Journal of Chromatography, 582 (1992) 258–262
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6537

Short Communication

Simple high-performance liquid chromatographic method for the determination of captopril in biological fluids

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(First received June 12th, 1992; revised manuscript received August 11th, 1992)

ABSTRACT

A rapid, simple and sensitive column-switching high-performance liquid chromatographic procedure for the determination of captopril in plasma and urine had been developed. *p*-Bromophenacyl bromide was used as a derivatizing reagent to react with captopril to form a product that showed ultraviolet-absorbing properties. For plasma samples the protein was removed with 6% perchloric acid before injection. The urine samples were directly injected into the chromatograph. The column-switching system was equipped with a pre-column (5 cm × 0.5 cm I.D.) packed with μBondapak C_{18} (37–50 μm) and an analytical column (15 cm × 0.5 cm I.D.) packed with YWG-C_{18}, 10 μm. Impurities were washed from the pre-column with 0.2% acetic acid and the retained substances were eluted into the analytical column with acetonitrile–water–acetic acid (35:65:0.4, v/v). Captopril was detected at 260 nm. The calibration curve was linear in the range 20–1000 ng/ml for plasma and 10–200 μg/ml for urine. The recoveries averaged 103.2 and 99.5% for plasma and urine, respectively. The coefficients of variation were all less than 10%.

INTRODUCTION

Captopril [1-D-3-mercapto-2-methyl-1-oxopropyl)-L-proline] is the first orally active inhibitor of angiotensin-converting enzyme, and is used for the treatment of hypertension [1] and congestive heart failure [2]. In order to study the pharmacokinetics of captopril in humans, a rapid, sensitive and selective assay for captopril in biological fluids is necessary. Radiochemical [3], gas chromatographic (GC) [4], gas chromatographic–mass spectrometric (GC–MS) [5] and high-performance liquid chromatographic (HPLC) [6–8] methods have been reported. These methods, based on liquid–liquid extraction, require much time and effort.

This paper describes a simple, rapid and accurate column-switching HPLC method to determine the plasma and urine concentrations of captopril with UV detection. An on-line pre-column is used to enrich the analyte and to clean up the samples without pre-extraction.

EXPERIMENTAL

Reagents

Captopril was obtained from Changzhou Pharmaceuticals (Changzhou, China). The derivatizing reagent, *p*-bromophenacyl bromide (p-

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0378-4347/92/$05.00

BPB), was a gift from Dr. Y. Kawahara (Sankyo, Japan). Methanol and acetonitrile (HPLC purity) were purchased from Wujin Chemicals (Shanghai, China). All other chemicals were of analytical-reagent grade.

Instruments

A Hitachi 655A Series liquid chromatograph, equipped with a Model 655A-12 pump, a Model 655-71 lower-pressure proportioning valve, a Model 655A-22 UV detector, a Model 655-71 processor B and a Rheodyne 7125 sample injector, was used. A Model YSB-2 pump and a Model 501 switching valve (Scientific Instrument Factory, Shanghai, China) were used to constitute the column-switching system.

Preparation of captopril-p-BPB

To 40 ml of methanol were added 0.43 g of captopril, 0.66 g of p-BPB and 0.4 g of triethylamine. The mixture was refluxed for 1 h and then evaporated to dryness under vacuum. Residues were dissolved in 100 ml of water alkalized (pH 10) with 2 *M* NaOH and washed four times with 25 ml of diethyl ether. The aqueous layer was acidified (pH 1) with 1 *M* HCl and extracted twice with 20 ml of diethyl ether. The organic layer was dried over $MgSO_4$ and then evaporated to give 500 mg of captopril-p-BPB as a clear oil.

Chromatographic conditions

The pre-column (5 cm × 0.5 cm I.D.) and analytical column (15 cm × 0.5 cm I.D.) were packed with μBondapak C_{18}, 37–50 μm (Waters Assoc., Milford, MA, USA) and silica gel YWG-C_{18}, 5 μm (Tianjing Chemical Reagent Factory, Tianjing, China), respectively. A 0.2% solution of acetic acid was used as the pre-treatment mobile phase at a flow-rate of 3 ml/min. The analytical mobile phase consisted of acetonitrile–water–acetic acid (35:65:0.4, v/v) at a flow-rate of 2 ml/min. Methanol was used as an eluent to wash the pre-column between injections. The UV detection wavelength was 260 nm, with the scale of 0.01 a.u.f.s., and the chart speed was 0.25 cm/min. The procedure were performed at room temperature.

Pre-treatment of samples

Captopril in plasma. To 1.5 ml of blood in a heparinized tube were added 50 μl of 0.1 *M* ascorbic acid and 0.1 *M* disodium ethylenediaminetetraacetate. After centrifugation, 0.5 ml of plasma was added immediately to a screw-cap centrifuge tube containing 50 μl of p-BPB (1 mg/ml). The tube was vortex-mixed for 30 s and allowed to stand at room temperature for 20 min. To the mixture were added 300 μl of 6% perchloric acid to precipitate the plasma protein. The mixture was vortex-mixed for 30 s and centrifuged for 10 min (10 000 *g*), and 500 μl of the supernatant were injected into the HPLC system.

Captopril in urine. To 50 μl of urine sample in a centrifuge tube were added 50 μl of p-BPB and

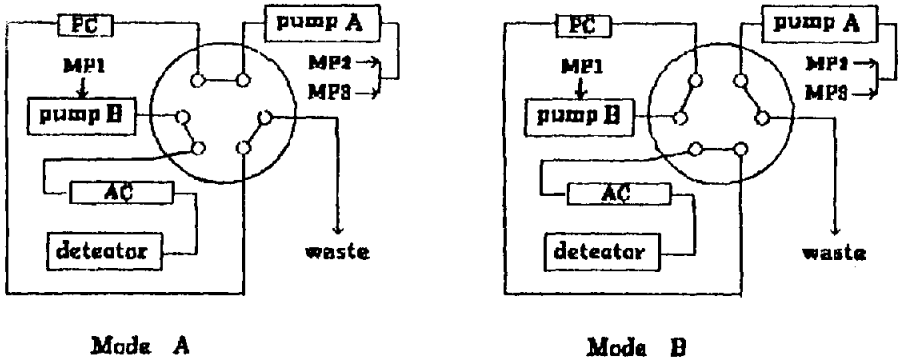

Fig. 1. Flow diagram of column-switching system for analysing captopril in plasma and urine. Mode A is for enriching and cleaning up, and mode B is for analysing. MP1 = acetonitrile–water–acetic acid (35:65:0.4, v/v); MP2 = 0.2% acetic acid; MP3 = methanol; PC = pre-treatment column; AC = analytical column.

600 μl of water. The mixture was vortex-mixed for 30 s and kept at room temperature for 20 min. Then 500 μl of the mixture were directly injected into the HPLC system.

Column-switching operation

The samples were cleaned with the pre-treatment mobile phase for 3 min, then the pre-column connection was switched from mode A to B (Fig. 1) to introduce the retained substances into the analytical column for separation and determination. After 2 min the pre-column connection was switched back to mode A, and the pre-column was washed for 2 min with methanol and recquilibrated for 2 min with pre-treatment mobile phase before the next injection. These operations were performed by the specified programs in the processor (Table I). Typical chromatograms for plasma and urine samples are shown in Fig. 2; the retention time of captopril–p-BPB was 9.5 min.

RESULTS

Calibration curves and detection limits

Drug-free plasma and urine spiked with increasing captopril concentration of 0, 20, 40, 100, 200, 400 and 1000 ng/ml for plasma and 0, 10, 20, 40, 100 and 200 μg/ml for urine were assayed as described above. The standard calibration curves were constructed by performing a linear regression analysis of the peak areas of captopril–p-BPB against the plasma or urine concentrations.

TABLE I

TIME SCHEDULE OF COLUMN-SWITCHING

MP1 = acetonitrile–water–acetic acid (35:65:0.4, v/v); MP2 = 0.2% acetic acid; MP3 = methanol.

Time (min)	Valve mode	MP1	MP2	MP3
0–3	A	On	On	Off
3–5	B	On	Off	Off
5–7	A	On	Off	On
7–9	A	On	On	Off
9	A	On	Off	Off

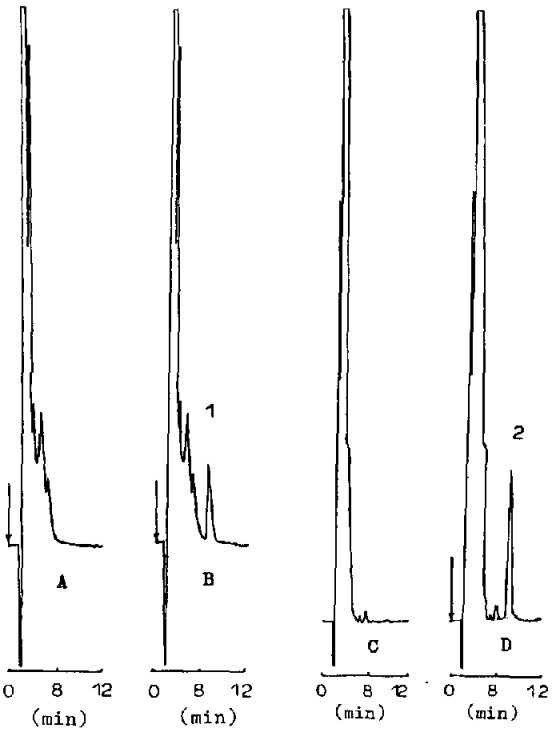

Fig. 2. Chromatograms of captopril in (A) blank plasma, (B) plasma from a volunteer, collected 1 h after oral administration of 50 mg of captopril, (C) blank urine and (D) from a volunteer collected 0–4 h after oral administration of 50 mg of captopril. Peaks: 1 = 457 ng/ml captopril in plasma; 2 = 51 μg/ml captopril in urine.

The correlation coefficients of the standard curves were 0.9925 and 0.9991 for plasma and urine, respectively. The assay limit was 10 ng/ml captopril in plasma (signal-to-noise ratio greater than 3).

Recovery and precision

Captopril was added to drug-free plasma or urine to provide samples of known concentration. The recovery and precision were determined by replicate analysis of each of these samples. The recovery was 103.2% for plasma and 99.5% for urine. The accuracy and precision are shown in Table II.

Application of the method

Plasma and urine concentrations were deter-

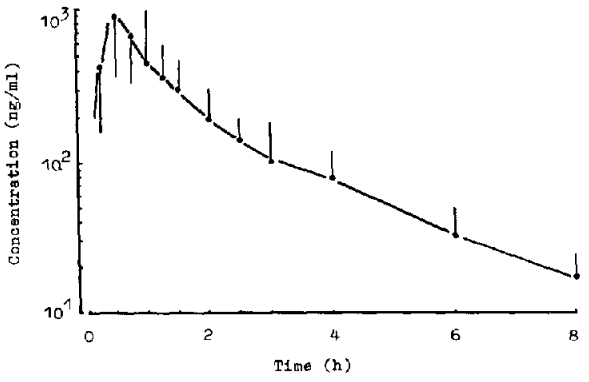

Fig. 3. Mean plasma level curve of captopril in volunteers after an oral dose of 50 mg (n = 8; mean ± S.D.).

mined in eight healthy volunteers, who received 50 mg of captopril each. Fig. 3 shows the mean plasma concentration–time curve of free captopril: the plasma concentration reached a maximum 0.62 ± 0.069 h after dosing with a level of 575 ± 19 ng/ml. The elimination half-life of captopril was 2.23 ± 0.79 h. The urinary excretion of free captopril for 12 h after dosing was 48.3 ± 8.9%.

DISCUSSION

Owing to the low plasma concentration and the absence of functional groups that absorb in the UV or visible region, the direct quantitation of captopril in plasma with a UV–visible detector was difficult. Reported HPLC methods [6–10] for assaying captopril in plasma were usually carried out as follows: (1) derivatization to form a captopril adduct with UV-absorbing or fluorescent properties; (2) extraction of captopril adducts with organic solvents; (3) evaporation and reconstitution; (4) injection for analysis. These procedures were time-consuming and laborious. Our method uses on-line column-switching to enrich and clean up the samples. The method is simple, rapid and accurate, and easy to operate.

Water was initially used as the pre-treatment mobile phase, but the peak of captopril–p-BPB was interfered with by system peaks, which were also present when the switching operation was performed without sample injection. The system peaks could be eliminated by using 0.2% acetic acid instead of water, and by washing the pre-column with methanol before each injection.

CONCLUSION

We have developed a rapid, simple, accurate and reproducible method for the determination of captopril in plasma and urine. This method will permit pharmacokinetic and pharmacodynamic studies of captopril in humans.

TABLE II

ACCURACY AND PRECISION OF THE METHOD FOR DETERMINATION OF CAPTOPRIL IN PLASMA AND URINE

Added	Within-day (n = 5)			Between-day (n = 5)		
	Found	S.D.	C.V. (%)	Found	S.D.	C.V. (%)
Plasma (ng/ml)						
40	42.4	3.1	7.2	41.8	3.3	7.9
100	99.8	4.9	4.9	98.8	5.2	5.3
400	401.3	13.5	3.4	402.5	19.3	4.8
Urine (μg/ml)						
20	20.5	1.0	4.9	21.2	1.29	6.1
40	40.3	1.3	3.2	40.6	1.94	4.8
100	96.5	2.2	2.3	97.7	3.32	3.4

ACKNOWLEDGEMENTS

We thank Changzhou Pharmaceuticals for its financial support. We also thank Dr. Y. Kawahara for his useful suggestions and the supply of derivatizing reagents and Dr. Y. K. Tam for his supply of reagent.

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