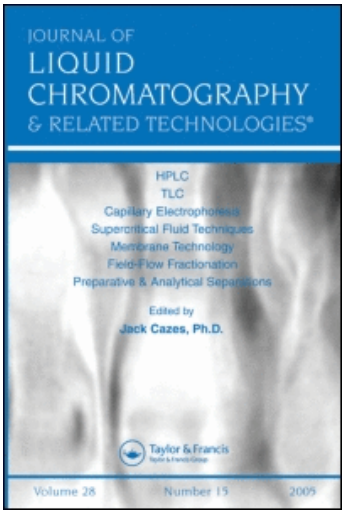

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THE DETERMINATION OF CAPTOPRIL IN PHYSIOLOGICAL FLUIDS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTRO-CHEMICAL DETECTION.

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ABSTRACT

A method has been devised for the measurement of the anti-hypertensive drug, captopril in physiological fluids. The drug is separated by chromatography on an ODS-column using a pH2 phosphate buffer/methanol (65:35) eluant and detected at a mercury electrochemical cell maintained at +0.07V v Ag/AgCl. The maximum sensitivity is greater than 1pmol injected onto the column. The application of the methodology to acid deproteinated plasma and urine from patients receiving captopril has been demonstrated.

INTRODUCTION

Captopril (SQ 14225, D-3-mercapto-2-methyl propanoyl-L-proline) is the first orally active inhibitor of angiotensin I converting enzyme (peptidyl dipeptidase hydrolase, kininase II, E.C.3.4.15.1) to become available for clinical use (1,2). It is a potent antihypertensive agent in both renovascular and essential hypertension (2), being of especial value in hypertension resistant to conventional drugs (3). Its use in con-

gestive cardiac failure also shows great promise (2,4).

Comparatively little is known of the pharmacokinetics and pharmacodynamics of captopril (5). Current methods for measurement of the drug in biological fluids are cumbersome, requiring extensive extraction and derivitisation prior to assay (6-11). As it has been shown that the predominant route of excretion is via the kidney (5), whose function is often impaired in hypertension, an assay for the drug in plasma might therefore be of value in optimising the dosage, especially as there is evidence that side-effects may be dose related (5,11).

Recently Rabenstein and Saetre (12) introduced a mercury electrode for the quantitation of cysteine and related thiols following separation on a cation exchange column. The oxidation reaction;

$$Hg + 2RSH \longrightarrow Hg(RS)_2 + 2H^+ + 2e$$

occurs at the electrode and should be applicable to any thiol.

We have therefore developed a method for the separation of captopril by reversed phase liquid chromatography followed by quantitation using an electrochemical detector.

EXPERIMENTAL

Materials

The chromatographic system consisted of a Altex 110 pump with 2m of 0.4mm bore stainless steel tubing provid-

ing additional pulse damping and a Bioanalytical Systems LC-4 electrochemical detector equipped with a Gold/Mercury cell. The Au/Hg cell was maintained at +0.07V versus Ag/AgCl reference electrode. A 100mm length and 5mm diameter column equipped with a syringe injector (HETP, Macclesfield, England) was slurry packed in the laboratory with 5µm ODS-Hypersil (Shandon, Runcorn, England). The column was connected to the electrochemical cell by 15cm of 0.15mm bore p.t.f.e. tubing. The output from the electrochemical cell was recorded on a 0-10mV servoscribe recorder.

Captopril was kindly donated by E.R. Squibb and Co. Potassium dihydrogen phosphate A.R. and orthophosphoric acid A.R. were obtained from BDH Chemicals and methanol from Rathburn Chemicals (Walkerburn, Scotland). Water was distilled and then de-ionised immediately prior to use.

The eluant, consisting of 100mmol/l aqueous KH_2PO_4 adjusted to pH2 with H_3PO_4 and containing 35% methanol, was filtered and then continuously degassed with oxygen-free nitrogen. The column was eluted at a flow rate of 1ml/min.

Samples

Stock captopril standards (1mmol/l) were prepared in 0.1M HCl. Captopril containing urine was collected directly into containers containing 1ml 6M HCl/100ml

urine. Freshly drawn heparinised blood was centrifuged at 4^{o} at 2000g for 5 min. Plasma proteins were immediately precipitated by the addition of 100µl sulphosalicylic acid (25mg/100µl) to 1ml of plasma. Following a further 5 min centrifugation the supernatant was rapidly frozen, then stored at -20^{o} until analysis. Immediately prior to analysis the supernatant was spun at 2000g for 5 min and 10 - 25µl were injected onto the column.

RESULTS

Although captopril could be directly oxidised at a glassy carbon electrode, the potential required for maximum response with the mercury electrode was substantially lower (Fig. 1). At voltages above +0.08V the decreased peak height was accompanied by a sharp increase in peak tailing. Sensitivity was increased 50-fold using the Hg-electrode. At the potential required with the glassy carbon electrode (1.2V) a number of other substances in plasma interferred with the chromatography whereas few significant peaks were found in normal plasma using the Hg-electrode.

When captopril standards were eluted with methanol: water mixture from ODS columns, the compound eluted as a triple peak (Fig. 2). This was believed to be a pH effect and when phosphate buffers of varying pH from 2 - 8 were studied, below pH3 only one major peak occurred

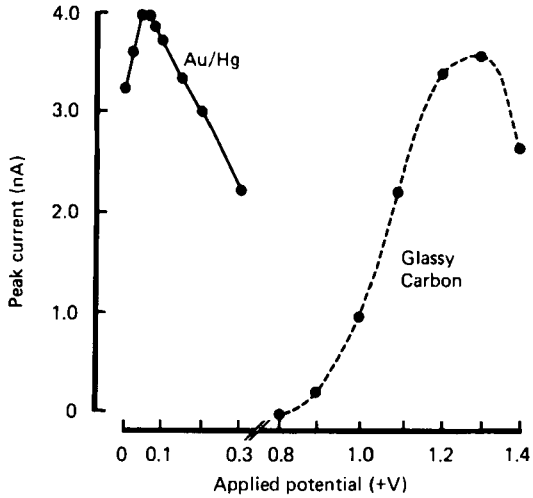

FIGURE 1. Hydrodynamic voltammogram for captopril on glassy-carbon and mercury electrodes, 5nmol and 100pmol was injected respectively.

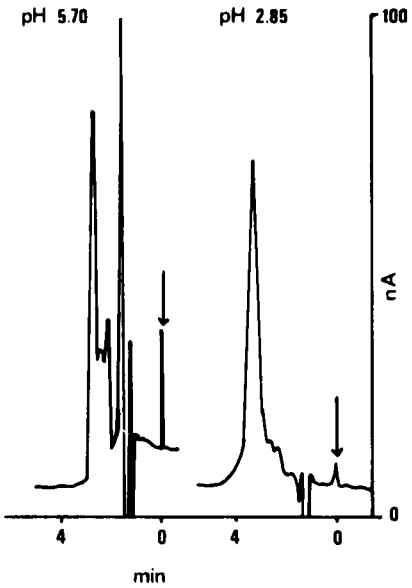

FIGURE 2. Effect of buffer composition on the elution profile of captopril. i) water/methanol 65:35 pH 5.70 and ii) 0.1M phosphate buffer/methanol 65:35 pH 2.85.

(Fig. 2). All subsequent studies were performed at pH2. Although a single peak was obtained it was relatively broad (N = 1300 plates) whereas the same column with the manufacturer's test mixture gave over 4500 plates. Attempts to improve the chromatography using ion pairing agents led to a rapid loss of sensitivity due to destruction of the mercury amalgam.

Fig. 3 AB shows standard chromatograms obtained following the injection of 10 and 2.5pmol of captopril under the conditions given in the methods. Maximum sensitivity $^{S}/_{N}$ = 2 was 400fmol. Reproducibility of the peak height following repeated injection of 100pmol was 99.8 ± 2.9mm (n=10). The detector response was linear in the range 0-1000pmol; a standard curve (0-100pmol) is shown in Fig. 4. Stock standard solutions (1mmol/l) prepared in 0.1M HCl were stable for at least 1 month when stored at 4^{o}, but it was found necessary to prepare working standards (1nmol/l) daily. It was necessary to remove plasma protein prior to sample application, most of the usual agents e.g. trichloroacetic acid, perchloric acid could be employed but sulphosalicylic acid was chosen on grounds of convenience.

Fig. 5 shows captopril in the plasma of a patient who had received 25mg of the drug 90 minutes earlier. The concentration was 240nmol/l of plasma. The captopril peak co- chromatographed with authentic compound added

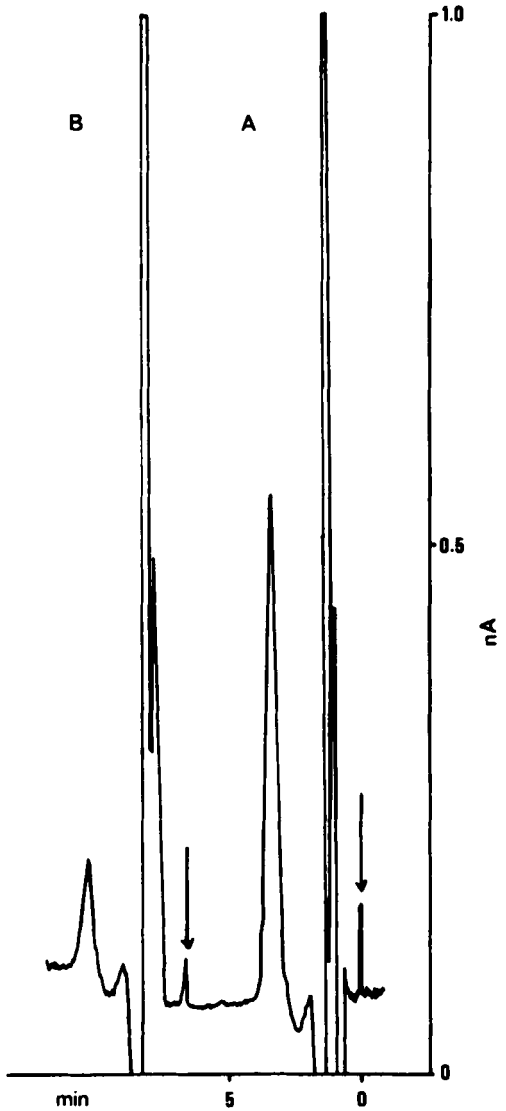

FIGURE 3. Chromatograms of A) 10pmol and B) 2.5pmol of captopril injected with conditions as in methods.

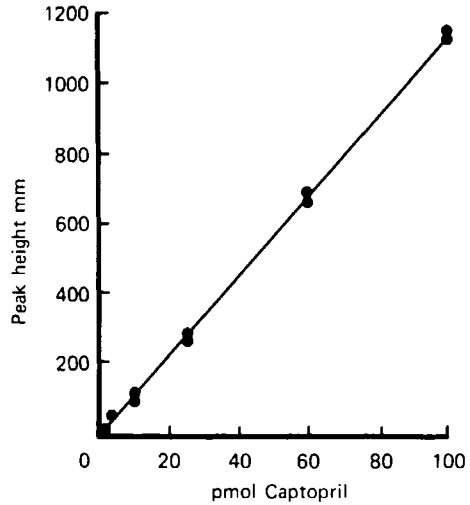

FIGURE 4. Standard curve for captopril.

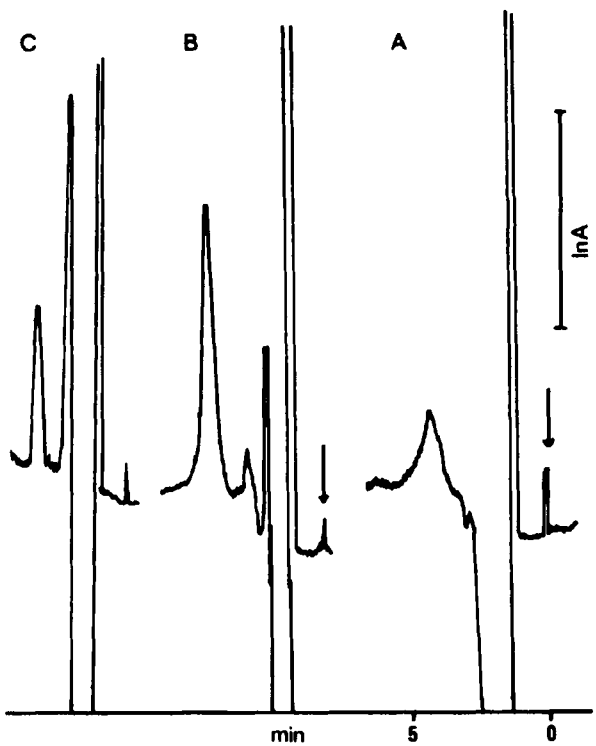

FIGURE 5. Captopril in physiological fluids. A) Plasma extract following 25mg dose. B) as A but with added captopril. C) Urine collected 1h after 6mg dose.

to the same plasma (Fig. 5B). Care was necessary to ensure that captopril was resolved from the large negative deflection. This deflection occurs in all plasma extracts and was not related to the protein precipitant used. At low methanol concentrations and at working potentials other than +0.07V it could broaden and interfere with quantitation. Recovery of captopril added to plasma and deproteinated immediately was 89 ± 4% (n=6). Captopril was rapidly lost from plasma if protein precipitation (and acidification) was delayed. In one experiment a 10% reduction in recoverable captopril occurred when deproteination was delayed for 15 minutes. Fig. 5C shows captopril in the urine collected from a patient who had received a 6mg dose one hour previously. The calculated concentration was 1.1μmol/l.

DISCUSSION

Thiols have always proved difficult to quantitate in physiological fluids although numerous spectrophotometric methods have been published. Difficulties are caused by the presence of naturally-occurring thiols and the instability of thiols in plasma and urine. Methods so far reported for captopril have therefore involved some form of chromatography prior to quantitation. GC (7,8), TLC (6) and HPLC (9,10) coupled with UV or fluorescent derivatisation (7.8.9.10), mass-spectroscopy (8) or radioisotopes have all been employed

to measure the drug in plasma. All the methodologies seem complex and time consuming. The mercury-based electrochemical detector used in the present method allows direct quantitation of the free thiol following a simple chromatographic step.

Difficulties such as oxidation to the disulphide during analysis and irreversible binding to column packings have been found to occur during the chromatography of thiols. In this study ODS-columns gave satisfactory separations only when low pH buffers were used. The large reduction in column efficiency found when captopril was chromatographed was acceptable only because the detector was highly specific. Even so sensitivity would be increased if a more efficient system could be found. Perlman and Kirschbaum (13) in a method published during the course of this study examined a number of packing materials in order to determine captopril in pharmaceutical preparations, and found problems of tailing, low efficiencies and split peaks. They recommended an ODS system, and ascribed the peak splitting to hindered rotation at the C-N bond. Monitoring the present system at their suggested wavelength (214nm) with a Cecil 212 detector gave a minimum level of detection of 50pmol, 100-fold less sensitive than the elctrochemical detector.

The sensitivity of the commercial mercury electrode used here has proved equivalent to that reported by Saetre and Rabenstein (12) for their mercury-pool electrode. With care the electrode response was reproducible and stable but at times sensitivity could fall dramatically. This was often reversed by pulsing the electrode with a voltage of +0.4V v Ag/AgCl to clean the mercury surface, but on other occasions a new mercury/gold amalgam was found necessary. Usually the amalgam was stable for two weeks before it required to be re-formed - a simple task.

The precautions outlined for the preparation of captopril containing fluids have not yet been rigorously determined but are those known to stabilise cysteine (14) and penicillamine (15) in plasma. Kawahara et al (9) have shown that below pH2 captopril solutions are stable and that at room temperature captopril is readily lost from plasma. The levels of captopril found in urine and plasma in this study are proportionately similar to those reported for D-penicillamine (16).

The mercury electrode will only detect free-SH groups it will not detect any metabolites of captopril where the -SH group is lost. By analogy with D-penicillamine (17) the following metabolites would be expe-

cted in the following order of decreasing abundance: captopril-cysteine disulphide, captopril disulphide, the S-methyl derivative and captopril-homocysteine disulphide. Unless the disulphides are reduced prior to analysis they will not be detected.

The method described can detect plasma and urinary captopril in man after a single oral dose of 6.25mg. It is thus sufficiently sensitive to allow measurement of plasma levels even with low-dose therapy and may have considerable application in monitoring captopril treatment as well as in pharmacokinetic studies. Clinical studies on captopril levels in man will be published in due course.

REFERENCES

1) Ondetti, M.A., Rubin, B., and Cushman, D.W. Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. Science 196, 441, 1977.

2) Atkinson, A.B., and Robertson, J.I. Captopril in the treatment of clinical hypertension and cardiac failure. Lancet 2, 836, 1979.

3) White, N.J., Yahaya, H., Ragagopalan, B., and Ledingham, J.G. Captopril and frusemide in severe drug resistant hypertension. Lancet 2, 108, 1980.

4) Dzau, V.J., Colucci, W.S., Williams, G.H., Curfman, G., Meggs, L., and Hollenberg, N.K. Sustained effectiveness of converting-enzyme inhibition in patients with severe congestive heart failure. N. Engl. J. Med., 302, 1373, 1980.

5) Heel, R.C., Brogden, R.N., Speight, T.M., and Avery, G.S. Captopril: A preliminary review of its pharmacological properties and therapeutic efficiency. Drugs, 20, 409, 1980.

6) Migdalof, B.H., Singhri, S.M., and Kripalani, K.J. Thin-layer radiochromatographic determination of captopril (SQ 14 224) and its disulfidodimer metabolite in blood. J. Liq. Chromatogr. 3, 857, 1980.

7) Matsuki, V., Fukuhara, K., Ho, T., Ono, H., Ohara, N., Yui, T., and Nambara, T. Determination of captopril in Biological fluids by Gas-Liquid chromatography. J. Chromatogr. 188, 177, 1980.

8) Funke, P.T., Ivashkiv, E., Malley, M.F. and Cohen, A.I. Gas chromatography/selective ion monitoring mass spectrometric determination of captopril in human blood. Anal. Chem. 52, 1086, 1980.

9) Kawahara, Y., Hisaoka, M., Yamazaki, Y., Inage, A., and Morioka, T. Determination of captopril in Blood and Urine by High Performance Liquid Chromatography. Chem. Pharm. Bull. 29, 150, 1981.

10) Jarrot, B., Plummer, O.H., Hooper, R., Miach, P. and Louis, W.J. Pharmacokinetics of captopril after acute and chronic administration to hypertensive subjects. J. Pharm. Sci., in press, 1981.

11) Editorial. Captopril: benefits and risks in severe hypertension. Lancet 2, 129, 1980.

12) Rabenstein, D.L. and Saetre, R. Mercury-based electrochemical detector for liquid chromatography for the detection of Glutathione and other sulphur-containing compounds. Anal. Chem. 49, 1036, 1977.

13) Perlman, S. and Kirschbaum, J. High-performance liquid chromatographic analyses of the antihypertensive drug Captopril. J. Chromatogr. 206, 311, 1981.

14) Perry, T.L. and Hansen, S. Technical pitfalls leading to errors in the quantitation of plasma amino acids. Clin. Chem. Acta 25, 53, 1969.

15) Bergstrom, R.F., Kay, D.R. and Wagner, J.G. The "in vitro" loss of penicillamine in plasma, albumin solutions and whole blood: Implications for pharmacokinetic studies of penicillamine. Life Sci. 27, 189, 1980.

16) Saetre, R. and Rabenstein, D.L. Determination of Penicillamine in Blood and Urine by High Performance Liquid Chromatography. Anal. Chem, 50, 276, 1978.

17) Perrett, D. The metabolism and Pharmacology of D. Penicillamine in man. J. Rheumatol. 8, Suppl 7, 41, 1981.