BIOMEDICAL CHROMATOGRAPHY, VOL. **12**, 57–60 (1998)

3-Bromomethyl-propyphenazone as a New Derivatization Reagent for High Performance Liquid Chromatography of Captopril and Hydrochlorothiazide with UV- Detection

Alaa Khedr[1] and Hosny El-Sherief[2]*

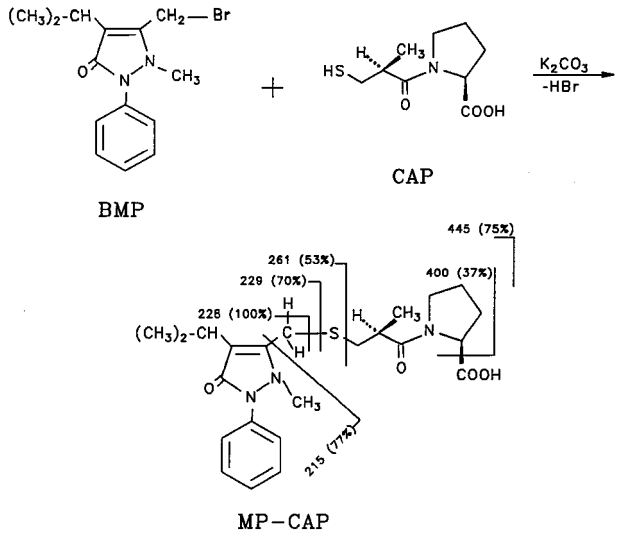
[1] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt
[2] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt

3-Bromomethyl-propyphenazone (BMP) was used as a derivatization reagent for the detection and quantification of captopril (CAP) and hydrochlorothiazide (HCT) by high performance liquid chromatography using Zorbax C8 column, and 0.05M sodium acetate, acetonitrile, methanol (14:17:4; pH 6.5) as mobile phase system with UV-detection at 254 nm. The cited reagent reacts with the mercapto and amino groups of CAP and HCT in acetone using anhydrous potassium carbonate as hydrobromide acceptor. The reaction was completed within 30 min for CAP and 60 min for HCT with heating at 105 ± 5°C in mini-reaction vial. The linear concentration ranges for both CAP and HCT were 8 to 160 and 6 to 140 ng per injection, respectively. The derivatized captopril was synthesized and confirmed with spectral analysis. This method was applied for determination of spiked captopril in human urine after extraction with Extrelut-20 column using ethyl acetate:isopropanol (85:15 v/v) as eluant. © **1998 John Wiley & Sons, Ltd.**

Biomed. Chromatogr. **12**, 57–60 (1998)

INTRODUCTION

Captopril (CAP) is a hypotensive drug which is commonly co-formulated together with hydrochlorothiazide (HCT) as diuretic. Sincce CAP has insignificant UV-absorption, many methods have been adopted to enhance its UV-absorption. The following substances have been reported as a derivatization reagents for HPLC analysis of CAP on reversed phase columns with UV-detection at 254–260 nm; p-Bromophenacyl bromide (Kawahara *et al*., 1981; Klein *et al*., 1990; Tian *et al*., 1992 and Jankowski *et al*., 1995), *N*-(4-benzoylphenyl)maleimide (Hayashi *et al*., 1985) and *N* - (4-dimethylamino - 3,5 - dinitrophenyl) - maleimide (Kawahara *et al*., 1981 and Klein *et al*., 1990). In addition, different fluorogenic derivatizing reagents have been reported for determination of CAP, among which; *N*-(1-pyrene)-maleimide (Jarrott *et al*., 1981), 7-fluorobenzofurazan-4-sulfonic acid (Boekens *et al*., 1988), ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (Ling *et al*., 1989), o-phthaldialdehyde in presence of secondary amine (Peterkova *et al*., 1990) and dicarbocyanine labels (Mank *et al*., 1994). Besides, most methods described for determination of HCT have not considered the combination with CAP (Kuo *et al*., 1990 and Ulvi *et al*., 1994). CAP and HCT have been simultaneously determined by HPLC, depending on measuring the UV-response at 220 nm (Jain, R. and Jain, C., 1991). 3-Bromomethyl-propyphenazone, have been synthesized by Meister Lucius and Brüning (1907), and used as a coupling agent for the synthesis of famprofazone and famprofazone analogues (Somogyi and Hofstetter, 1951 and Rücker *et al*., 1982). In this work, BMP as derivatization reagent reacted with mercapto and amino groups of captopril and hydrochlorothiazide, respectively (Scheme 1). The derivatized compounds have shown maximum UV-absorption at 243 and 284 nm. The reaction conditions were optimized to ensure complete derivatization of both compounds.



Scheme 1. Reaction of BMP with CAP and mass spectral schematic MP-CAP, *m/z* (abundance %).

EXPERIMENTAL

Apparatus. The HPLC system consisted of an UV-8 model II Spectrophotometer detector (TSK TOYO SODA, Japan), a Gynkotek pump (model 300, Germering, Germany), a Shimadzu integrator (C-R 3A, Chromatopac, Kyoto, Japan) and a Du Pont Column Oven (Du Pont Instrument, Wilmington, USA). The

* Author to whom correspondence should be addressed.

CCC 0269–3879/98/020057–04 $17.50

Received 6 January 1997
Accepted 30 January 1997

column used was Du Pont Zorbax C8 (4.6 mm i.d. × 25 cm, 6 μm particle diameter). The sample loop was 10 μL. The Chromatographic peaks were UV-scanned with the use of Photodiodarray detector (Tsp Thermo Separation Products, Spectromonitor 5000, Connecticut, USA) controlled with LCtalk program (LCtalk version 2.03 software, copyright 1993 by Thermo Separation Products, Connecticut, USA). Heating block was constructed personally from aluminum, designed for half insertion of the reaction vials and heated over controlled temperature hot plat. Screw capped borosilicon mini-reaction vials 1-mL with TFE liners were used for sample preparation (Alltech, GmbH, Unterhaching, Germany). ^{1}H NMR spectrum was recorded on JEOL JNM-EX 270 MHz Spectrometer (Tokyo, Japan), sample was dissolved in acetone using TMS as internal standard. EI-mass spectral analysis was recorded on JEOL JMS-SX 102 AQQ, 70 eV (Tokyo, Japan). Infrared spectrum was recorded on IR-470 spectrometer (Schimadzu Corporation, Tokyo, Japan).

Materials. Captopril and hydrochlorothiazide were gifts from Bristol-Myers Squibb (Cairo, Egypt). Propyphenazone was obtained from Caeser & Loretz (Hilden, Germany). Deionized-distilled water in glass was used for preparation of 0.05 M sodium acetate. Acetonitrile, methanol, acetone (all HPLC grade), TLC-silica gel 60 F254 aluminum sheets, silica gel GF254 and Extrelut-20 bags were perchased from E-Merck (Darmstadt, Germany). All other solvents used were analytical grade. 3-Bromomethyl-propyphenazone (BMP) was synthesized from propyphenazone and bromine, according to Meister Lucius and Brüning, 1907, recrystallized from chloroform:diethylether (1:2 v/v), and tested for its purity by TLC and HPLC analysis. The structure of synthesized BMP was confirmed by ^{1}H NMR and IR analysis.

Mobile phase. Mobile phase was prepared by mixing 140 mL 0.05 M sodium acetate, 170 mL acetonitrile and 40 mL methanol. Then adjusted to pH 6.5 with few drops of acetic acid. The column oven was adjusted at 35°C to resume day to day temperature variation.

Standard solutions. Ten milligrams of BMP was dissolved in 10 mL acetone, and kept in dark using aluminum foil. Ten milligrams from CAP and HCT were dissolved in 10 mL acetone. One milliliter from this solution was diluted to 10 mL with acetone to be used for preparation of calibration BMP-derivatized solution.

Synthesis of captopril-BMP derivative (MP-CAP). Into a 50 mL round flask, 4.34 g of captopril (0.02 mol) was dissolved in 20 mL acetone containing 2 g anhydrous potassium carbonate. This solution was heated to 60°C, and 6.18 g of 3-bromomethyl propyphenazone (dissolved in 10 mL acetone, 0.02 mol) was added dropwise. Double sided water condenser was constructed and the reaction mixture was refluxed with stirring for 2 h. Finally, the reaction mixture was cooled and dried under vacuum at 50°C together with stream of nitrogen gas. The oily residue was mixed with 50 mL distilled water, acidified with acetic acid and shaken with 150 mL $CHCl_3$. The organic layer was separated, dried over anhydrous sodium sulphate, and evaporated to dryness under vacuum with stream of nitrogen. The oily yellowish residue was purified with preparative TLC using *n*-hexane:isopropanol:acetone (6:1:1 by vol) as developing system (yield, 52%). The derivatized captopril and excess reagent were eluted at R_f: 0.45 and 0.75, respectively, and located by viewing under UV-lamp at 254 nm. The structure of derivatized captopril was confirmed by ^{1}H NMR 270 MHz (mp = 50°C, uncorrected). IR; = 1658 cm^{-1} (amidic C = O), 1746 cm^{-1} (carboxylic C = O).

Derivatization of CAP and HCT. The derivatization of both compounds were carried separately and in mixture using borosilicate screw capped mini-reaction vials of 1-mL capacity. Certain volume from both CAP and HCT (3–80 μL) was transferred to a reaction vials, mixed with 50 μL BMP (1 mg/mL), mixed with ca. 1 mg anhydrous K_2CO_3, and adjusted to 200 μL with acetone. The vials were capped and allowed to stand in heating block at 105 ± 5°C for 30 min (for CAP only) or 60 min (for HCT or CAP and HCT in mixture). The reaction mixture was cooled, dried under gentle stream of nitrogen gas, reconstituted in 500 μL acetonitrile and shaken for 2 min. Ten microliter was finally injected for HPLC analysis at 254 nm with flow-rate of 1 mL/min. This procedure was repeated five times for nine different concentrations from both CAP and HCT.

Extraction of CAP from spiked human urine. One litre urine collected from healthy persons, free from drugs and food preservatives, was spiked with 0.5 mg captopril. Ten milliliters from this solution was diluted to 15 mL with water and extracted with Extrelet-20 columns, using 60 mL ethylacetate:isopropanol (85:15 v/v) as eluant. The eluant was concentrated by evaporation under vacuum at 50°C, filtered with syringe filtration disc and transferred quantitatively to the reaction vial. The solution was then dried under stream of nitrogen gas, reconstituted in 100 μL acetone, and 100 μL BMP solution (1 mg/mL) was added. This mixture was mixed with ca. 1 mg anhydrous K_2CO_3, and proceed as under derivatization. This procedure was repeated five times using five different concentrations of spiked urine parallel with non-spiked urine samples from the specified persons.

RESULTS AND DISCUSSION

Captopril has insignificant UV-absorbance at 200 nm, so its derivatization was an essential requisite for UV detection by high pressure liquid chromatographic analysis.

Factors affecting derivatization

The derivatization reaction was more preferable to proceed in a minimal amount of dry acetone than chloroform or dichloromethane. However, it was better to dissolve the final reaction mixture in acetonitrile to avoid large peak corresponding to acetone eluted at retention time of 2–3.5 min. Anhydrous potassium carbonate was found to enhance the derivative formation in quantitative yield.

Monitoring of the reaction mixture for HCT:BMP indicated that relative peaks eluted at 12, 14.4 and 27.7 min. This may be contributed to the presence of three amino centers which could be of different reactivity toward BMP. Therefore fully derivatized HCT was completely attained after heating for 50 min, and eluted as single peak at 27.7 min. Meanwhile for CAP, having only one reactive centre, no reaction byproducts appeared and fully derivatized product was achieved after heating for 20 min. Accordingly, optimum reaction time was found to be 30 and 60 min at 105 ± 5°C for CAP and HCT, respectively (Figs. 1 and 2). The reagent and final reaction product were stable for more than one week if kept in dark at room temperature. The molar concentration of BMP (100 μL, 1 mg/mL) must be four times more concentrated as CAP or HCT, which was sufficient for derivatization of sample urine extract [Figure 1(b)].

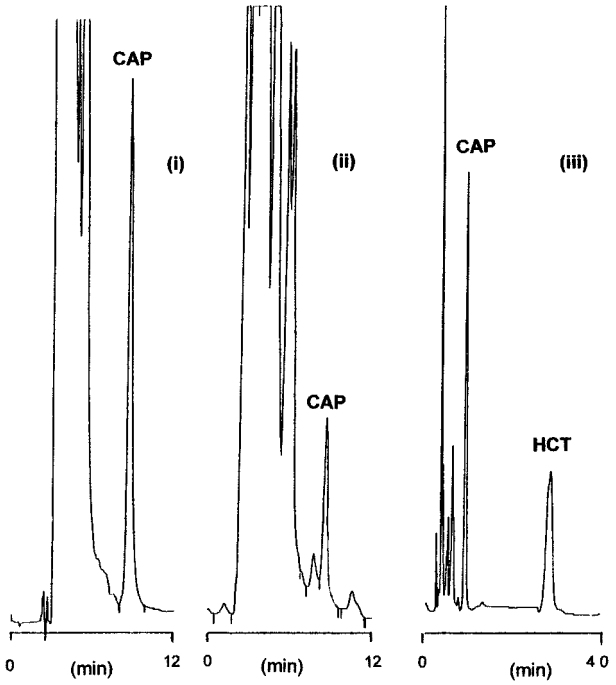


Figure 1. Chromatograms of BMP—derivatives each of; captopril (a); captopril from spiked urine (b) and mixture of captopril and hydrochlorothiazide (c).

Chromatographic conditions

The cited mobile system was found to be suitable for the analysis of BMP derivatives, using Zorbax-C8 column. However, Zorbax-ODS, was not suitable for the analysis of HCT derivative due to high retention time and band broadening. However, upon using Zorbax-C8 column, underivatized HCT was eluted at 3.3 min, but fully derivatized HCT was eluted isocratically at 27.7 min with band broadening [Figure 1(c)]. This broadening could be avoided with gradient elution by increasing the percentage of acetonitrile:methanol mixture from 55% at 9 min to 100% at 16 min. With this procedure the derivatized HCT could be eluted at 14 min as sharp narrow peak with a precise quantitative results (SD, 2.3, for 10 replicate experiments).

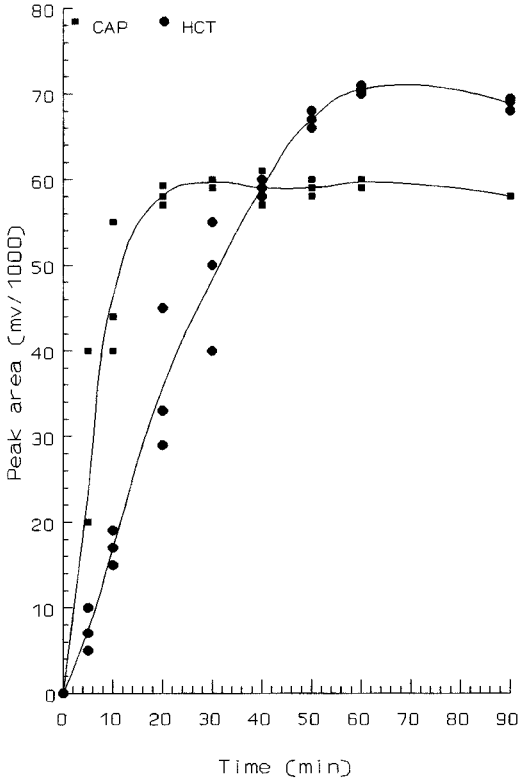
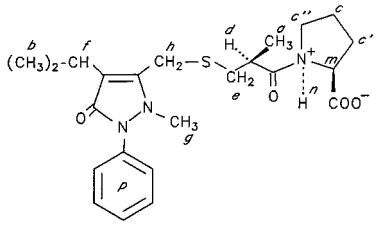


Figure 2. Reaction time course of CAP and HCT with BMP at 110°C (both, 70 ng/injection).

Table 1. ^{1}H NMR data of MP-CAP (270 MHz).



Proton	σ (ppm)	Integration (xH)
a	1.15 (*d*)	3H
b	1.25 (*d*)	6H
c and c′	2.00–2.15 (*m*)	4H
d	2.68 (*m*)	1H
e	2.80 (*m*)	2H
f	2.90 (*m*)	1H
g	3.05 (*s*)	3H
c″	3.67 (*t*)	2H
h	3.84 (*s*)	2H
m	4.48 (*t*)	1H
n	5.16 (*q*)	1H
p	7.35 (*m*)	5H

Linear calibration range

The calibration curves for CAP and HCT were linear over the range from 8 to 160 ng and 6 to 140 ng per injection, respectively ($r = 0.994$–0.999). The relative standard deviation of the peak area for ten replicate measurements of both CAP and HCT at 70 ng per injection was 0.9–2.3. The detection limits attained were 3 and 2 ng per injection for both compounds respectively, at a signal-to-noise ratio of 5.

Extraction of CAP from urine

The recovered amount of CAP from spiked human urine was found to be $100 \pm 0.5\%$, by the use of Extrelut-20 columns, without need of sample pretreatment. The amount of BMP required for complete derivatization of urine extract was 100 μL (1 mg/mL). Figure 1(b), show the derivatized extracted urine spiked with CAP. Figure 1 (a and b), indicates the derivatized CAP eluted at 8.6 min, intense peak at 2–3.5 min corresponding to acetone and excess reagent peak at 5.6 min.

Structural confirmation of MP-CAP

The derivatized CAP was investigated by UV-scan, ^{1}H NMR, IR, MS, TLC and HPLC analysis. Two UV-absorption maxima were recorded at 284 and 243 nm for MP-CAP which were identical with that of BMP itself (289 nm). Thin layer chromatography of MP-CAP and BMP have shown R_f values of 0.45 and 0.75, respectively

using *n*-hexane:isopropanol:acetone as developing system (15:2.5:2.5 by vol). The separated spots were scratched, dissolved in the mobile system and filtered with syringe filteratin disc for HPLC analysis, which confirm the same reaction product. Infrared spectrum (in KBr disc) of MP-CAP was compared with that of CAP and BMP, where, S-H was not observed (at 2500 cm^{-1}), in addition to strong bands at 1746 cm^{-1} with broad band at 3475 cm^{-1} corresponding the carboxylic group, and strong band at 1658 cm^{-1} corresponding the amidic carbonyl group of both CAP and BMP moieties. Table 1, shows the ^{1}H NMR data of TLC-purified MP-CAP, which confirm alkylation at SH- functional group of CAP. Besides, mass spectral analysis has shown base peak at 228 *m/z*, corresponding to heterocleavage of CAP moiety, in addition to other characteristic fragment ion peaks (scheme 1).

Conclusively, 3-hydroxymethyl-propyphenazone (BMP) could be used as a derivatization reagent for HPLC detection and determination of captopril (CAP) in urine with UV-detection. The sensitivity of this method was comparatively higher than those using *p*-bromophenacyl bromide, *N*-(4-benzoylphenyl)maleimide, or *N*-(4-dimethylamino-3,5-dinitrophenyl)maleimide for CAP (Kawahara *et al*., 1981; Hayashi *et al*., 1985; Klein *et al*., 1990; Tian *et al*., 1992 and Jankowski *et al*., 1995). Also, CAP and HCT mixture could be qualitatively and quantitatively analysed.

REFERENCES

Boekens, H., Foullois, M. and Mueller, R. F. (1988). *Fresenius Z. Anal. Chem.* **330**, 431.

Hayashi, K., Miyamoto, M. and Sekine, Y. (1985). *J. Chromatogr., Biomed. Appl.* **39**, 161.

Jain, R. and Jain, C. L. (1991). *Indian Drugs.* **28**, 380.

Jankowski, A., Skorek, A., Krzysko, K., Zarzycki, P. K., Ochocka, R. J. and Lamparczyk, H. (1995). *J. Pharm. Biomed. Anal.* **13**, 655.

Jarrott, B., Anderson, A., Hooper, R. and Louis, W. J. (1981). *J. Pharm. Sci.* **70**, 665.

Kawahara, Y., Hisaoka, M., Yamazaki, Y., Inage, A. and Morioka, T. (1981). *Chem. Pharm. Bull.* **29**, 150.

Klein, J., Colin, P., Scherer, E., Levy, M. and Koren, G. (1990). *Ther. Drug Monit.* **12**, 105.

Kuo, B.-S., Mandagere, A., Osborne, D. R. and Hwang, K.-K. (1990). *Pharm. Res.* **7**, 1257.

Ling, B. L., Baeyens, W. R. G., Marysael, H., Straggier, K. and De Moerloose, P. (1989). *J. Liq. Chromatogr.* **12**, 3135.

Mank, A. J. G., Lingeman, H. and Gooijer, C. (1994). *J. High Resolut. Chromatogr.* **17**, 797.

Meister Lucius and Brüning in Höchest, AM. Patentschrift Nr. 206637, Klasse 12p, Gruppe 8, Patentiert im Deutschen Reiche vom 3 October 1907 ab.

Peterkova, M., Matousova, O. and Rejholec, V. (1990). *Cesk. Farm.* **39**, 80; *Anal. Abstr.* (1990). **5G58.**

Rücker, G., Mrongovius, R. and Neugebauer, M. (1982). *Arch. Pharm.* (Weinheim), **315**, 839.

Somogyi, J. C. and Hofstetter, E. Geistlich Söhne, AG. für Chemische Industrie, Patentschrift Nr. 275620, Swiss, 1 sept. 1951.

Tian, W. R., Gao, S. and Wang, S. X. (1992). *Yaoxue Xuebao.* **27**, 613; *Anal. Abstr.* (1993). **8G165**, 1091.

Ulvi, V. and Keski-Hynnila, H. (1994). *J. Pharm. Biomed. Anal.* **12**, 917.

