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Determination of Captopril in Human Plasma with Precolumn Derivatization Using Solid Phase Extraction and HPLC

M.R. Hadjmohammadi*, K. Kamel and F. Khajooei nezhad
Department of chemistry, Mazandaran University, Babolsar, Iran

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A rapid, accurate and sensitive method for the determination of captopril in human plasma was developed by solid phase extraction and high performance liquid chromatography (HPLC), using precolumn derivatization of captopril with chromophore label *o*-phethaldialdehyde (OPA). The extraction of captopril from human plasma was carried out by an amino propyl cartridge. A 0.01 M solution of HCl in methanol showed the best recovery and was chosen for elution of captopril in cartridge. This methanolic solution was applied to react with aqueous solution of OPA and glycine as a coderivatization reagent. The process of derivatization was completed within 2 min at room temperature. The derivatized captopril was injected into a reverse phase HPLC system. Mobile phase was consisted of water:acetonitrile:trifluoroacetic acid (85:15:0.1 v/v/v) with a flow rate of 1 ml min^{-1} and detector was used at 345 nm. Linear dynamic range and limit of detection were found as 0.1-6 ppm and 0.1 ppm, respectively.

Keywords: Captopril, Precolumn derivatization, SPE, HPLC

INTRODUCTION

Captopril, (s)-1-(3-Mercapto-2-methyl-L-oxo-propyl)-L-proline (Fig. 1), belongs to a group of angiotensin-converting enzyme inhibitors that are used for the treatment of hypertension [1] and congestive heart failure [2]. Determination of captopril in plasma has been problematic due to its relative instability [3,4]. Captopril can not absorb at useful UV-Vis spectral region and is readily converted into its disulfide dimmer and forms disulfide conjugates with endogenous thiol compounds [5]. To measure free or uncharged captopril concentration, a derivatization technique in which a chromophore or a fluorophore is introduced to render this UV-transparent molecule detectable must be performed [6].

Several methods have already been reported for the quantitative determination of free captopril in biological fluids including gas chromatography/mass spectrometry (GC/MS) [7], radioimmunoassay (RIA) [8,9], HPLC [10-12] and electrochemical methods [13]. Some of the reported methods require expensive equipment and other methods are very laborious. A major drawback of some existing methods is their need to large sample volumes [14]. The derivatization reagent *o*-Phethaldialdehyde (OPA) has already been used for the derivatization of primary amines and amino acids, and selective derivatization of thiols when an amine is added as a coderivatization reagent [15,16]. The derivatization reaction of

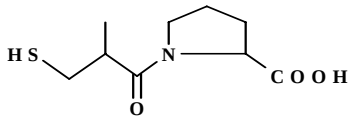

Fig. 1. The structure of captopril.

*Corresponding author. E-mail: hadjmr@umz.ac.ir

captopril in the presence of OPA and glycine as a coderivatization reagent has been reported in the literature [17].

In this work, *o*-phethaldialdehyde as derivatization reagent was combined with solid phase extraction as a pretreatment procedure. The presented method is rapid, sensitive, and easy to perform and require only 50 µl of biological samples per assay.

EXPERIMENTAL

Chemicals

Captopril was purchased from sigma (St. Louis, USA). *tri*-Butylphosphine (TBP), *o*-Phethaldialdehyde (OPA), glycine, HPLC grade methanol and acetonitrile were obtained from Fluka (Buchs, Switzerland). Trifluoroacetic acid was obtained from Merck (Darmstadt, Germany). Water was doubly distilled, deionized and filtered through a 0.45 µm Millipore filter. All other reagents were analytical grade.

Instrumentation and Conditions

The HPLC system comprised of a LC pump series 10, model 7125 manual injector with a 10-µl loop, a LC-95 UV-Vis spectrophotometer as detector (all from Perkin-Elmer, Norwalk, CT, USA), a AR-55 linear recorder (Pye Unicam, Holland) and a C_{18} (250 × 4.6 mm, 5 µm) column. The pH meter used for adjustment of the mobile phase's pH was model 3030 (JenWay, Ltd., UK).

Appropriate conditions for the determination of captopril, obtained using its standard solution in 15% acetonitrile in water containing 1% trifluoroacetic acid. In this solution, the absorption signal of captopril has no interference with the signals of other compounds present, and at least 20 min elution time was necessary prior to the next injection. The optimum flow rate, injection volume and λ_{max} were 1 ml min^{-1}, 10 µl and 345 nm, respectively.

Sample Preparation from Human Plasma

Captopril was taken orally by a volunteer. To 10 ml of the blood of this volunteer, was added 10 ml TBP (1000 ppm). The sample was then centrifuged immediately for 5 min at a rate of 2000 rpm, and its plasma was separated. To remove the excess of TBP from plasma, it was extracted twice with 1 ml n-hexane. Then, the supernatant plasma was passed through an aminopropyl cartridge under vacuum. To remove the interferences, the cartridge was washed with 3 ml of water and 1 ml of diethyl ether. The analyte was then eluted with 2 ml of 0.01 M solution of HCl in methanol. The collected captopril in methanolic solution of HCl was finally derivatized immediately with OPA and injected into chromatographic system.

Derivatization Procedure

All stock standard solutions of captopril were prepared in methanol. The *o*-phthaldialdehyde solution was made by dissolving 50 mg of drug in 50 ml water (1000 ppm). Standard aqueous solution of glycine was prepared at a concentration of 0.01 M. To derivatize captopril with OPA, 20 µl of standard solution of captopril or plasma sample which passed through the cartridge, was pipetted into a flask. Subsequently, 2 ml of OPA, 3 ml of phosphate buffer of pH 8.5 and 100 µl of 0.01 M glycine were added and mixed for 2 min at room temperature. Finally, 10 µl of the derivatized captopril was injected into the HPLC system.

RESULTS AND DISCUSSION

For selection of the best solvent for extraction of captopril from cartridge, eight eluting solvents were examined. The solvents used included dichloromethane, diethyl ether, acetic acid, acetonitrile, ethanol, methanol, 0.01 M solution of HCl in ethanol and methanol. After loading captopril samples (standard and real samples) into the cartridge, 3 ml of each solvent was passed through the cartridge. Then, each sample was derivatized (as mentioned above) and injected into the chromatographic system. The results summarized in Table 1 reveal that a 0.01 M solution of HCl in methanol has the best recovery for captopril and thus was selected for further studies.

In the next step, after loading captopril sample into the cartridge, 0.5, 1, 2, 3, 4, 5 ml volumes of the 0.01 M solution of HCl in methanol were passed through the cartridge. Each volume was repeated for five times. Then each sample was derivatized and injected into chromatographic system. The results are shown in Table 2. As is obvious a volume of 2 ml 0.01 M solution of HCl in methanol revealed the best recovery

Table 1. Effect of Nature of Eluting Solvent on the Recovery of SPE Procedure (n = 5)[a]

Eluting solvent	Recovery (%)	RSD (%)
Dichloromethane	50	4.0
Diethyl ether	51	4.0
Acetic acid	77	3.0
Acetonitrile	82	2.8
Ethanol	89	2.2
Methanol	95	2.0
0.01 M Solution of HCl in ethanol	95	2.5
0.01 M Solution of HCl in methanol	98	2.1

[a]Conditions: captopril concentration, 100 ppb; volume of eluting solvent in each stage, 3 ml; cartridge, aminopropyl from Varian.

Table 2. Effect of Volume of 0.01 M Methanolic Solution of HCl on the Recovery of SPE Procedure (n = 5)[a]

Volume (ml)	Recovery (%)	RSD (%)
0.5	89	3.2
1.0	92	3.0
2.0	99	3.0
3.0	96	4.0
4.0	95	4.8
5.0	90	4.8

[a]Conditions as Table 1.

and was chosen for the sample assays.

To determine the breakthrough volume, different volumes of a 0.1 ppm solution of captopril in methanol were passed through the cartridge. Then, the collected analyte was derivatized and injected into the chromatographic system and the height of captopril peak was measured (Fig. 2). As it is obvious from Fig. 2, maximum peak height was observed until a solution volume of 250 ml and decreased drastically at larger sample volume. Thus, the breakthrough volume of the system is 250 ml.

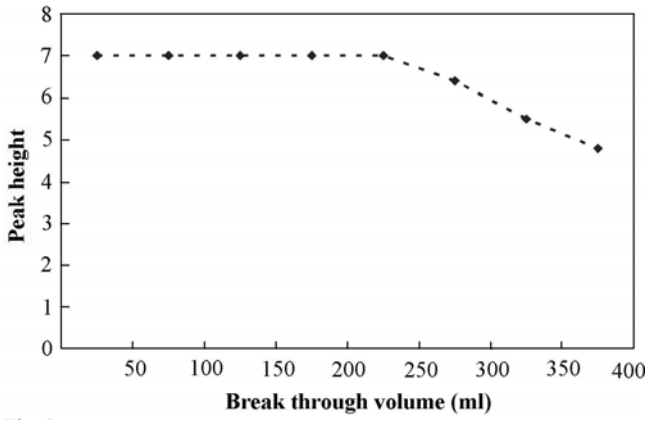

Fig. 2. Variation of HPLC peak height of captopril with volume. Conditions: captopril concentration, 0.1 ppm; mobile phase, water/acetonitrile/trifluoroacetic acid (85/15/0.1 v/v/v); column, C_{18} (250 × 4.6 mm, 5 μm); pH of derivatized sample, 8.5; pH of mobile phase, 3.5; Flow rate, 1 ml min^{-1} at room temperature.

Known amounts of captopril were added to known portions of the plasma samples of a healthy volunteer and extracted using the method described above and the captopril concentrations were determined using a calibration curve. The correlation coefficient relative to captopril solutions (n = 5) with concentrations ranging between 0.1-6 ppm was 0.993 and relative error for a 1 ppm solution of captopril was 3.4%. The repeatability precision values, expressed as the mean relative standard deviation (RSD), for each concentration was calculated from five independent extractions of captopril standard samples. At the spike levels of 1, 2 and 6 ppm, the repeatabilities were 0.73%, 0.34% and 0.84%, respectively, indicating good performance of the method developed in this work.

The captopril residues in blood of volunteers were determined using standard addition method. Figure 3 shows typical chromatograms of sample solutions extracted from the plasma samples of healthy and volunteers. At the experimental conditions used, the retention time of captopril was about 8.5 min, and its identification in the sample solution was carried out by comparison of the retention time of the standard. Results showed that the amount of captopril in the unhealthy

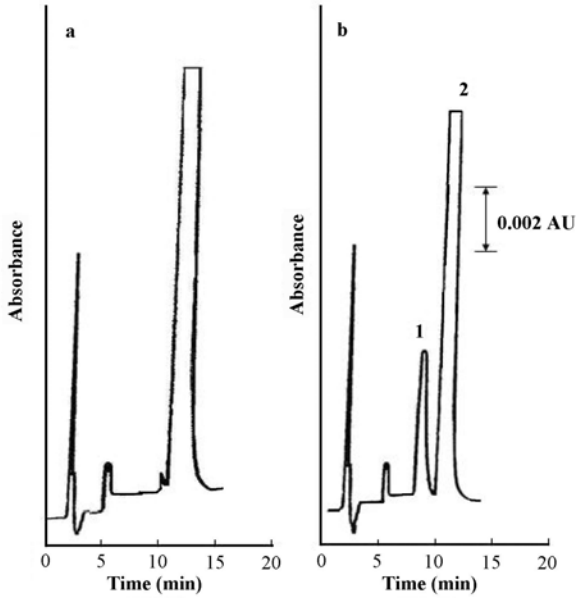

Fig. 3. Chromatograms obtained for separation of derivatized captopril in Healthy volunteer blood sample (blank solution) (a) and in Blood sample of volunteer that used captopril (b). Peak 1, derivatized captopril; peak 2, *o*-phethaldialdehyde. Conditions as Fig. 2.

blood after 1 h was found to be 1 ppm. The linear dynamic range and limit of detection were 0.1-6 ppm and 0.1 ppm, respectively. The limit of detection was calculated using $3s_b/m$ equation.

CONCLUSIONS

The proposed method is rapid, sensitive and accurate with a good recovery of captopril in plasma and requires a small volume of biological samples (50 µl). The combination of solid phase extraction for pretreatment of captopril sample from the matrix with HPLC for its analysis showed high recoveries, low limit of detection and wide linear dynamic range.

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