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Determination of captopril in human serum by high performance liquid chromatography using solid-phase extraction

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Abstract

A rapid, simple and sensitive assay was developed for determination of captopril in human serum. We employed silica gel cartridge for efficient extraction of captopril adduct from human serum. Captopril was trapped with *p*-bromophenacyl bromide (*p*BPB) to give captopril–*p*BPB adduct. A 4-ml benzene extract of 1 ml acidified serum was passed through 1 ml silica gel cartridge. Potential interfering compounds were removed with 4-ml benzene wash. The captopril–*p*BPB adduct was eluted with 0.5 ml acetonitrile. Of this acetonitrile solution (100 µl) was injected on an ODS reverse phase HPLC column (chromatography conditions; mobile phase; acetonitrile–water–acetic acid (225:270:5, v/v/v), flow rate; 1 ml min^{-1}, detection; UV at 263 nm). It is found that this method is accurate and does not require time consuming evaporation-concentration steps. Recovery exceeds 94% and analytical responses are linear over captopril concentration range from 50 up to 1000 ng ml^{-1}. The coefficients of variation from 108 ng ml^{-1} to 605 ng ml^{-1} varied between 3.7–7.7% and the relative error did not exceed 3.7%. Therefore, this method can be used for routine clinical monitoring and in pharmacokinetic studies of captopril. © 1997 Elsevier Science B.V.

Keywords: Captopril; Reverse phase chromatography; Solid phase extraction; *p*–Bromophenacyl bromide; Thiosalicylic acid

1. Introduction

Captopril belongs to a group of angiotensin-converting enzyme inhibitors that are used for the treatment of hypertension [1] and congestive heart failure [2]. Quantitation of its serum concentration has been problematic due to its relative instability. Captopril is readily converted into its disulfide dimer and forms disulfide conjugates with endogenous thiol compounds [3].

To measure free or unchanged captopril concentrations a chemical stabilizer or derivatization reagent must be added to biological samples to prevent the formation of the disulfide [4]. Several methods for determination of captopril in plasma have been described recently, such as; HPLC

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methods [5–10], radiochemical [11], GC [12], and GC-MS [13]. These methods are based on liquid–liquid extraction and evaporation–concentration steps that are time consuming and require careful attention to achieve adequate sensitivity. Here we report a simple, rapid and accurate HPLC method using an efficient and fast extraction by solid phase silica gel cartridge for determination of captopril in biological samples.

2. Experimental

2.1. Materials

The captopril working standard was provided from Darou-pakhsh Pharm. Co. *p*-Bromophenacyl bromide was synthesized according to the reported procedure [14] and purified by column chromatography on silica gel with dichloromethane, followed by recrystalization from ethanol. Thiosalicylic acid and HPLC grade acetonitrile were purchased from Fluka (Switzerland).

Silica gel solid phase columns (1 ml) were obtained from Siva (USA) and washed with 1 ml benzene prior to use. Captopril–*p*BPB adduct and thiosalysilic acid–*p*BPB adduct (I.S.) were prepared as described by Kawahara et al. [5]. Other reagents and chemicals were of analytical grade.

2.2. Solid phase extraction

For extraction, 1 ml of serum that was added to a 10- ml screw-cap glass tube, was acidified with 0.2 ml HCl (1 N), and extracted twice with 2 ml of benzene. The organic layer was passed through a silica gel that has been previously connected to a vacuum elution system. The cartridge was washed with another 4 ml benzene. Then captopril–*p*BPB adduct was eluted with 0.5 ml of acetonitrile.

2.3. Instruments and chromatographic conditions

A liquid chromatograph system (Pye Unicam, model PU 4030) equipped with an injector (7121 Rheodyne), pump (PU 4011), UV- detector (PU 4110 UV/Vis), Spheri-5 ODS, 5 μm, 100 × 4.6 mm column and Spheri-5 ODS-GU guard column (Applied Biosystems, US) were used. The mobile phase consists of acetonitrile–water–acetic acid (225:270:5, v/v/v) and flow rate was 1 ml min^{-1}. The solvents were mixed, filtered, degassed by ultrasonication before use. The column effluent was monitored by a UV-absorption measurement at 263 nm, chromatograms were traced on a chart recorder with chart speed 300 mm h^{-1} and peak height ratio measurements were used for quantitation. The procedures were performed at room temperature.

2.4. Sample preparation

Blood (10 ml) was collected in 15 ml plastic tubes containing 30 mg *p*BPB. The tube was vortexed for 30 s and was left at room temperature for at least 15 min to allow reaction between captopril and *p*-BPB to be completed as suggested by Giudicelli et al [15]. The serum was separated, added to a 10-ml screw-cap glass tube and stored at −20°C. At the time of assay the serum was thawed and 1 ml of the sample was transferred to another 10-ml screw-cap glass tube. Captopril–

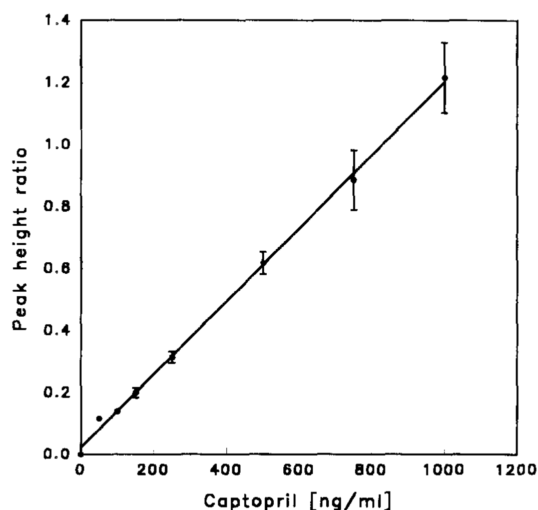

Fig. 1. Captopril calibration curve. The correlation coefficient is 0.998. Each point is the mean of at least six determinations and S.E. of mean is indicated by vertical bars.

Table 1
Reproducibility and accuracy for captopril

Spiked value (ng ml^{-1})	Number of samples	Assay value (ng ml^{-1}) (mean ± S.D.)	Coefficient of variation (%)	Accuracy (%)
Within-day				
108	6	111.5 ± 8.6	7.7	3.2
225	5	223 ± 15	6.7	−0.9
345	6	360.6 ± 19.3	5.3	4.5
605	6	627.3 ± 23.2	3.7	3.6
Day-to-day				
260	6	257.6 ± 16.0	6.2	−0.9
530	5	550 ± 33.6	6.1	3.7

*p*BPB adduct was extracted as described above. Then, 50 µl of I.S. solution (10 µg ml^{-1} in acetone) was added to the eluted acetonitrile solution and made up to exactly 1 ml with mobile phase. A 100-µl sample was injected into the chromatograph.

3. Results and discussion

3.1. Solid phase extraction

There are two general methods for extraction of drugs from biological samples; liquid–liquid and solid-phase extractions. Published methods for determination of captopril in serum samples employed liquid–liquid extraction, which is time consuming and needs extensive work. For a faster method with sufficient accuracy and precision, we have used solid phase extraction that has not been reported for captopril assays. This method has several advantages: (a) it is much faster (it takes usually 15 min compared to 50 min for liquid extraction procedures); (b) it has higher accuracy and precision; (c) it requires a small amount of serum; and (d) it does not require evaporating benzene under nitrogen which releases a hazardous organic solvent into air.

3.2. Calibration

Calibration samples were prepared using drug-free serum. Serum samples (1 ml) were spiked with captopril at known concentrations (0, 50, 100, 150, 250, 500, 750, 1000 ng ml^{-1}) and were assayed as described above. A least-squares linear regression evaluation of the peak height ratio (Y) versus concentration (X) relationship gave $Y = 0.00115\ X + 0.04358$ with a correlation coefficient of 0.998 (Fig. 1).

3.3. Reproducibility and accuracy

Reproducibility and accuracy were determined for five or six spiked serum samples with respect to a calibration graph (Table 1). The within-day coefficients of variation were 3.7–7.7%. The day-to-day coefficients of variation for analysis of the same serum samples on 3 days over a period of 2 weeks were 6.2 at 260 ng ml^{-1} ($n = 6$) and 6.1 at 530 ng ml^{-1} ($n = 5$). The accuracy of the method expressed as the mean deviation of all concentrations from the theoretical value ranged from −0.9 to 4.5%.

Table 2
Extraction yield of captopril from spiked serum samples ($n = 3$)

Spiked value (ng ml^{-1})	Extraction yield mean (%)	Coefficient of variation (%)
250	99.5	5.1
500	99.6	2
750	99.8	1.4

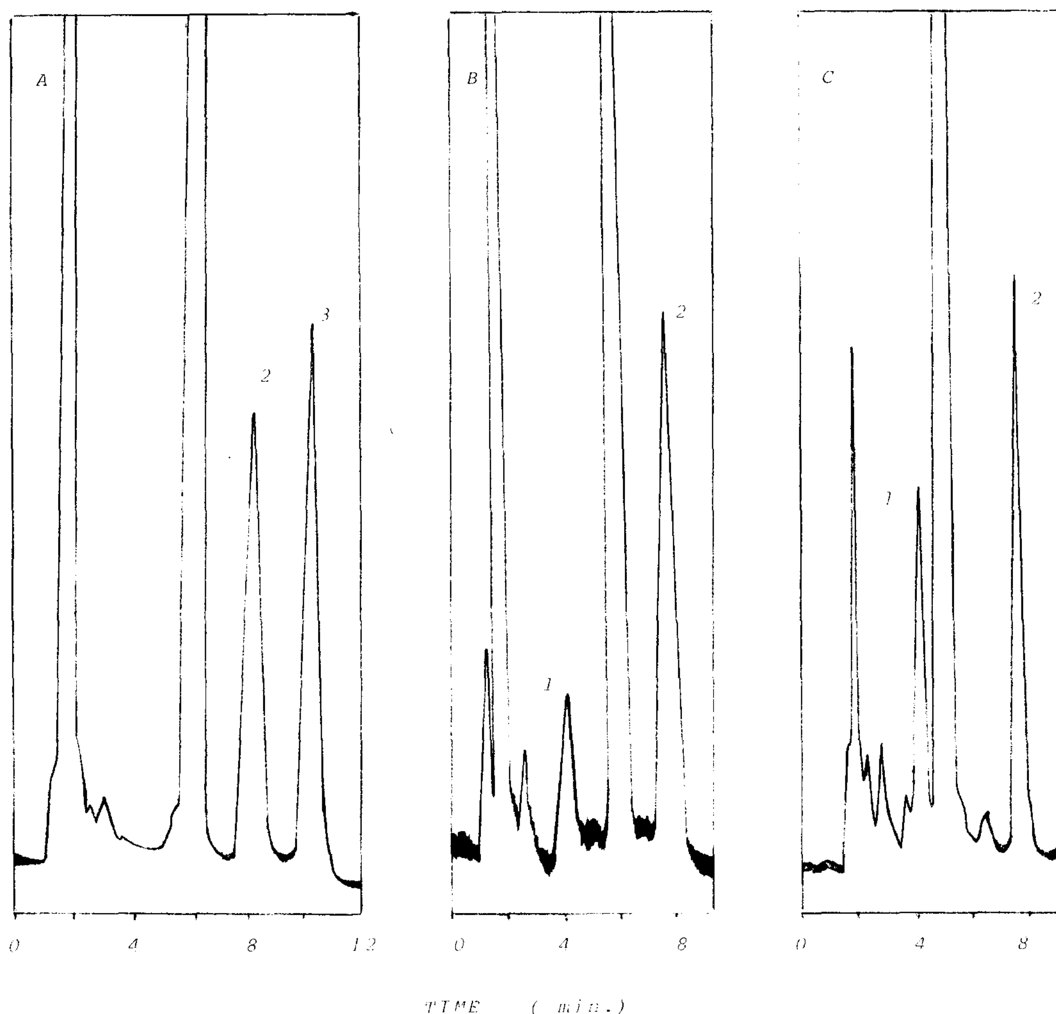

Fig. 2. Chromatograms of captopril: (A) blank serum spiked with I.S. (500 ng ml^{-1}) and p-BPB (3mg ml^{-1}); (B) serum spiked with captopril–p-BPB adduct (equivalent to 250 ng ml^{-1} captopril) adduct and I.S (500 ng ml^{-1}); (C) serum from a volunteer half an hour post administration of captopril tablet (50 mg). Peaks: 1 = captopril–pBPB; 2 = I.S.; 3 = p-BPB.

3.4. Recovery

The recovery of captopril was estimated as follows, control serum samples spiked with 250, 500, 750 ng ml^{-1} captopril were determined as described. The recovery was calculated by comparing the peak ratios of control serum samples with those of equivalent samples of captopril–pBPB.

As shown in Table 2, captopril was recovered quantitatively in the range of 94.4–104.6%. These recoveries are comparable with other reported methods [5–10] and are an indication that the reaction between captopril and pBPB will be completed within 15 min.

3.5. Chromatography

In reverse-phase HPLC determination of captopril, the mobile phase is commonly a ternary mixture of acetonitrile–water–acetic acid. In this study various combinations of this mixture were

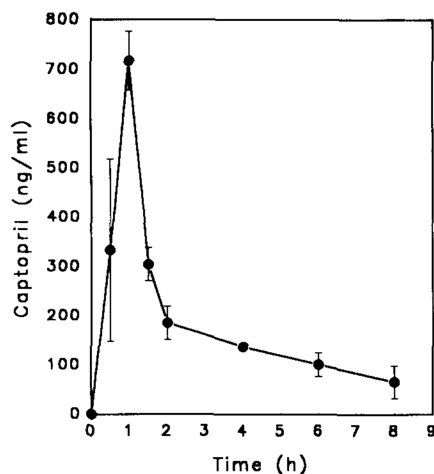

Fig. 3. Mean serum level curve of captopril in volunteers after an oral dose of 50 mg. Each point is the mean of three persons and S.E. of mean is indicated by vertical bars.

examined. A mixture of acetonitrile–water–acetic acid (225:270:5) gave the best separation of peaks, and was selected for our assay.

Typical chromatograms of a blank serum, a sample serum spiked with captopril–*p*BPB and I.S., and a serum from a volunteer is shown in Fig. 2. The retention times of captopril–*p*BPB and the internal standard adduct were 4 and 8 min, respectively. No interfering peaks were found in several blank serum samples examined. The detection limit was 15 ng ml^{-1} (signal to noise ratio >3) which is well below the drug concentration expected in biological specimens from patients who had been given therapeutic doses of captopril.

Table 3
Individual pharmacokinetic parameter after oral administration of 50 mg captopril (Squibb)

Subject	T_{max} (h)	C_{max} (ng ml^{-1})	AUC_{0-8} (ng.h ml^{-1})[a]
A	1	747.2	1665.1
B	1	801.0	1369.2
C	1	603.9	1318.7
Mean	1	717.37	1451.0

[a]Area under curve.

3.6. Application to biological samples

The proposed method was applied to the determination of captopril in serum samples obtained from three healthy, volunteers who orally received tablets containing 50 mg of captopril (Squibb). Blood samples were taken at 0, 0.5, 1, 1.5, 2, 4, 6 and 8 h after administration. Fig. 3 shows the mean serum concentration–time curve of the three subjects and Table 3 gives the parameters calculated from data in Fig. 3. Captopril was readily absorbed from this preparation and reaches the maximum concentration of ~717 ng ml^{-1} after 1 h post administration. These values are in agreement with the previous reports [16].

4. Conclusion

The method described is sufficiently simple, sensitive and rapid for the determination of captopril at therapeutic concentrations in human serum. It can be used for routine clinical monitoring and in pharmacokinetic studies.

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