*Journal of Chromatography*, 578 (1992) 203–206
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6396

# Determination of dioxopiperazine metabolites of quinapril in biological fluids by gas chromatography–mass spectrometry

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(First received January 14th, 1992; revised manuscript received March 31st, 1992)

ABSTRACT

The dioxopiperazine metabolites of quinapril in plasma and urine were extracted with hexane–dichloroethane (1:1) under acidic conditions. Following derivatization with pentafluorobenzyl bromide and purification of the desired reaction products using a column packed with silica gel, the metabolites were analysed separately by capillary column gas chromatography–electron-impact mass spectrometry with selected-ion monitoring. The limits of quantitation for the metabolites were 0.2 ng/ml in plasma and 1 ng/ml in urine. The limits of detection were 0.1 ng/ml in plasma and 0.5 ng/ml in urine, at a signal-to-noise ratio of $>3$ and $>5$, respectively. The proposed method is applicable to pharmacokinetic studies.

## INTRODUCTION

Quinapril HCl, 2-{2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl}-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid monohydrochloride, is a new potent and orally active angiotensin-converting enzyme inhibitor (ACEI) [1,2]. Quinapril is hydrolysed to the pharmacologically more active metabolite quinaprilat, and also additionally converted into the dioxopiperazine metabolites [3].

We have already reported the use of gas chromatography–negative-ion chemical ionization mass spectrometry (GC–NICI-MS) for the determination of quinapril and quinaprilat in biological fluids [4]. In order to characterize the pharmacokinetics of administered quinapril, it is necessary to determine not only quinapril and quinaprilat but also two other dioxopiperazine metabolites. However, for the determination of the dioxopiperazine metabolites of ACEI, the earlier methods are still unsatisfactory in the area of sensitivity [3,5].

This paper describes the development of a highly sensitive method for the determination of the dioxopiperazine metabolites of quinapril in plasma and urine by means of GC combined with electron-impact (EI) ionization MS.

## EXPERIMENTAL

### *Chemicals and reagents*

The metabolites, PD109488 (I) and PD113413 (II), were supplied by Warner-Lambert/Parke-Davis (Ann Arbor, MI, USA, Fig. 1). The dioxopiperazine analogues of CI-907 and CI-907 diacid [6] (used as the internal standards for compounds I and II, respectively) were synthesized

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0378-4347/92/$05.00 

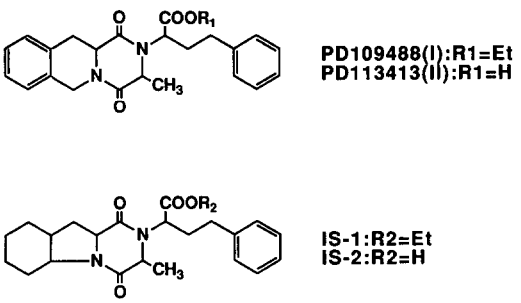


Fig. 1. Structures of the dioxopiperazine metabolites and the internal standards.

by the Chemistry Division of our Research Laboratories. Pentafluorobenzyl bromide (PFB-Br) and diisopropylethylamine were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All other chemicals (Nacalai Tesque, Kyoto, Japan) were of analytical-reagent grade and used without further purification. Silica gel, Kieselgel 60 (Merck, Rahway, NJ, USA) packed in a Pasteur pipette (25 mm × 6 mm I.D., Corning, Huntsville, AL, USA), was used without further activation.

*Gas chromatography–mass spectrometry*

A JEOL JMS-DX300 GC–MS system (Tokyo, Japan) equipped with a fused-silica capillary column (Ultra-2, 10 m × 0.32 mm I.D., 0.52 μm film thickness, Hewlett-Packard, Palo Alto, CA, USA) was used. Compounds I and II were assayed separately under different GC conditions. The column temperature was held initially at 272°C (compound I) or 286°C (compound II) for 1 min, then programmed at 16°C/min to 310°C (compound I) or 316°C (compound II). The injection port, interface and ion source were kept at 300, 280 and 280°C, respectively. The ionization energy was 70 eV and the emission current was 300 μA. Helium was used as the carrier gas at a flow-rate of 1.0 ml/min. The sample was injected by using a splitless solvent-cut injector (JEOL, Tokyo, Japan).

*Preparation of working standard and calibration curves*

Both compounds I and II were dissolved in methanol at a concentration of 25 μg/ml. The solution was diluted with methanol to produce the working standard (100 ng/ml), which was stored at 4°C until assay. Calibration curves in the range 0.2–10 ng per tube were constructed by analysing the sample containing 5 ng internal standard (I.S.) per tube. Quantitation was based on the peak-area ratios of the compounds and their I.S. *versus* absolute amounts of compounds I and II. The best-fit straight lines were determined by the least-squares method.

*Sample preparation*

To 1 ml of plasma or urine were added 5 ng of the I.S., 1 ml of 1 *M* HCl and 2.5 ml of hexane–dichloroethane (1:1). The vial contents were mixed for 15 min and centrifuged (1500 *g* for 5 min), and the organic layer was evaporated to dryness under vacuum. The residue was dissolved in 50 μl of dimethylformamide and treated with 5 μl of diisopropylethylamine and 2 μl of PFB-Br. After standing for 15 min at an ice-cold temperature, the sample was evaporated to dryness under vacuum. The residue was dissolved in 2 ml of hexane–ethyl acetate (5:1) and applied to a column (25 mm × 6 mm I.D.) of silica gel, which was preconditioned with hexane–ethyl acetate (5:1). The column was washed with 4 ml of the same solvent, then the sample was eluted with 5 ml of hexane–ethyl acetate (1:1). The eluate was evaporated to dryness under vacuum, and the residue was reconstituted in 50 μl of ethyl acetate, and 5 μl of the resulting solution were injected into the GC–MS system.

## RESULTS AND DISCUSSION

Typical EI mass spectra of compound I and the PFB derivative of compound II are illustrated in Fig. 2. Molecular ions of both compounds were observed. For selected-ion monitoring (SIM) analysis, fragment ions at *m/z* 316 (compound I), 468 (the PFB derivative of compound II), 308 (I.S. 1) and 460 (the PFB derivative of I.S. 2), which were formed by the elimination of the phenylethyl moiety, were used. The SIM chromatograms of the extracts from plasma and urine are shown in Figs. 3 and 4. The chromato-

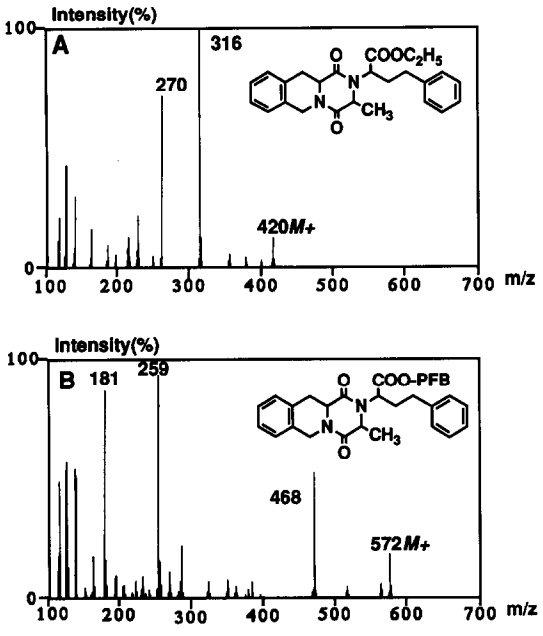


Fig. 2. EI mass spectra of PD109488 (A) and the PFB derivative of PD113413 (B).

graphic analysis was complete within 4 min, and no interfering peaks derived from endogenous components were observed.

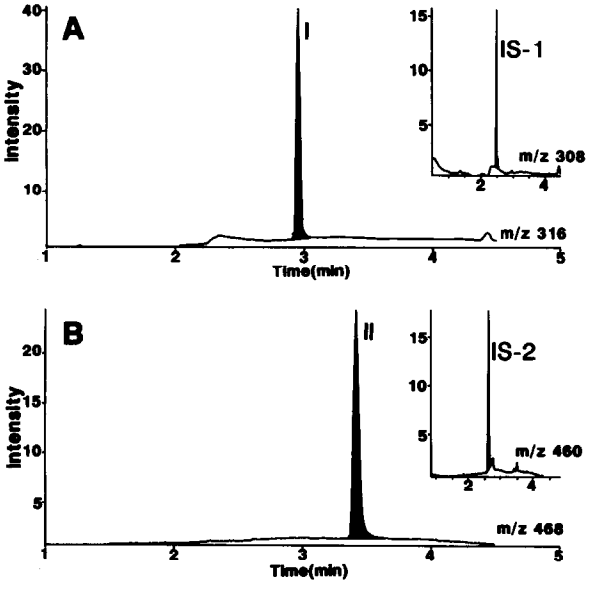


Fig. 3. SIM chromatograms of human plasma spiked with 10 ng/ml PD109488 (A) and PD113413 (B).

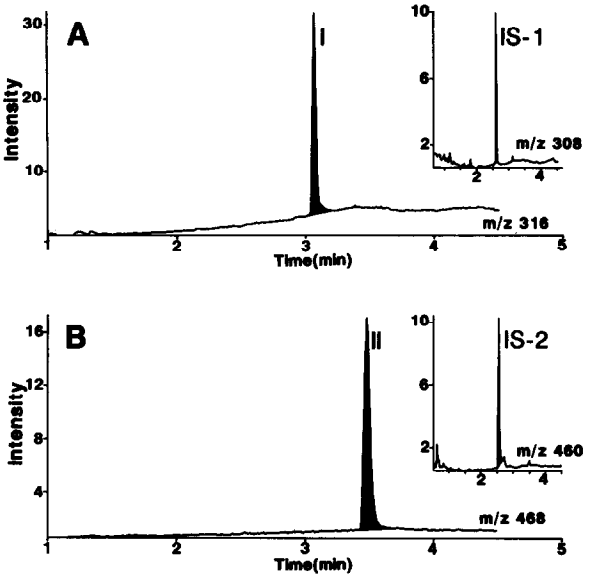


Fig. 4. SIM chromatograms of human urine spiked with 50 ng/ml PD109488 (A) and PD113413 (B).

We had to eliminate quinapril and quinaprilat from biological fluids prior to the assay, because they are chemically converted into their dioxopiperazine forms during sample preparation. By using the extraction method with hexane–dichloroethane (1:1) at a pH less than 3, the dioxopiperazine metabolites were quantitatively extracted, and quinapril and quinaprilat were completely retained in the biological fluids.

The calibration curves were obtained in the range 0.2–10 ng. The equations of the regression lines for compounds I and II were $y = 1.26x -$

TABLE I

RECOVERY OF PD109488 AND PD113413 IN PLASMA

Values are mean ± S.D., $n = 9$; mean values over three days.

| Concentration added (ng/ml) | Recovery (%) | |
|---|---|---|
| | PD109488 | PD113413 |
| 0.2 | 96.1 ± 5.4 | 108.2 ± 6.0 |
| 0.5 | 94.2 ± 2.4 | 100.8 ± 4.6 |
| 2 | 91.7 ± 2.5 | 99.9 ± 3.4 |
| 10 | 93.0 ± 5.3 | 100.9 ± 4.5 |
| Total | 93.7 ± 4.2 | 102.5 ± 5.6 |

TABLE II

RECOVERY OF PD109488 AND PD113413 IN URINE

Values are mean ± S.D., $n$ = 6; mean values over three days.

| Concentration added (ng/ml) | Recovery (%) | |
|---|---|---|
| | PD109488 | PD113413 |
| 1 | 94.6 ± 5.6 | 95.8 ± 3.4 |
| 2.5 | 89.6 ± 8.2 | 95.8 ± 2.2 |
| 10 | 92.5 ± 1.9 | 93.7 ± 3.2 |
| 50 | 96.8 ± 4.4 | 98.4 ± 3.4 |
| Total | 93.4 ± 5.8 | 95.9 ± 3.4 |

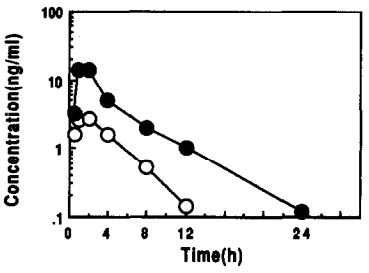


Fig. 5. Typical plasma concentration–time profiles for PD109488 (○) and PD113413 (●) after oral administration of 10 mg of quinapril.

0.020 ($r$ = 0.9998) and $y = 0.737x - 0.0081$ ($r$ = 0.9998), respectively. Tables I and II show the quantitative total-assay recovery over a three-day period. The limits of quantitation were 0.2 ng/ml in plasma and 1 ng/ml in urine. The limits of detection were 0.1 ng/ml in plasma and 0.5 ng/ml in urine, at a signal-to-noise ratio of $>3$ and $>5$, respectively. The sensitivity of the proposed method is 50–100 fold higher than those reported previously [3,5].

The present method was applied to the measurement of the dioxopiperazine metabolites in a human volunteer receiving 10 mg of quinapril orally. The results are shown in Fig. 5. We obtained the exact pharmacokinetic parameters by using our method.

## CONCLUSION

The proposed method permits the quantitative determination of the dioxopiperazine metabolites of quinapril at a concentration down to 0.2 ng/ml in plasma and 1 ng/ml in urine. The method is specific, reproducible and highly sensitive, and hence is useful for pharmacokinetic studies.

## ACKNOWLEDGEMENTS

The authors thank T. Hamasaki, K. Kishiro and R. Takamatsu for helpful discussions during these studies and K. Ikeda for the synthesis of the internal standards.

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