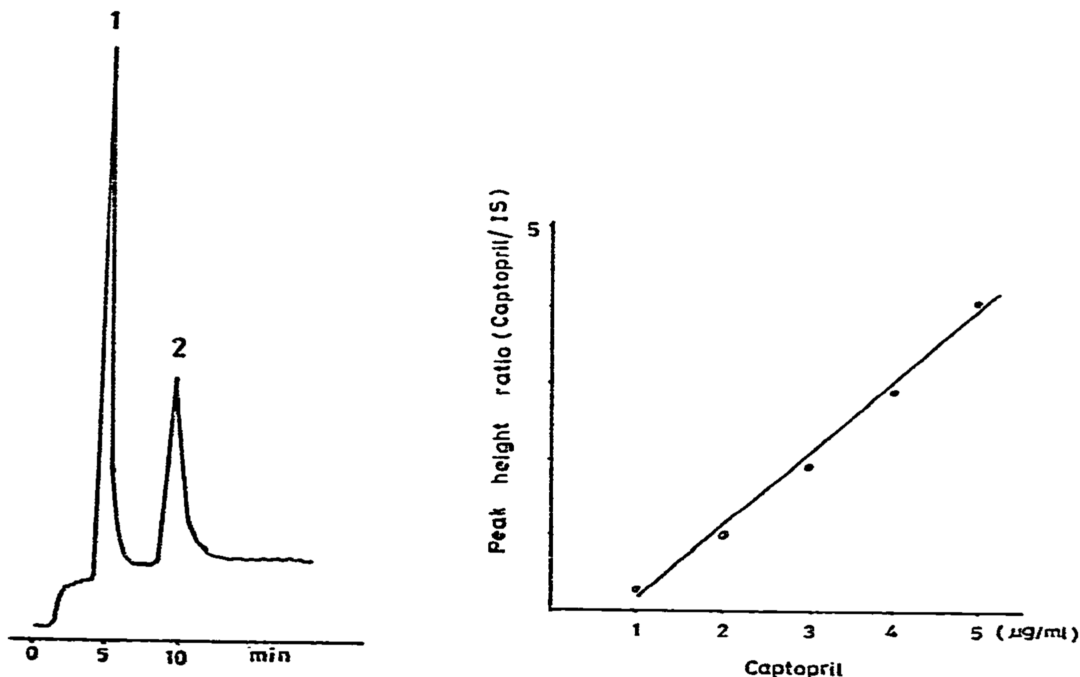

Fig. 4. Gas chromatogram of captopril (1) and captopril–NHM internal standard (2).

Fig. 5. Calibration graph for captopril.

to the samples and the recovery was measured by the proposed procedure (Table I). The mean values obtained for the blood and urine samples were 81.3% and 100%, respectively. A satisfactory recovery was also confirmed with captopril–NEM added to the blood and urine specimens. In addition, it was demonstrated that captopril–NEM in the biological fluids was stable for at least 5 days when stored in a refrigerator.

TABLE I

RECOVERY OF CAPTOPRIL AND CAPTOPRIL–NEM ADDED TO BLOOD AND URINE

Compound	*Amount added (μg)*	*Blood (n = 5)*		*Urine (n = 3)*	
		Amount found (μg)	*Recovery (%)*	*Amount found (μg)*	*Recovery (%)*
Captopril	8.5	6.9	81.3	8.5	100.0
Captopril–NEM	6.2	5.2	84.0	6.0	97.0

The amounts of captopril in blood and urine were determined after intravenous administration of 1 mg/kg to three dogs. The change in the blood level of captopril is illustrated in Fig. 6. The blood concentration decreased markedly after 3 min and no unchanged captopril was detectable after 2 h. The half-life was calculated to be 19.5 min by the linear regression method. Simultaneously, the cumulative urinary excretion of captopril was also determined (Table II). Half or more amount of the administered captopril was excreted in the urine within 1 h and the mean recovery of unchanged captopril after 24 h was 74%.

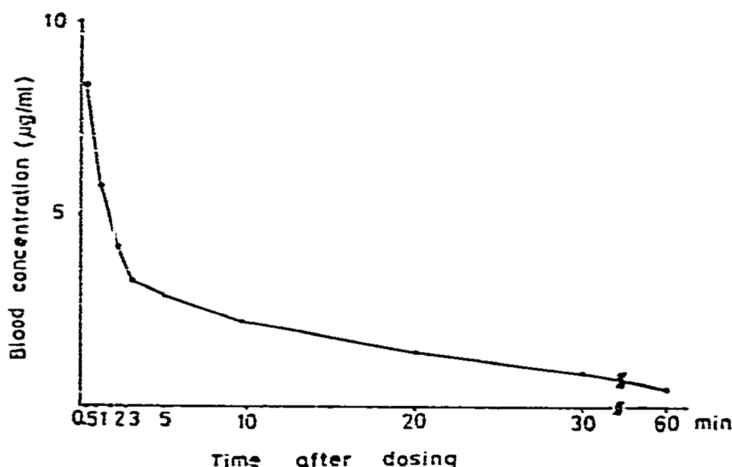

Fig. 6. Change in blood level of captopril in dogs. Each point represents the mean of values obtained with three dogs. A single dose of captopril (1 mg/kg) was administered intravenously to each dog.

The proposed method for the determination of captopril in biological fluids was satisfactory with respect to both sensitivity and reliability in animal experiments. It should be noted that derivatization of captopril into the NEM adduct prevents its oxidative degradation and facilitates its separation from biological materials.

TABLE II

URINARY EXCRETION OF UNCHANGED CAPTOPRIL IN DOGS

A single dose of captopril (1 mg/kg) was administered intravenously to each of two dogs.

Time (h)	*Amount excreted (% of dose)*	
	Dog 1	*Dog 2*
0–1	61.2	53.9
1–2	5.5	11.3
2–6	7.4	7.9
6–24	n.d.*	0.9
0–24 (total)	74.1	74.0

* Not detectable.

It is hoped that the proposed method for the quantitation of captopril will provide a more precise knowledge of the pharmacokinetics of this drug. Further quantitative studies on the metabolic fate of captopril in living animals are being conducted and the details will be reported elsewhere.

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