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Trace analysis of quinapril and its active metabolite, quinaprilat, in human plasma and urine by gas chromatography–negative-ion chemical ionization mass spectrometry

Nobuharu Goto, Taeko Kamata and Kiyoteru Ikegami

Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., 3-7-25, Koyata, Iruma-shi, Saitama 358 (Japan)

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ABSTRACT

A highly specific and sensitive method for the simultaneous determination of quinapril and its active metabolite, quinaprilat, in human plasma and urine by gas chromatography–negative-ion chemical ionization mass spectrometry is described. Quinapril and quinaprilat were extracted from human plasma and urine by using a Bond-Elut C₁₈ cartridge. The plasma or urine extract was treated with pentafluorobenzyl bromide followed by trifluoroacetic anhydride to convert quinapril and quinaprilat into their pentafluorobenzyl-trifluoroacetyl derivatives, which were analysed by a selected-ion monitoring method using deuterium-labelled internal standards. The limits of quantitation for both quinapril and quinaprilat were 0.05 ng/ml in plasma and 0.5 ng/ml in urine. The proposed method is applicable to pharmacokinetic and clinical pharmacological studies with satisfactory reliability.

INTRODUCTION

Quinapril HCl, 2-{2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl}-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid monohydrochloride (I, Fig. 1) is a potent and orally active angiotensin-converting enzyme inhibitor (ACEI) [1,2]. Quinapril is a prodrug which, on absorption, is hydrolysed to the pharmacologically more active metabolite, quinaprilat (II) [3].

Quantitation of quinapril and quinaprilat has been accomplished by high-performance liquid chromatography [4] and by gas chromatography (GC) [5]. These earlier methods had limits of detection of order of 5–20 ng/ml in plasma and 50–

100 ng/ml in urine, so that the plasma concentrations of quinapril and quinaprilat in human volunteers could be measured for only *ca.* 5 and 24 h, respectively, after administration of a 20-mg dose of quinapril. In order to establish accurate pharmacokinetic and pharmacodynamic parameters, a more sensitive method is required.

This paper describes the development of a

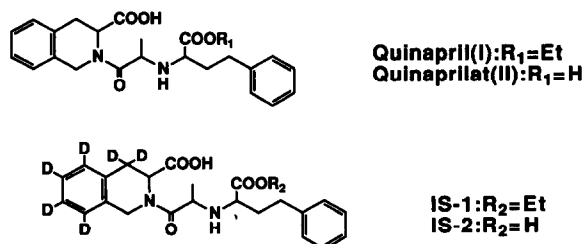


Fig. 1. Structures of quinapril, quinaprilat and the internal standards.

Correspondence to: Dr. N. Goto, Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., 3-7-25, Koyata, Iruma-shi, Saitama 358, Japan.

highly sensitive and specific method for determination of quinapril and quinaprilat in human plasma and urine by means of gas chromatography–negative-ion chemical ionization mass spectrometry (GC–NICI–MS).

EXPERIMENTAL

Chemicals and reagents

Quinapril HCl and its active metabolite quinaprilat were supplied by Warner-Lambert/Parke-Davis (Ann Arbor, MI, USA). The corresponding deuterium-labelled internal standards (I.S. 1 and I.S. 2, respectively, Fig. 1) were synthesized by the Chemistry Division of our Research Laboratories. A Bond-Elut C₁₈ cartridge and a vacuum-extraction manifold (Vac Elut) were obtained from Analytichem International (Harbor City, CA, USA). Silica gel, Kieselgel 60 (Merck, Rahway, NJ, USA) was used without further activation. Pentafluorobenzyl bromide (PFB-Br), trifluoroacetic anhydride (TFAA) and diisopropylethylamine were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All other chemicals (Wako, Osaka, Japan) employed were of analytical-reagent grade and used without further purification.

Gas chromatography–negative-ion chemical ionization mass spectrometry

A VG TRIO-1 quadrupole mass spectrometer (VG Masslab, Altrincham, Cheshire, UK) equipped with a Hewlett-Packard 5890A gas chromatograph (Palo Alto, CA, USA) was used. A fused-silica capillary column (Ultra-1, 10 m × 0.32 mm I.D., 0.17 μm film thickness, Hewlett-Packard) was directly inserted into the ion source. Helium was used as the carrier gas at a head pressure of 9807 Pa. The samples were injected with a splitless solvent-cut injector (G L Science, Tokyo, Japan). The injector, interface and ion source temperature were kept at 300, 295 and 200°C, respectively. The column temperature was initially held at 200°C for 2 min, then programmed at 70°C/min to 300°C. The mass spectrometer was operated in the NICI mode, with an ionization energy of 70 eV and an emission cur-

rent of 350 μA. Methane was used as the reagent gas.

Working standards

Working standards of compounds I and II were freshly prepared on the day of use. Compounds I and II were dissolved in 0.5% NaHCO₃ at a concentration of 100 μg/ml as the free bases. After the sample was diluted with distilled water, we obtained the working standards at concentrations of 30 and 3 ng/ml. Working standards of the I.S. were prepared similarly.

Sample preparation

Sample preparation was done by a modification of the method described by Ferry *et al.* [5]. Briefly, to 1 ml of plasma or 0.1 ml of urine, the I.S. solution was added. The sample was diluted with 1 ml of 0.2 M KCl–HCl solution (pH 0.8) and applied to a Bond-Elut C₁₈ cartridge, which was placed on a Vac Elut. After successive washing with 1 ml of 0.007% HCl and twice with 3 ml of hexane, the sample was eluted twice with 0.4 ml of chloroform–2-butanol (2:1). The eluate was evaporated to dryness under a stream of nitrogen at 40–50°C, and the residue was treated for derivatization as described below.

Derivatization procedure

The residue was treated with 50 μl of dimethylformamide, 5 μl of diisopropylethylamine and 4 μl of PFB-Br, and then kept at an ice-cold temperature for 20 min. The reaction mixture was evaporated to dryness under a stream of nitrogen at 40–50°C. The residue was dissolved in 1 ml of hexane–ethyl acetate (4:1) and applied to a column (20 mm × 7 mm I.D.) of silica gel, which was preconditioned with 2 ml of hexane–ethyl acetate (4:1). The column was washed with 3 ml of hexane–ethyl acetate (4:1), and the sample was eluted with 2.0 ml of 0.2% diisopropylethylamine in hexane–ethyl acetate (1:1). The eluate was evaporated to dryness under a stream of nitrogen at 40–50°C. The residue was treated with 1 ml of 0.2 M KCl–HCl solution and 4 ml of cyclohexane. The vial contents were then mixed and centrifuged (1500 g for 5 min), and the upper organic

layer was discarded. Then, 1 ml of 1 M $\text{NaHCO}_3\text{--Na}_2\text{CO}_3$ buffer (pH 9.5) was added to the aqueous phase, and the alkaline products were extracted with 3 ml of hexane–2-butanol (98:2). The organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.25 ml of ethyl acetate, treated with 0.5 ml of 5% TFAA in hexane, and left at 60°C for 15 min. The sample was evaporated to dryness under a stream of nitrogen, and the residue was dissolved in hexane–ethyl acetate (10:1) and applied to a column (20 mm \times 7 mm I.D.) of silica gel, which was preconditioned with 2 ml of hexane–ethyl acetate (10:1). The column was washed with 2 ml of hexane–ethyl acetate (10:1), and the sample was eluted with 2 ml of hexane–ethyl acetate (4:1). The eluate was evaporated to dryness under a stream of nitrogen, the residue was reconstituted in 0.1 ml of acetone, and 1–5 μl were injected into the GC system.

Calibration

Calibration curves were prepared daily by adding known amounts of compounds I and II to 1 ml of human plasma or 0.1 ml of human urine.

Plasma samples in the range 0–0.3 ng/ml contained 50 pg of I.S. 1 and 1 ng of I.S. 2; those in the range 0.1–30 ng/ml contained 1 ng of each I.S. The standard daily responses were collected and consecutively cumulated to calculate an overall least-squares linear regression of the peak-area ratio *versus* drug concentrations.

Urine samples in the range 0–0.5 ng/ml of compound I and 0–50 ng/ml of compound II contained 1 ng of each I.S.; those in the range 5–200 ng/ml of compound I and 20–1000 ng/ml of compound II contained 10 ng of each I.S. The samples were analysed daily and calculated as described above.

RESULTS AND DISCUSSION

Derivatization with PFB-Br

The PFB derivative of a carboxylic acid possesses excellent qualities for GC–MS analysis, particularly NICI-MS analysis [6–9]. However, derivatization with PFB-Br for quantitation at

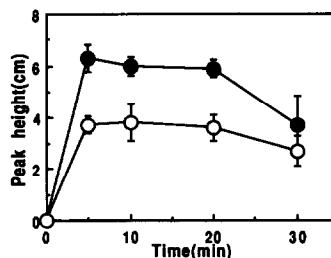


Fig. 2. Time-courses for the derivatization of quinapril (○) and quinaprilat (●) with PFB-Br at an ice-cold temperature. A 50-ng amount of quinapril and quinaprilat was treated with 4 μl of PFB-Br in the presence of 5 μl of diisopropylethylamine. Results are shown as mean \pm S.D., $n = 3$.

the picogram level has yet to be achieved without a side-reaction.

Initially, suitable conditions for the quantitative derivatization of compounds I and II with PFB-Br were examined. The reaction rate was influenced by the concentrations of diisopropylethylamine and PFB-Br. When treated with 4 μl of PFB-Br in 10% diisopropylethylamine–dimethylformamide at an ice-cold temperature, the yields reached a plateau at 5 min as illustrated in Fig. 2. When derivatization was carried out at a higher temperature, *ca.* 60–80°C, the yields were reduced and the interfering peaks were markedly increased. Under the conditions described in the experimental section, compounds I, II and the I.S. were quantitatively converted into their mono- or diPFB derivatives.

Gas chromatography–negative-ion chemical ionization mass spectrometry

The NICI mass spectra of the PFB–TFA derivatives of compounds I and II and the I.S. are illustrated in Fig. 3. Molecular ions were observed for only some compounds. To ensure the specificity of SIM, fragment ions in the high mass region, m/z 533 (I), 539 (I.S. 1), 685 (II) and 691 (I.S. 2) were used, which were formed by the elimination of the PFB moiety from the respective molecular ion. The PFB–TFA derivatives show good chromatographic behavior, and amounts of the derivatives equivalent to femto-gram level of compounds I and II in plasma could be detected (Fig. 4).

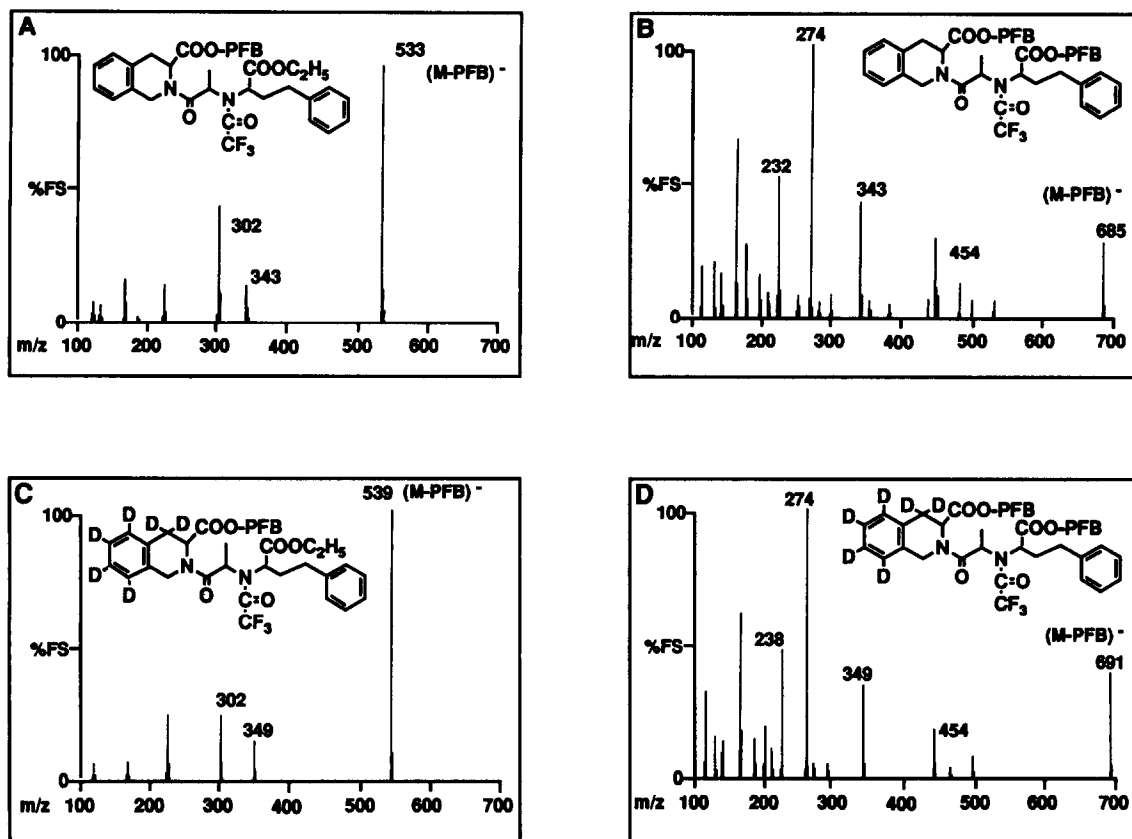


Fig. 3. Methane NICI mass spectra of PFB-TFA derivatives of quinapril (A), quinaprilat (B) and their deuterium-labelled analogues (C and D).

Limit of quantitation

The by-products derived from PFB-Br and TFAA during the derivatization could not be eliminated by the back-extraction method [5]. Further purification steps were incorporated into the derivatization procedure where, after the reaction with PFB-Br and subsequently with TFAA, the sample was twice treated with silica gel column chromatography. No interfering peaks were observed, and compounds I and II were quantitatively determined at a concentration level down to 0.05 ng/ml in plasma and 0.5 ng/ml in urine, with a good precision and accuracy. The limits of detection were below 0.02 ng/ml in plasma and 0.1 ng/ml in urine (Table I).

Among the various methods for the determination of ACEI, the present method is ten-fold

more sensitive than previous ones using GC-MS or radioimmunoassay [10,11].

Calibration curves for compound I in plasma showed a linear response in the low range (0–0.3 ng/ml) and the high range (0.1–10 ng/ml). Typ-

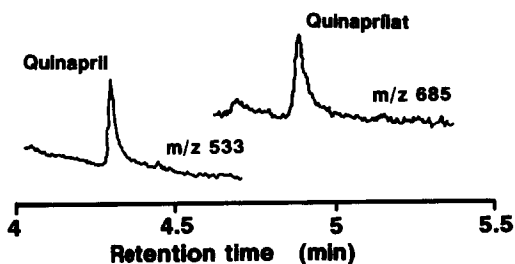


Fig. 4. SIM chromatograms showing the limit of detection. The plasma sample containing 2 pg/ml quinapril and quinaprilat was extracted.

TABLE I

LIMIT OF QUANTITATION OF PLASMA AND URINE ASSAY

The first values are for quinapril and the second for quinaprilat.

Actual concentration (ng/ml)	Calculated concentration ^a (mean, <i>n</i> = 5) (ng/ml)	Precision (C.V., %)	Accuracy (%)
<i>Plasma</i>			
0	0.00568 / -0.00210	18.4 / 12.6	- / -
0.02	0.0268 / 0.0218	7.7 / 11.8	34.0 / 9.0
0.05	0.0479 / 0.0490	7.1 / 5.8	- 4.2 / -2.0
0.1	0.0992 / 0.105	3.1 / 4.7	- 0.8 / 5.0
0.5	0.501 / 0.510	2.2 / 1.2	0.2 / 2.0
1.0	0.979 / 0.979	4.4 / 0.65	- 2.1 / -2.1
2.0	2.02 / 2.00	4.3 / 1.8	1.0 / 0.0
<i>Urine</i>			
0	-0.0723 / 0.147	10.9 / 6.1	- / -
0.1	0.101 / 0.0780	11.8 / 3.1	1.0 / -22.0
0.5	0.516 / 0.532	3.2 / 4.7	3.2 / 6.4
5.0	5.11 / 4.75	1.4 / 3.8	2.2 / -5.0
10	9.94 / 10.1	2.1 / 3.6	- 0.6 / 1.0

^a Calculated from the least-squares line for plasma: quinapril, $y = 0.606x + 0.00706$, $r = 0.9988$ ($n = 35$); quinaprilat, $y = 0.811x + 0.0150$, $r = 0.9997$ ($n = 35$). Calculated from the least-squares line for urine: quinapril, $y = 1.28x + 0.0393$, $r = 0.9996$ ($n = 25$); quinaprilat, $y = 1.83x + 0.260$, $r = 0.9981$ ($n = 25$).

ical equations for the calibration curves were $y = 28.7x + 0.154$, $r = 0.9980$ (in the low range) and $y = 1.49x + 0.0529$, $r = 0.9987$ (in the high range). For compound II in plasma, the equation

was $y = 1.71x - 0.00695$, $r = 0.9996$, in the range 0.3–30 ng/ml. The calibration curves for compound I in urine showed a linear response in the low range 0–5 ng/ml ($y = 1.38x + 0.0269$, r

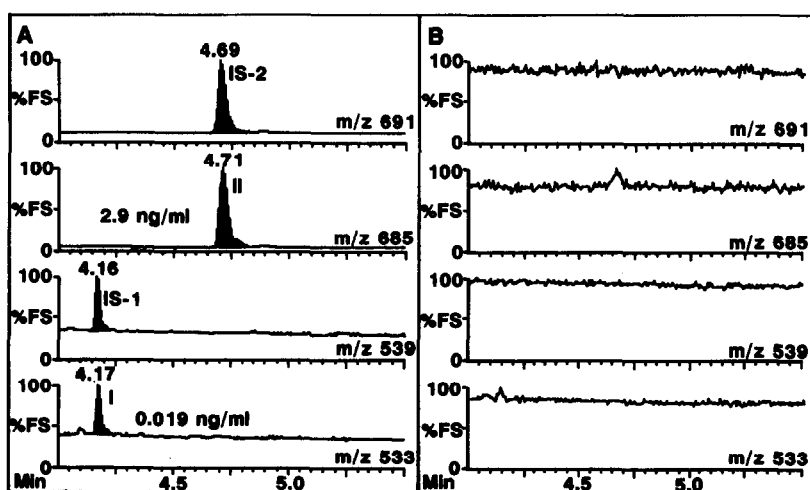


Fig. 5. SIM chromatograms of (A) a human plasma sample, 24 h after a single oral 10-mg dose of quinapril, and (B) drug-free plasma.

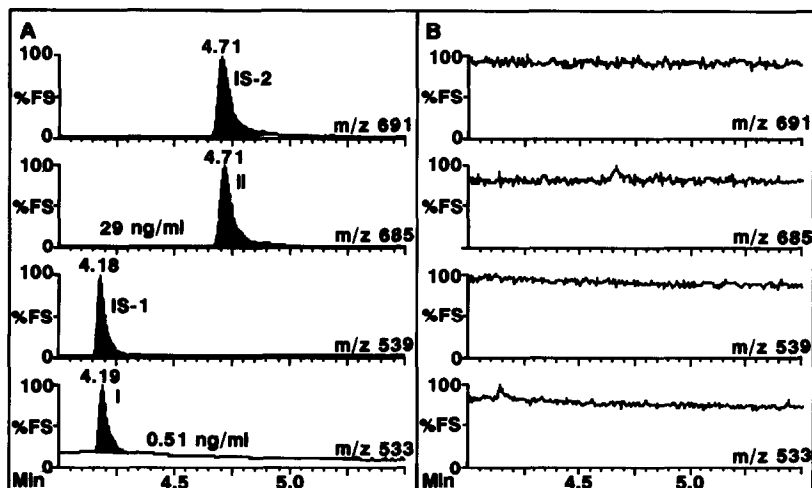


Fig. 6. SIM chromatograms of (A) a human urine sample, 36–48 h after a single oral 10-mg dose of quinapril, and (B) drug-free urine.

= 0.9996) and in the high range 50–2000 ng/ml ($y = 0.124x + 0.0853$, $r = 0.9998$). For compound II in urine, the linear calibration ranges were 20–500 ng/ml (in the low range), which corresponds to the regression equation $y = 1.76x + 0.0498$, $r = 0.9977$, and 200–10 000 ng/ml (in the high range), which corresponds to the regression equation $y = 0.215x - 0.183$, $r = 0.9984$.

The method was used to assay plasma and urine samples from healthy volunteers after the administration of a single 10-mg dose of compound I. The SIM chromatograms with no interfering peaks for plasma and urine samples are shown in Figs. 5 and 6. Plasma concentration–time profiles for compounds I and II are shown in Fig. 7.

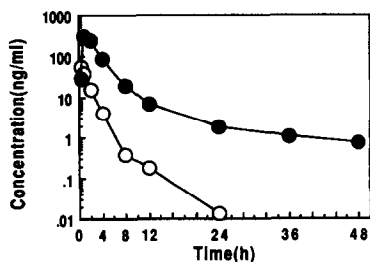


Fig. 7. Typical plasma concentration–time profiles for quinapril (○) and quinaprilat (●) after oral administration of a single 10-mg dose of quinapril.

CONCLUSION

A GC–NICI–MS method for the determination of compound I and II in human plasma and urine has been developed for concentrations down to 0.05 ng/ml in plasma and to 0.5 ng/ml in urine. The method is suitable for studies of the pharmacokinetics and pharmacodynamics of compounds I and II.

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