



CAPSULES

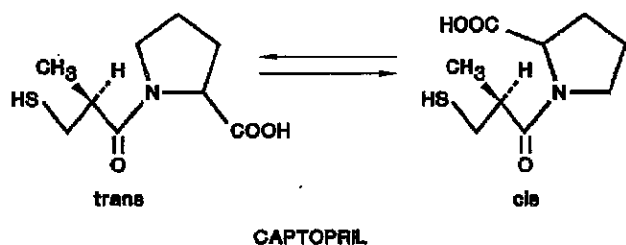
notes and applications from Bioanalytical Systems, Inc.

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Captopril and Captopril Disulfide In Plasma and Urine

Purpose

Captopril, 1 [2(S)-3-mercapto-2-methyl-1-oxopropyl]-L-proline, was specifically designed (1,2) to competitively inhibit angiotensin-converting enzyme. It is an orally active antihypertensive agent (3,4) and may be effective in treating congestive heart failure. A method to detect the parent drug and its disulfide metabolites in plasma or urine is desired.



Existing Methods

The quantitation of captopril in biological fluids has been carried out by gas-chromatography(5), gas chromatography-mass spectrometry(6), radio thin-layer chromatography(7), and liquid chromatography with derivatization(8).

LCEC Method

Liquid chromatography with electrochemical detection furnishes a rapid, sensitive method for measuring captopril levels in plasma and urine. A dual Hg/Au electrode scheme is utilized, wherein eluting thiols and disulfides are detected in the same injection. The method is also applicable to disulfide metabolites formed from the reaction of the drug with a physiological thiol.

Captopril is excreted in the urine of treated patients, at mid - $\mu\text{g/mL}$ levels. Its disulfide metabolites, including captopril disulfide and captopril-cysteine mixed disulfide, are present at low $\mu\text{g/mL}$ concentrations.

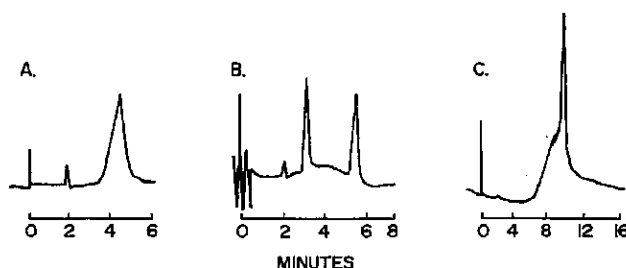


Figure 1. Chromatograms of captopril standards with varied chromatographic conditions: A) Biophase ODS 5 μm , 25% CH_3CN / 75% 0.1 M monochloroacetate, pH 3.0; B) Biophase ODS 5 μm , 10% CH_3OH / 90% 0.2 M perchlorate, 0.005 M citrate, pH 6.5; and C) Biophase Cyano, 0.1 M phosphate, pH 2.0.

Conditions

System: A BAS 200 liquid chromatograph is ideal for the assay since it contains the built-in deoxygenation utilities required for dual Hg/Au electrode operation. Otherwise, if O_2 remains in the mobile phase, background currents become prohibitively high and preclude low detection limits and long electrode lifetimes. The BAS 200 is unique in that no gas-permeable (plastic) tubing is used up to the detector; hence it is easy to maintain deoxygenated conditions.

A BAS 400 system may be adapted to deoxygenated operation by adding user-supplied glassware with mild heating and sparging. Construction details are provided in the manuals.

Column: Biophase ODS 5 μm (BAS P/N MF6017), 250 x 4.6 mm

Mobile Phase: 20-25% CH_3CN : 80-75% 0.1 M sodium phosphate buffer (pH 2.0), depending on column conditions. Deoxygenate.

Flow Rate: 1.3 - 1.5 mL/min

Temperature: 40°C

Detector Conditions: Upstream Hg/Au electrode: -1.0 V, 5-10 μA f.s. Downstream Hg/Au electrode: +0.15 V, 2-200 nA f.s., as necessary depending on sample.



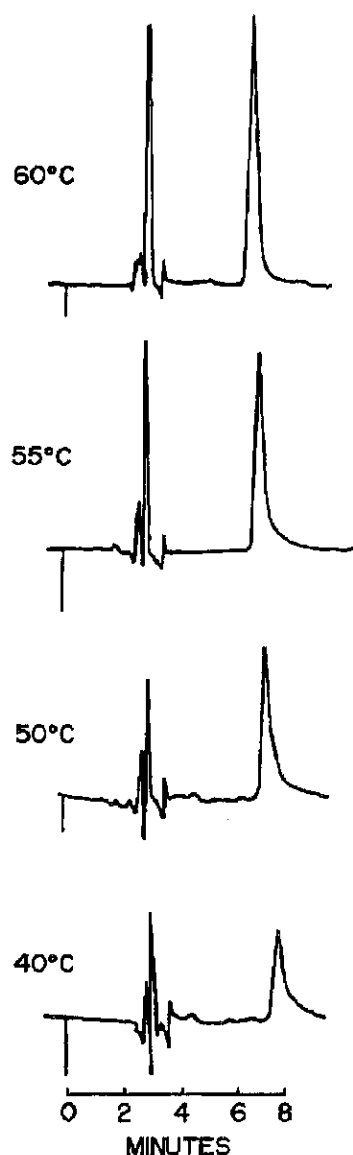
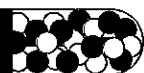


Figure 2. Effect of increased temperature on captopril peak shapes. Biophase ODS 5 μ m column, 25% CH_3CN / 75% 0.1 M phosphate, pH 2.0. Each chromatogram represents an injection of the same standard solution of captopril.

The electrode is prepared as described in LCEC Application Capsules 171 or 192.

Reagents

1M HClO_4 . Place 100-300 mL of deionized, distilled water in a 500 mL volumetric flask. Carefully add 43 mL of 70% HClO_4 . Dilute to volume using deionized water.

0.1 M HClO_4 . Mix 9 mL of 70% HClO_4 with approximately 100 mL of deionized, distilled water in a 1 liter

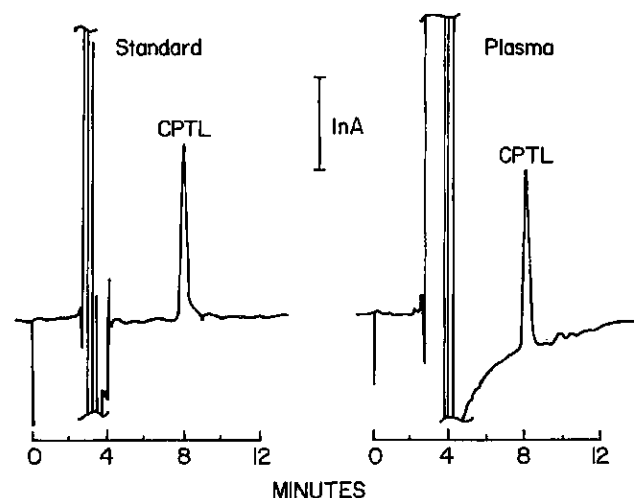


Figure 3. LCEC chromatograms for captopril, with conditions as in F2: (A) standard, 25 pmoles captopril injected and (B) spiked plasma prepared as in text, 25 pmoles injected.

volumetric flask. Add 0.5 g Na_2EDTA and dilute to 1 liter with deionized water. Warm and stir to dissolve EDTA.

Captopril Standard Solutions. Dissolve 67.8 mg captopril in 25 mL acetonitrile. Store in freezer. Prepare fresh standards daily by diluting 1-20 μ L aliquots of stock standard to 100 mL with 0.1 M HClO_4 , producing captopril concentrations of 0.125 to 2.5 μ M.

Plasma Procedure

1. To 500 μ L of fresh plasma in a 1.5 mL centrifuge tube, immediately add 200 μ L 1 M HClO_4 to precipitate proteins. Vortex briefly and let stand 10 minutes.
2. Centrifuge at 1500 x g or higher for 5 minutes.
3. Transfer supernatant into a BAS Microfilter with 0.2 μ m RC-58 membrane. Centrifuge at 1500 x g to obtain an injectable filtrate.
4. Inject 100 μ L of filtrate.

Urine Procedure

1. To 1.0 mL urine add 200 μ L of a solution containing 1 M HClO_4 and 3 g/L disodium EDTA.
2. Centrifuge at 1500 x g for 3 minutes and inject supernatant.

Chromatography

An extensive study was required to optimize chromatographic conditions for captopril, which demonstrates quite unusual behavior under routine chromatography. Octadecylsilane (Biophase ODS), cyano (Biophase Cyano), and macroreticular styrene-divinyl-benzene copolymer (Hamilton PRP-1) columns were tested with a variety of mobile phase pH's and temperatures. The chromatograms shown in F1 were selected to illustrate the unique characteristics of captopril.

The observed behavior is thought to be due to hindered rotation about the "peptide" bond of captopril, which causes the molecule to have cis and trans conformation. Apparently the cis and trans rotamers have different retention characteristics, but are slowly interconverting on the LC time scale, producing unusual peak shapes and multiplets of peaks. A helpful discussion of this phenomenon for proline dipeptides similar to captopril has appeared in the literature(11).

Mobile phase pH and temperature were the factors ultimately responsible for satisfactory chromatography of captopril. The optimum performance was obtained with a Biophase ODS column and pH 2.0 mobile phase. The effects of increasing temperature with this system are described in F2, showing the improvement in peak shape that resulted from operating at 40-60°C.

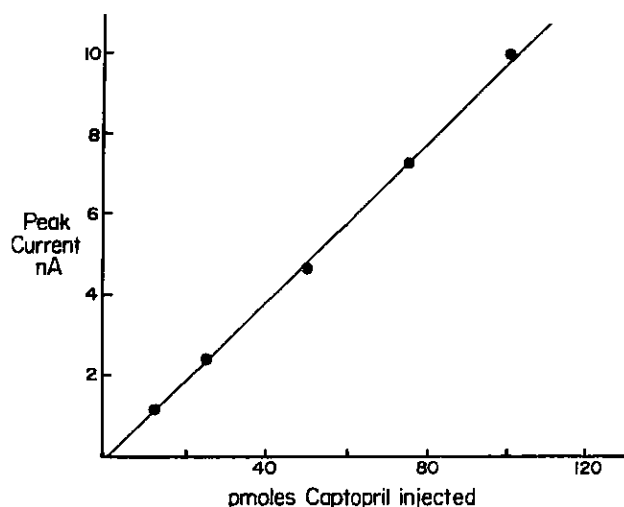


Figure 4. Linearity of LCEC for captopril, (correlation coefficient = 0.999, slope = 0.1 nA/pmole, y-intercept = - 0.20 nA).

Captopril disulfide elutes farther out due to its increased hydrophobicity. As long as captopril is cleanly separated from early peaks in the run, disulfide elution may be accelerated by increasing the acetonitrile concentration.

Assay Performance

Chromatograms of a standard solution of captopril and of a fresh plasma sample spiked with captopril are shown in F3. The sample was spiked to a concentration of 250 nmoles/L, which was reported to be a reasonable therapeutic level(9). The plasma chromatogram is clean and free from interferences even at this high sensitivity (5.4 ng injected) because of the extreme selectivity inherent in using the Hg/Au electrode at these potentials. Only biological thiols and halide ions will produce a signal, and these polar species are rapidly eluted before captopril.

The linearity of the system is illustrated by the standard curve for captopril detailed in F4. Detection limits were 2.0 picomole injected (0.4 ng), with a S/N of 3. This corresponds to a plasma concentration of 20 nmoles/L.

F5 shows a chromatogram of urine spiked with 10 µg/mL captopril (46 nmoles/mL) and 20 µg/mL captopril disulfide (46 nmoles/mL). The LCEC system would also provide peaks for the mixed disulfides of captopril, cysteine, and glutathione in real clinical samples.

Conclusions

Direct electrochemical detection greatly simplifies the assay of plasma levels of captopril. The high degree of selectivity allows samples to be injected after only precipitation of proteins. Perrett and Drury(9) have also reported determination of captopril levels in urine using LCEC.

Another paper(12) took an interesting alternate approach to captopril determination by LCEC. The thiol group was derivatized with an electroactive reagent, then a series of extractions and evaporations led to an injectable solution. The detection limits were slightly higher than the direct detection method. The extensive sample cleanup was required because they were not utilizing the selective mercury-sulfhydryl reaction.

References

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CAPTOPRIL IN URINE

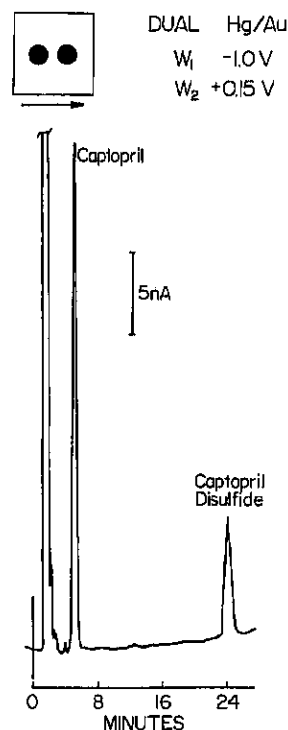


Figure 5. Dual Hg/Au chromatograms of acidified urine spiked with captopril (10 $\mu\text{g/mL}$) and its disulfide (20 $\mu\text{g/mL}$).

