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# Determination of captopril in human blood by gas chromatography–negative-ion chemical ionization mass spectrometry with [$^{18}O_4$]captopril as internal standard

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## ABSTRACT

A method for the quantitative measurement of captopril in human blood is described. Blood was immediately treated with N-ethylmaleimide to prevent oxidative degradation. The carboxyl moiety was derivatized to the pentafluorobenzyl ester, which shows excellent properties for negative-ion chemical ionization mass spectrometry. A stable isotope-labelled standard was prepared from the intact target molecule in quantitative yield by exchanging the oxygen atoms of the free carboxylic acid and the imide moiety against $^{18}O$. The detection limit under negative-ion chemical ionization conditions is ca. 100 times lower than under electron-impact or positive-ion chemical ionization conditions, therefore only very small amounts of the original sample have to be analysed. The method was applied to be quantitative determination of unchanged captopril in human plasma after oral administration of a 25-mg dose.

## INTRODUCTION

Captopril, 1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline, is a potent inhibitor of angiotensin-converting enzyme [1–3]. It is widely used as an antihypertensive drug [4]. Metabolism has been shown to occur entirely by con-

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jugation via the thiol group as thiomethyl derivatives or disulphide conjugates with other thiol-containing compounds (cysteine, glutathione, etc.) [5–8]. Quantitation of captopril has been accomplished by thin-layer chromatography [6], high-performance liquid chromatography (HPLC) [9–12], spectrofluorometry [13], gas chromatography (GC) [14,15] and gas chromatography–mass spectrometry (GC–MS) [16–20]. The thiol group of captopril is readily oxidized, thus derivatization [generally by forming the Michael adduct with N-ethylmaleimide (NEM)] has to be carried out immediately after sampling. Though relatively stable, the NEM derivative may also be oxidized by solvent impurities during extraction and sample clean-up.

This paper describes a method for the determination of free captopril in human blood with GC–negative-ion chemical ionization (NICI) MS, using a stable-isotope-labelled standard, whose fragment ions do not interfere with the quantitation of captopril.

## EXPERIMENTAL

### *Materials*

NEM and diisopropylethylamine were purchased from Sigma (Munich, F.R.G.). Pentafluorobenzyl bromide was obtained through Supelco (Crans, Switzerland). $^2H_2{}^{18}O$ and $H_2{}^{18}O$ were from Ventron (Karlsruhe, F.R.G.). All other solvents and reagents of analytical grade were from Merck (Darmstadt, F.R.G.).

### *Gas chromatography*

A Finnigan 9610 GC was used with a DB-5 fused-silica capillary column from J&W (Rancho Cordova, CA, U.S.A.) (15 m × 0.25 mm I.D., 0.25 μm film thickness). The column was directly connected to the ion source of the mass spectrometer. Helium was used as a carrier gas. The splitless Grob-injector was kept at 290°C. The capillary column was kept at 180°C for 12 s, and then the temperature was raised to 320°C at 40°C/min. The final temperature was held until elution was complete. After 12 s the split valves were opened for 3 min to purge the injector.

### *Mass spectrometry*

A Finnigan 4500 mass spectrometer was used. NICI spectra were recorded with methane as a moderating gas at an ion source pressure of 0.3 Torr. The electron energy and emission current were set at 120 eV and 0.25 mA, respectively. In the multiple-ion detection (MID) mode the sampling time was set at 200 ms per mass.

*Preparation of standard solutions*

Stock solutions of the NEM derivative of captopril were prepared by dissolving 50 mg of captopril in 10 ml of phosphate buffer (0.1 *M*, pH 7.4) containing 250 mg of NEM and leaving the solution for 10 min at room temperature. Working solutions were obtained by dilution with acetone. Standard solutions were stored at −20°C.

*Pentafluorobenzyl ester formation*

Pentafluorobenzyl (PFB) esters were prepared by reaction with 100 μl of PFB bromide solution in acetonitrile (7%, w/w) and 20 μl of diisopropylethylamine at room temperature for 30 min.

*Methyl ester formation*

Methyl esters were prepared by treating the sample with a solution of diazomethane in diethyl ether–methanol (9:1, v/v) for 15 min at room temperature.

*Stable isotope labelling of captopril–NEM*

A 2-mg amount of the captopril–NEM adduct was dissolved in 100 μl of 5 *M* hydrochloric acid in $H_2{}^{18}O$ (prepared by bubbling in HCl gas into $H_2{}^{18}O$ until saturation, and dilution of the resulting 12 *M* HCl) and diluted with 100 μl of acetonitrile. The solution was carefully flushed with nitrogen and heated to 65°C in a tightly sealed vial for 20 h. After cooling, the solvent was removed on a rotary evaporator, and the residue was dissolved in methanol. An aliquot was derivatized with PFB bromide, and the isotopic distribution was checked by GC–NICI-MS. Stock solutions were standardized against unlabelled substance and stored at −20°C. The stability against back exchange of the $^{18}O$ atoms was checked by spiking a blank blood sample with the labelled standard and analysing it after 24 h at room temperature.

*Sample preparation*

Blood was collected by vacutainer, and 1 ml was immediately treated with 2 ml of a 0.5% solution of NEM in phosphate buffer (0.1 *M*, pH 7.4); the solution was mixed and left at room temperature for 15 min. After centrifugation (10 min, 1500 *g*), the supernatant liquid was collected and frozen at −20°C until analysis. Then 50 μl of a methanolic solution of the $^{18}O$-labelled standard (60 ng) was added to 1 ml of the thawed sample and mixed thoroughly. After the addition of 2.5 ml of ethyl acetate, the mixture was vortexed and centrifuged, and the organic layer was discarded. Next 4 ml of ethyl acetate were added along with 0.8 g of sodium chloride and 100 μl of 1 *M* hydrochloric acid, and the mixture was vigorously shaken. After centrifugation, the ethyl acetate layer was transferred to a clean glass vial, and the solvent was removed under a stream of nitrogen at 50°C. Traces of water were removed azeotropically with

dichloromethane. The dry residue was derivatized as described above, the reagent was removed under nitrogen, and the derivative was dissolved in 50 $\mu$l of benzene. An aliquot (0.5–1.0 $\mu$l) was subjected to GC–NICI-MS.

A calibration curve was established by spiking blank samples with the appropriate amounts of unlabelled captopril–NEM and processing them as described above. Quantitation was achieved by comparing the peak areas of unlabelled compound ($m/z$ 341) and internal standard ($m/z$ 349).

*Stability of captopril in blood*

To check the stability of captopril in blood, a sample drawn 1 h after oral administration of 25 mg of captopril to a volunteer was treated with the NEM buffer solution at 0, 10, 20, 30, 40 and 60 min after sampling.

## RESULTS AND DISCUSSION

In order to find a derivative with both good chromatographic properties and high sensitivity, as well as low fragmentation under NICI conditions, methyl esters and PFB esters of captopril–NEM have been investigated. The electrophoric nature of the substituted N-ethylsuccinimide, formed by Michael addition of thiols with NEM, has already been recognized [21]. Thus, a prominent response of both derivatives should be expected with NICI-MS. The NICI mass spectra of the methyl ester and the PFB ester are shown in Fig. 1 and Fig. 2a, respectively. The spectrum of the methyl ester is dominated by the fragment ion at $m/z$ 125, which accounts for more than 95% of the total ion current of the molecule and can be attributed to the NEM moiety. The deriv-

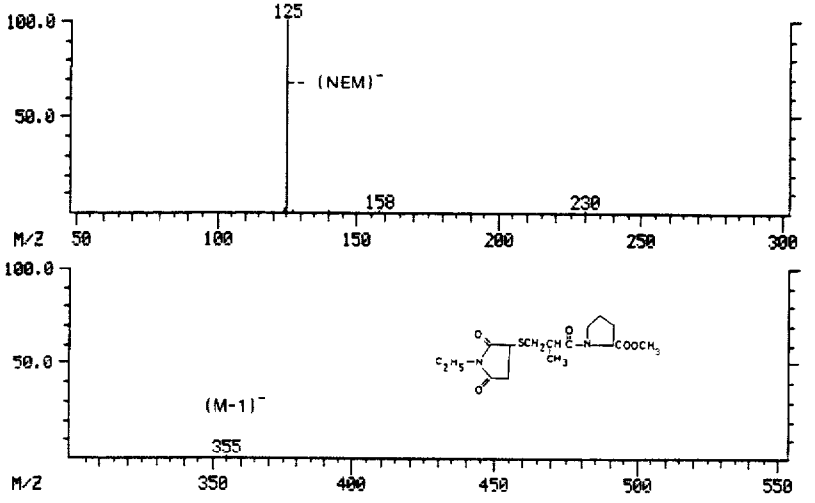


Fig. 1. Partial NICI mass spectrum of captopril–NEM methyl ester

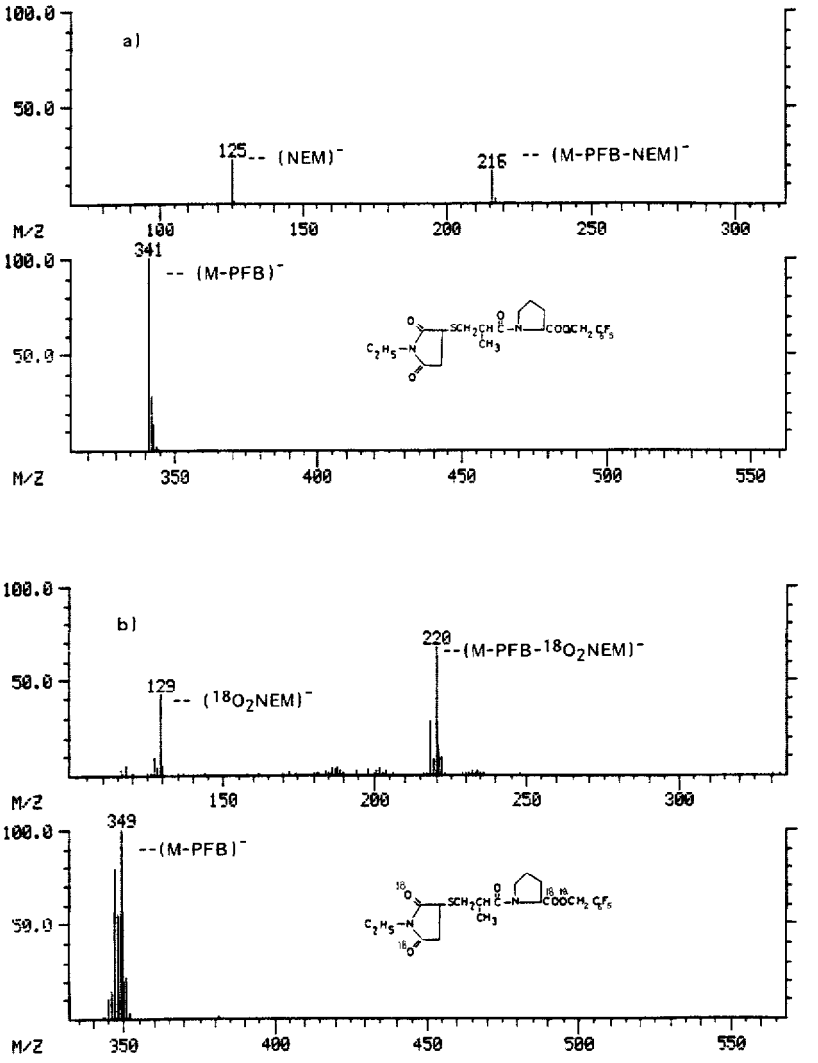


Fig. 2. Partial NICI mass spectrum of (a) unlabelled and (b) $^{18}O_4$-labelled captopril–NEM-PFB derivative.

ative, however, is not suitable for quantitative determinations out of biological matrices, since the same fragment ion is formed by other thiol groups and interference from endogenous compounds is very high at $m/z$ 125. The NICI mass spectrum of the PFB ester shows a fragment ion at $m/z$ 341, which results from loss of the PFB group. Inasmuch as both the carboxylate anion detected and the pentafluorotropylium cation formed are stabilized by resonance, this "quasimolecular ion" is of very high relative abundance and thus highly useful

for quantitative MS. Two fragment ions of minor abundance are at $m/z$ 125 and $m/z$ 216, which derive from the NEM group and by neutral loss of NEM after PFB elimination, respectively. Derivatization with NEM leads to the formation of two diastereoisomers, which are separable by GC (Fig. 3).

To achieve rapid access to a stable isotope-labelled standard, a method was developed for exchanging at least two oxygen atoms for $^{18}O$, starting from the intact target molecule. Because captopril is readily oxidized in solution, the NEM adduct was used. Efforts to obtain isotope incorporation by base-catalysed hydrolysis of the methyl ester in $H_2{}^{18}O$, as described earlier for prostaglandins and related compounds [22,23], failed owing to sample degradation and incomplete labelling. Acid-catalysed conversion led to a four-fold labelled product with no unlabelled species detectable. The NICI mass spectrum of its NEM-PFB derivative is shown in Fig. 2b. The fragment ions show an increase of 4 daltons for the NEM moiety ($m/z$ 129) and the carboxyl group (indicated by $m/z$ 220), thus suggesting an exchange reaction in both the carboxyl and the imide functions. This can be explained by acid-catalysed hydrolysis with subsequent ring opening, followed by intramolecular acid-catalysed re-formation of the cyclic imide. The reaction yielded a multiple labelled compound with no unlabelled species detectable. The ratio of the intensities of the fragment ions at $m/z$ 220, 218 and 216 reflects the $^{18}O$ content of the carboxylic acid group at the proline moiety. Thus, 70% are double-labelled, 30% are single-labelled, and no unlabelled material is present. The isotope distribution remained constant under the experimental conditions for captopril measurement. The isotopic composition of the labelled product is given in Table I.

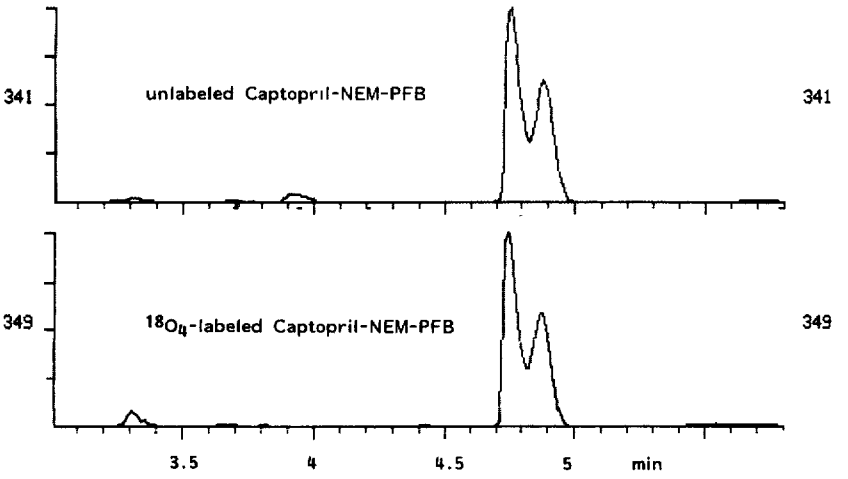


Fig. 3. Typical multiple ion detection mass chromatogram obtained after GC–NICI-MS analysis of a blood sample drawn from a volunteer 1 h after oral administration of 25 mg of captopril. The drug was analysed as its NEM adduct PFB ester.

TABLE I

ISOTOPIC COMPOSITION OF CAPTOPRIL-NEM AFTER ACID-CATALYSED OXYGEN EXCHANGE

| *m/z* | Percentage of total for (M−PFB) |
|---|---|
| 341 | 0.00 |
| 343 | 0.11 |
| 345 | 5.41 |
| 347 | 41.96 |
| 349 | 52.52 |

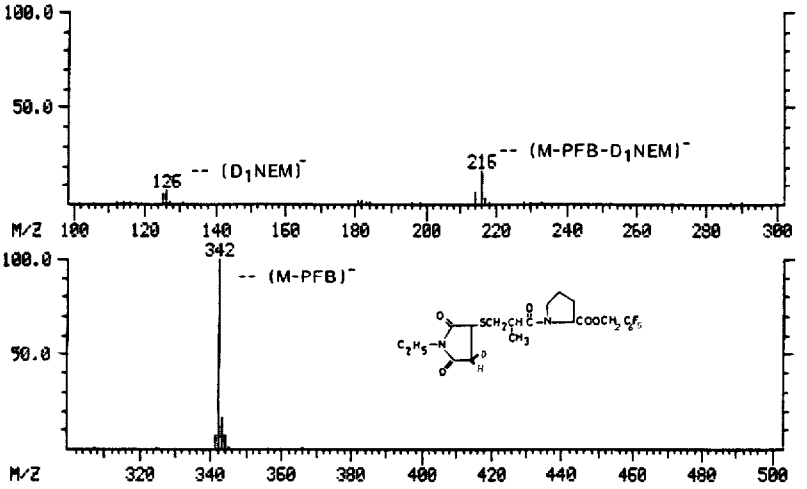


Fig. 4. Partial NICI mass spectrum of captopril after formation of its NEM adduct in $^2H_2O$ and esterification with PFB bromide.

Back-exchange studies revealed that there was no loss of label after 24 h in blood at room temperature.

Additional labelling can be achieved by carrying out the NEM adduct formation in $^2H_2O$. In this case, hydrogen at the thiol group is readily exchanged against deuterium, which adds to the double bond of NEM in the course of Michael addition. The mass spectrum of monodeuterated captopril–NEM-PFB ester is shown in Fig. 4. By combining these two procedures, an increase of 9 daltons can be achieved.

When biological samples have to be processed, it is of critical importance to add the NEM reagent immediately after sampling. Alterations of captopril levels measured as a function of delay in adding the NEM solution are shown in Table II.

TABLE II

STABILITY OF CAPTOPRIL IN HUMAN BLOOD

A blood sample drawn 1 h after administration of 25 mg of captopril to a volunteer was treated with the NEM buffer solution after the time delay indicated.

| Addition of NEM (delay in min) | Value measured (ng/ml) |
|---|---|
| 0 | 53.5 |
| 10 | 44.1 |
| 20 | 37.8 |
| 30 | 34.3 |
| 40 | 30.9 |
| 60 | 21.6 |

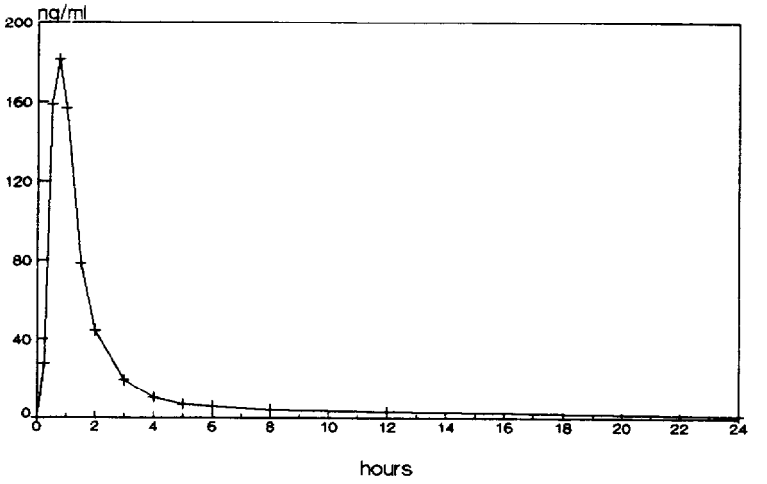


Fig. 5. Mean blood levels after oral administration of 25 mg of captopril to twelve volunteers.

A typical multiple ion detection mass chromatogram obtained after analysis of a sample drawn 1 h after oral administration to a human volunteer is shown in Fig. 3. A calibration graph was established, covering the range 0–300 ng/ml of blood. The coefficient of correlation ($r^2$) was 0.9999. Coefficients of inter- and intra-assay variation were calculated to be 2.13% and 1.15% at a concentration of 30 ng captopril per ml blood, respectively. The lower limit of detection was found to be 500 pg per ml blood. Detection limits in the low picogram range can be obtained by analysing more sample from a more concentrated solution.

The method was applied to the measurement of captopril in human volunteers receiving 25 mg of the drug orally. Blood samples were drawn at 0, 0.25,

0.30, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 h after administration of the drug. The results are shown in Fig. 5.

## CONCLUSIONS

The use of NICI-MS for detection of captopril as its NEM-PFB derivative is highly advantageous owing to the high sensitivity and specificity towards the quasimolecular ion of the compound. The multiple-labelled product shows an isotopic composition that remains constant throughout the captopril assay and is devoid of any unlabelled species. Basically, this method can be applied to the measurement of captopril metabolites as well. Owing to the high sensitivity of the assay, only very small aliquots of the original sample have to be processed and analysed, thus avoiding lengthy purification procedures. Since the $^{18}O$-labelled standard is readily available, a stable isotope dilution assay can be performed, thereby avoiding loss of sample relative to the standard.

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## REFERENCES

1 R.R. Vollmer, J.A. Boccagno, D.N. Harris and V.S. Murthy, Eur. J. Pharmacol., 51 (1978) 39.
2 B. Rubin, R.J. Laffan, D.G. Kotler, E.H.O. Keefe, D.A. DeMaio and M.E. Goldberg, J. Pharmacol. Exp. Ther., 204 (1978) 271.
3 D.N. Harris, C.L. Heran and H.J. Goldberg, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 37 (1978) 718.
4 R.C. Heel, R.N. Brogden, T.M. Speight and G.S. Avery, Drugs, 20 (1980) 409.
5 O.H. Drummer, B. Jarrott and W.J. Louis, Clin. Exp. Pharmacol. Physiol. Suppl., 7 (1982) 81.
6 K.J. Kripalani, D.N. McKinistry, S.M. Singhvi, D.A. Willard, R.A. Vukovich and B.H. Migdalof, Clin. Pharmacol. Ther., 27 (1980) 636.
7 B.K. Park, P.S. Grabowski, J.H.K. Jeung and A.M. Beckenridge, Biochem. Pharmacol., 31 (1982) 1755.
8 T. Ikeda, T. Komai, K. Kawai and H. Shindo, Chem. Pharm. Bull., 29 (1981) 1416.
9 K. Hayashi, M. Miyamoto and Y. Sekine, J. Chromatogr., 338 (1985) 161.
10 Y. Kawahara, M. Hisaoka, Y. Yamazaki, A. Inage and T. Morita, Chem. Pharm. Bull., 29 (1981) 150.
11 B. Jarrott, A. Anderson, R. Hooper and W.J. Louis, J. Pharm. Sci., 70 (1981) 665.
12 K. Shimada, M. Tanaka, T. Nambara, Y. Imai, K. Abe and K. Yoshinaga, J. Chromatogr., 227 (1982) 445.
13 E. Ivashkiv, J. Pharm. Sci., 73 (1984) 1427.
14 Y. Matsuki, K. Fukuhara, T. Ito, H. Ono, N. Ohara and T. Yui, J. Chromatogr., 188 (1980) 177.

15 M.S. Bathala, S.H. Weinstein, F.S. Meeker, jr., S.M. Singhvi and B.H. Migdalof, J. Pharm. Sci., 73 (1984) 340.
16 O.H. Drummer, B. Jarrott and W.J. Louis, J. Chromatogr., 305 (1984) 83.
17 P.T. Funke, E. Ivashkiv, M.F. Malley and A.I. Cohen, Anal. Chem., 52 (1980) 1086.
18 A.I. Cohen, R.G. Devlin, E. Ivashkiv, P.T. Funke and T. McCormick, J. Pharm. Sci., 71 (1982) 1251.
19 E. Ivashkiv, D.N. McKinstry and A.I. Cohen, J. Pharm. Sci., 73 (1984) 1113.
20 Y. Matsuki, T. Ito, K. Fukahara, T. Nakamura, M. Kimura and H. Ono, J. Chromatogr., 239 (1982) 585.
21 M. Jemal and A.I. Cohen, Anal. Chem., 57 (1985) 2407.
22 H.J. Leis, E. Malle, R. Moser, J. Nimpf, G.M. Kostner, H. Esterbauer and H. Gleispach, Biomed. Environ. Mass Spectrom., 13 (1986) 483.
23 R.C. Murphy and K.L. Clay, Methods Enzymol., 86 (1982) 547.