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HPLC Determination of Captopril in Human Plasma and Its Pharmacokinetic Study

Ke Li,* Li Tan and Ji-hong Zhou

Department of Instrumental Analysis, Box 65, Jinling Hospital, Nanjing 210002, The Peoples Republic of China

A simple and sensitive reversed-phase liquid chromatographic method has been developed and validated for the analysis of captopril in human plasma and the study of the pharmacokinetics of the drug in human body. Captopril was stabilized by forming an adduct with *p*-bromophenacyl bromide. The adduct formed and 4-chloro-2-nitroaniline (internal standard) were extracted with ethyl acetate:benzene (1:1), and then measured by HPLC using a Spherisorb C_{18} column as stationary phase and a water:acetonitrile: acetic acid mixture (44:55:0.2, v/v/v) as mobile phase. Captopril was quantified by absorbance at 258 nm. The method proved to be linear in the clinical range of 5–500 ng/mL. The lower limit of detection of captopril in plasma was 2 ng/mL. Intra-day and inter-day coefficients of variation of assay for captopril in plasma were 5.8%–8.5% ($n=7$) and 8.0%–9.5% ($n=5$), respectively. The recoveries of captopril were 90%–98% for plasma. The data obtained was fitted with 3P87 program on computer to study the pharmacokinetics. The results showed that the disposition of captopril was conformed to a two-compartment open model with $T_{max}=0.56$ h, $C_{max}=266.5$ ng/mL and $AUC_{0\to\infty}=380.3$ ng·h/mL. The method has been used to determine captopril in plasma samples from ten volunteers and provided data on the pharmacokinetics of the drug. The results inferred that captopril is absorbed rapidly and had a relatively short half-life time in healthy individuals.

INTRODUCTION

Captopril is the first orally active inhibitor of angiotensin-converting enzyme and has been widely used for the treatment of hypertension and congestive heart failure (Cleland *et al.*, 1991; Pennock *et al.*, 1993).

Only the free captopril is pharmacologically active. However, captopril contains a sulphydryl group and binds readily to albumin and other plasma proteins to convert into its disulphide dimer or disulphide conjugates (Duchin *et al.*, 1988). To measure free or unchanged captopril concentration, a chemical stabilizer must be added to the biological samples to prevent the formation of captopril disulphides. This makes it difficult to determine free or unchanged captopril concentration for clinical drug monitoring and pharmacokinetic studies.

A few procedures have been reported for quantitation of captopril in human plasma, including radioimmunoassay (Wong *et al.*, 1981), gas chromatography (Matsuki *et al.*, 1980) and gas chromatography-mass spectrometry (Ito *et al.*, 1987). Nevertheless, some procedures are rather cumbersome and involve specialized, expensive equipment usually not available in a clinical setting. In recent years, high-performance liquid chromatography (HPLC) (Pereira and Tam, 1988; Colin and Scherer, 1989; Klein *et al.*, 1990; Wakabayashi *et al.*, 1994) has been extensively used to determine captopril in biological samples. This report describes a simple, rapid and sensitive HPLC procedure for the determination of free captopril in plasma by using *p*-bromophenacyl bromide as a coupling and stabilizing reagent. It has been used to determine captopril in human plasma samples from ten volunteers who had taken captopril tablets and provided data on the pharmacokinetics of the drug.

* Author to whom correspondence should be addressed.

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EXPERIMENTAL

Apparatus. The HPLC system used consisting of a Waters Model 510 pump, a U6K injector, a 490E programmable multiwavelength detector operated at 258 nm and 0.010 AUFS and a Baseline 810 Chromatography Workstation (Waters Assoc., Milford, USA). Chromatographic separations were carried out on a Spherisorb C_{18} column (250×4.6 mm ID; particle size 10 μm; Dalian Elite Scientific Instruments Co. Ltd., Dalian, P R China) at ambient temperature.

Standards and reagents. Captopril (99.6%) was a gift from Changzhou Pharmaceutical Factory (Changzhou, P R China). 4-Chloro-2-nitroaniline (internal standard) and *p*-bromophenacyl bromide (*p*-BPB) were supplied by Shanghai Chemical Reagents Factory (Shanghai, P R China) and Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan), respectively. HPLC-grade acetonitrile and methanol (Institute for Fine Chemical Engineering of Huaiyin Plastic Product Factory, Jiang Su, P R China) were used to prepare the mobile phase. All chemicals, except where otherwise stated, were of analytical grade, and water used in this assay was doubly distilled. Captopril tablets were provided by the Changzhou Pharmaceutical Factory.

Preparation of the standard captopril-*p*-BPB adduct for the assay. Captopril (0.43 g) and *p*-BPB (0.66 g) were dissolved together in 40 mL of methanol. Triethylamine (0.40 g) was then added and the mixture was refluxed for 1 h. Vacuum-evaporation of the solvent gave an oil that was dissolved in 100 mL of water. The solution obtained was alkalinized to pH 10 by a dropwise addition of 2 M NaOH. Then it was washed four times with 25 mL ethyl acetate. The organic layer was discarded whereas the aqueous one was brought back to pH 1 by addition of 1 M HCl. This was finally extracted three times by ETOAc (20 mL), dried over $MgSO_4$ and evaporated *in vacuo* to get a clear oil.

The ultraviolet spectra of the adduct showed that the wave-

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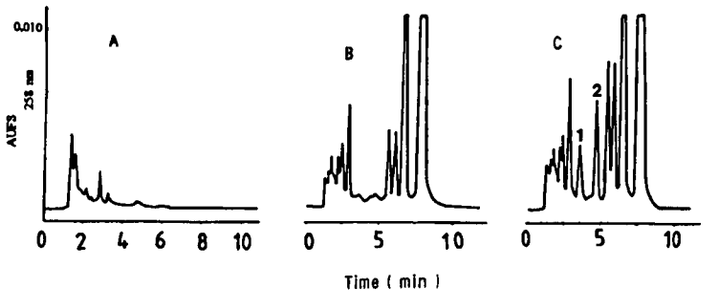

Figure 1. Chromatograms of (A) blank plasma; (B) *p*-BPB in human plasma; (C) captopril, *p*-BPB, and internal standard in human plasma. 1, Captopril; 2, 4-chloro-2-nitroaniline (internal standard).

lengths of the maximum and minimum were at 257.4 nm and 234.0 nm, respectively. Thin layer chromatography performed on a silica gel GF_{254} gave an R_f of 0.45, using a benzene-acetic acid 3:1 mixture as a solvent.

Mobile phase. The mobile phase consisted of acetonitrile, deionized water and acetic acid (44:55:0.2, v/v/v). This solution was passed through a 0.45-μm membrane filter (Millipore, Bedford, MA, USA) and was then degassed before use. The flow-rate of mobile phase was 1.40 mL/min.

Preparation of solutions. Captopril, captopril-*p*-BPB and 4-chloro-2-nitroaniline stock standard solutions (1.00 mg/mL) were prepared by dissolving 100 mg of captopril, captopril-*p*-BPB and 4-chloro-2-nitroaniline in 100 mL of methanol and kept in a refrigerator, respectively. The extracting solvent consisted of ethyl acetate and benzene (1:1).

Analytical procedure. Freshly drawn blood (2.5 mL) was mixed with 50 μL of a solution of EDTA (0.1 mol/L) and ascorbic acid (0.1 mol/L). The mixture was immediately centrifuged at 4000 r/min for 10 min.

An aliquot of 1.0 mL of the plasma was added to a chemically clean screw-capped glass tube containing 150 μL of *p*-BPB (1 mg/mL) and 20 μL of 4-chloro-2-nitroaniline (50 μg/mL) solution. The tube was vortexed for 30 s and left at room temperature for 15 min. Following this, 200 μL of 2 mol/L HCl was added and vortexed for 10 s. Then 5 mL of a 1:1 mixture of ethyl acetate:benzene were poured in the tube as an extracting solvent and vortex mixed for 2 min. A 4.5 mL aliquot of the organic layer was collected and evaporated to dryness with nitrogen at 50°C, and then 100 μL of acetonitrile was added to dissolve the residue. After 20 s vortex mixing, 25 μL of the sample solution were injected into the HPLC system.

RESULTS

Chromatographic separation

Figure 1 shows typical chromatograms of human plasma samples. Under the chromatographic conditions described, captopril and 4-chloro-2-nitroaniline had retention times of approximately 3.6 min and 4.7 min, respectively. It can be seen, from Fig. 1, that good separation and detectability of captopril in human plasma were obtained with minimal interference from plasma components. Hence, it is relatively easy to estimate the peak-area with accuracy.

Precision

The data for studies of within-day reproducibility, evaluated by assaying seven plasma samples containing different concentration of captopril, and between-day reproducbility, evaluated by assaying the same concentration seven times over a 5-day period, were summarized in Table 1. The range of percentage of relative standard deviation (% RSD) was from 5.8% to 8.5% for within-day analyses and from 8.0 to 9.5% for between-day analyses, respectively.

Linearity and detection limit of method

A series of whole blood samples containing 5, 10, 20, 50, 100, 200 and 500 ng/mL of captopril was prepared to study the relationship between the ratio of peak-area of captopril to 4-chloro-2-nitroaniline and the concentrations of captopril under selected conditions. The results showed that the peak-area ratio was linearly related to the captopril concentration for the range of 5–500 ng/mL. The linear equation for the concentration versus the ratio of peak-area was $Y=3.17\times10^{-3}\,X-0.02$ with a correlation coefficient of 0.9998. The detection limit was 2 ng/mL.

Extraction efficiency and recovery

Ether, benzene, ethyl acetate, hexane:isopropanol (95:5), benzene:ethyl acetate (1:1) and ether:hexane (1:1) solvent systems were used to extract the captopril adduct, respectively. The results showed that benzene:ethyl acetate (1:1) solvent system was found to be very efficient. Extraction efficiencies of captopril-*p*-BPB and the internal standard were determined by comparing peak-areas of the analytes from extracted standards from plasma to those from a chromatographic standard solution prepared in acetonitrile at the equivalent concentration and chromatographed directly. The extraction efficiency of captopril-*p*-BPB and the internal standard from plasma were 100.6±4.6% (mean±SD, $n=7$) and 89.2±5.6% ($n=7$), respectively.

Table 1. Within-day and between-day precision of the method

Concentration added (ng/mL)	RSD (%) Within-day ($n=7$)	RSD (%) Between-day ($n=5$)
20	8.5	9.5
100	6.2	8.0
400	5.8	8.7

Table 2. Recovery of captopril from spiked human plasma ($n=7, \bar{x}\pm s$)

Concentration added (ng/mL)	Concentration measured (ng/mL)	Recovery (%)	RSD (%)
20	18.02±1.32	90.1±6.6	7.3
100	91.00±6.00	91.0±6.0	6.6
400	393.20±14.00	98.3±3.5	3.6

To establish the conversion and extraction recovery, drug-free plasma was spiked with 20, 100 and 400 ng/mL captopril. The peak-heights of the captopril-*p*-BPB adduct formed and extracted was compared with the peak-height of captopril-*p*-BPB at same concentrations in acetonitrile injected directly into the HPLC. The data obtained for different concentrations of captopril were summarized in Table 2.

Application

Ten healthy male Chinese volunteers aged 24.7±s5.5 and weighing 59.7±s8.3 kg entered the study. All volunteers gave their written consent and underwent a physical examination. There were no abnormal findings in liver and kidney functions in particular. After 12 h of overnight fasting, the volunteers received a single oral dose of 30-mg captopril. Blood samples (2.5 mL) were taken before medication and after 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 h. Figure 2 illustrates the profile of plasma concentration versus time for captopril in the ten volunteers.

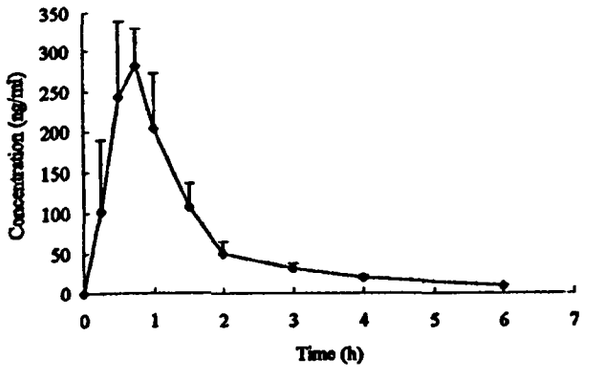

Figure 2. Mean plasma concentration–time curve after oral administration of 30 mg of captopril to ten healthy volunteers. ($n=10$, $x\pm s$).

Table 3. Pharmacokinetic parameters of captopril after administering an oral dose of single 30 mg to ten healthy Chinese volunteers

	$T_{a1/2}$ (h)	$T_{\alpha1/2}$ (h)	$T_{\beta1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	V (L)	$AUC_{0-\infty}$ (ng·h/mL)
$\bar{x}$	0.31	0.18	2.18	0.56	266.5	35.9	380.3
±SD	0.09	0.09	0.48	0.17	83.8	23.5	49.4

DISCUSSION

The effect of the composition of mobile phase on the chromatographic separation was investigated in this study. The results indicated that the optimum resolution for captopril, 4-chloro-2-nitroaniline and endogenous substance in plasma was obtained when the mobile phase was composed of acetonitrile, water and acetic acid (53:47:0.2 v/v/v). The retention times of the captopril were obviously prolonged with decreasing acetonitrile content. However, the rentention time of 4-chloro-2-nitroaniline changed only slightly with varying acetonitrile content.

The pharmacokinetics of captopril were studied in ten healthy Chinese volunteers. After a single oral administration of 30 mg captopril, the data obtained was fitted with a 3P87 program on computer. Table 3 shows the pharmacokinetic parameters of ten volunteers who had captopril administered orally. The results suggest that the disposition of captopril is conformable to a two-compartment open model. Peak concentration in plasma occurred 0.56 h after ingestion and the mean peak concentration achieved was 266.5 ng/mL. This implies that captopril is rapidly and well absorbed in healthy individuals. Moreover, it had been found that captopril had a relatively short half-life time ($T_{1/2}$=2.18 h). This may be due to its free sulphydryl group forming mixed disulphides with endogenous thiol-containing compounds.

CONCLUSION

The method provided excellent recovery and good precision, and is simple and reliable in both chromatographic conditions and sample preparation. Furthermore, the analytical procedure is easy to handle and is very suitable for the routine determination of a large number of samples because of the short time between the two injections. It has been successfully applied to the analysis of the pharmacokinetic study of captopril in whole blood samples obtained from ten healthy volunteers during the participating in a clinical trial of captopril single oral dose. The results implied that captopril is absorbed rapidly and had a relatively short half-life in healthy individuals.

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