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Note

Determination of quinapril and its active metabolite in human plasma and urine by gas chromatography with electron-capture detection

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Quinapril is a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor currently being studied in patients for treatment of hypertension and congestive heart failure. Quinapril is deesterified in vivo to the diacid (Fig. 1) ([3*S*-[2[*R**(*R**)]],3*R**]-2-[2-[(1-carboxy-3-phenylpropyl)amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid) (CI-928, I) [1], which is the compound primarily responsible for drug efficacy. Quantitation of the ACE inhibitors enalapril and ramipril has been conducted by radioimmunoassay (RIA) or ACE inhibition assay [2–4]. These techniques are not selective for the parent drugs. To evaluate clinical pharmacokinetic studies of quinapril, a quantitative gas chromatographic (GC) method with electron-capture detection (ECD) was developed. The method allows simultaneous quantitation of both parent drug and active diacid metabolite concentrations in plasma and urine, and is sufficiently sensitive to measure concentrations in human plasma to 10 ng/ml and urine to 50 ng/ml.

EXPERIMENTAL

Materials

The hydrochloride salt of quinapril (CI-906), the monohydrate salt of I, and internal standard (1-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-1H-indole-2-carboxylic acid) (CI-907, II; Fig. 1), were synthesized by Warner-Lambert/Parke-Davis (Morris Plains, NJ, U.S.A.). Solvents used in the analysis were from J. T. Baker (Phillipsburg, WI, U.S.A.) or Burdick & Jackson (Muskegon, MI, U.S.A.) and were glass-distilled or petrochemical

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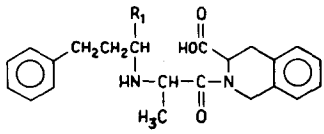
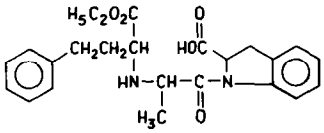


QUINAPRIL: $R_1 = COOC_2H_5$
I (CI-928): $R_1 = COOH$

(a)



INTERNAL STANDARD
II (CI-907)

(b)

Fig. 1. Chemical structures of (a) quinapril and I and (b) internal standard (II).

grade. Dry chemicals were supplied by either J. T. Baker or Sigma (St. Louis, MO, U.S.A.). The water used during sample analysis was purified using a Millipore (Milford, MA, U.S.A.) cation–anion exchange resin with a 0.45-μm filter. Diazomethane was prepared from the Diazald Generator Kit (Aldrich, Milwaukee, WI, U.S.A.).

Preparation of standards and solutions

A stock solution containing both quinapril and I was prepared in methanol–water (1:19) and a stock solution of internal standard was prepared in water. From working standards, plasma calibration standards from 10 to 1000 ng/ml, and urine calibration standards from 50 to 2000 ng/ml were prepared.

Blood was collected in heparinized containers from healthy, drug-free volunteers fasted for a minimum of 10 h. Plasma was separated and frozen until use. Urine was collected from several non-fasted human volunteers to generate a drug-free pool.

Sample preparation

Bond Elut® C_{18} disposable cartridges were preconditioned with one column volume of methanol, two column volumes of water and two column volumes of 0.2 *M* potassium chloride–0.2 *M* hydrochloric acid solution at 20 kPa using an Analytichem vacuum suction system. Aliquots (1 ml) of plasma or urine unknown or standard were pipetted into 16×100 mm disposable culture tubes. A 50-μl volume of a 10 μg/ml internal standard solution and 1 ml of 0.2 *M* potassium chloride–0.2 *M* hydrochloric acid solution were added to each tube. Samples were transferred to the cartridges and pulled through at a rate of 1.5 ml/min. Cartridges were washed with two column volumes of water and dried for 15 min at 60 kPa.

Samples were eluted into 100×16 mm culture tubes with two 0.7-ml volumes

of chloroform–2-butanol (2:1). Eluates were methylated with 0.5 ml of diazomethane for 5 min at room temperature and evaporated to dryness at 40°C in a vortex evaporator. Samples were acidified with 1 ml of 0.2 *M* potassium chloride–0.2 *M* hydrochloric acid solution, washed with 5.5 ml of cyclohexane, vortexed for 10 min at medium speed, and centrifuged at 1000 *g* for 5 min. The cyclohexane layer was separated and discarded. Samples were basified with 1 ml of 1.0 *M* sodium carbonate buffer, extracted into 5.5 ml of hexane–2-butanol (98:2), vortexed at medium speed for 10 min, and centrifuged at 1000 *g* for 5 min. The organic layer was transferred to a 100×16 mm culture tube and evaporated to dryness using a vortex evaporator. After addition of 250 μl of ethyl acetate and 1 ml of hexane-containing 5% trifluoroacetic anhydride, samples were placed in a 42°C temperature block for 15 min. Samples were then evaporated to dryness using a vortex evaporator and the residue was dissolved in 200 μl of 2-butanol. The samples were vortexed, transferred to a 300-μl injection vial, and 1 μl was injected into the gas chromatograph.

Apparatus and instrumental parameters

Analyses were performed on a GC system consisting of Hewlett-Packard (HP) 5890 equipped with a 15-mCi ^{63}Ni linear electron-capture-detector, an HP 7672A autosampler, and an HP 3392 integrator (Avondale, PA, U.S.A.). The column was a 30×0.32 mm I.D. fused-silica capillary column with 0.25 μm bonded methyl silicone (DB-1®, J. & W. Scientific, Rancho Cordova, CA, U.S.A.).

The carrier gas was helium at 2.5 ml/min for the plasma assay and 2.0 ml/min for the urine assay and the make-up gas was nitrogen at 28 ml/min. For the plasma assay, the injection port temperature was 220°C and the detector temperature was 350°C. Column oven temperature was maintained at 220°C for 2.5 min, then programmed from 220 to 300°C at 7.0°C/min, and increased from 300 to 307°C at 2.5°C/min. For the urine assay, the injection port temperature was 280°C and the detector temperature was 350°C. Column oven temperature was held at 230°C for 3.5 min, programmed from 230 to 285°C at 8.0°C/min, then increased from 285 to 305°C at 3.0°C/min and held for 2.5 min at 305°C.

RESULTS AND DISCUSSION

Representative chromatograms for plasma and urine samples are given in Fig. 2. No interfering peaks were observed. The retention times for quinapril, I and II were 15.6 min, 15.4 min and 14.2 min, respectively.

Peak-area ratios were proportional to quinapril or I concentrations over the range of 10–1000 ng/ml for plasma and 50–2000 ng/ml for urine. Standard curves were fitted using a second degree polynomial equation of the form $Y=B_0+B_1\cdot X+B_2\cdot X^2$ using a weighting factor of 1/concentration. Excellent fits were observed for the plasma and urine calibration curves ($r=0.9970$–0.9999).

The lower limit of detection for quinapril and I was 10 ng/ml in plasma and 50 ng/ml in urine. Samples below these concentrations were reported as below the quantifiable limit.

System repeatability was determined by ten replicate injections of a 200 ng/ml

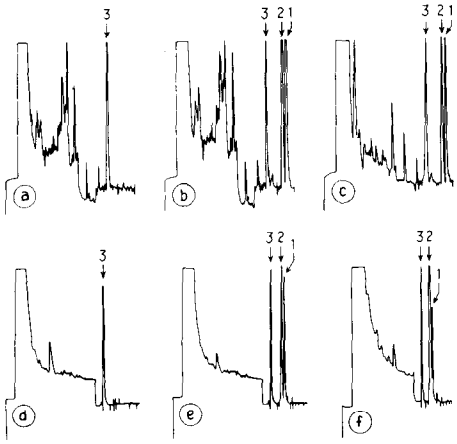


Fig. 2. Representative chromatograms of (a) drug-free pooled human plasma with internal standard (3); (b) human plasma standard containing 500 ng/ml quinapril (1) and I (2); (c) human plasma sample following a single 40-mg quinapril oral dose containing 284 ng/ml quinapril and 312 ng/ml I; (d) drug-free pooled human urine; (e) human urine standard containing 250 ng/ml quinapril and I; and (f) human urine sample following a single 40-mg quinapril oral dose containing 164 ng/ml quinapril and 623 ng/ml I.

plasma standard and a 500 ng/ml urine standard. The relative standard deviations (R.S.D.) were 5.96 and 5.87% for quinapril and I in plasma, respectively, and 2.01 and 1.90% for quinapril and I in urine. The R.S.D. values for extraction repeatability based on ten independently processed standards were 3.8 and 11.6% for quinapril and I in plasma, resepctively, and 5.6 and 4.97% for quinapril and I in urine.

Precision of plasma standards (expressed as R.S.D. for back-calculated concentrations) was determined over four days at nine concentrations and ranged from 2.78 to 18.5% with a mean of 7.37% for quinapril and from 3.25 to 15.6% with a mean of 6.96% for I. Precision of urine standards was determined over three days at nine concentrations and ranged from 1.45 to 14.0% with a mean of 4.38% for quinapril and from 1.98 to 10.0% for I. Accuracy was determined by comparing the known drug concentrations with those determined. The relative error for plasma samples ranged from −8.2 to 14.4% for quinapril and from −5.8 to 6.0% for I. The relative error for urine samples ranged from −8.4 to 6.8% for quinapril and from −11.0 to 7.6% for I.

Recent assay methods for similar ACE inhibitors in plasma have used organic extraction with high-performance liquid chromatography (HPLC) [5] and organic extraction combined with GC–ECD [6]. The present method, however, has a ten-fold lower sensitivity limit than these methods and can be used to assay both plasma and urine samples. A GC method with nitrogen detection was developed for measuring ramipril and its diacid metabolite in urine [7]. The method was considered unsuitable for quantifying plasma concentrations due to insufficient sensitivity. ECD was used in the present method to improve sensitivity. Additionally, chromatographic interferences were decreased by replacing the silica solid phase extraction with a cyclohexane wash followed by organic extrac-

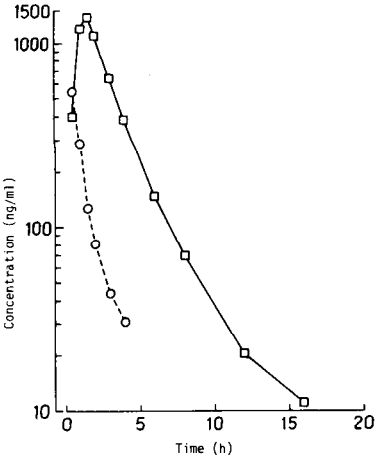


Fig. 3. Mean quinapril and I plasma concentration–time profiles; ○ = quinapril; □ = I.

tion. Sensitivity of the present method is similar to previously described ACE inhibition enzyme assay methods [4], but the present method is preferred due to higher sample throughout as well as specificity for the parent drug and its active diacid metabolite.

Application of the method

The method was used to assay plasma and urine samples collected from healthy volunteers after single 40-mg quinapril capsule administration. Representative chromatograms from the analysis of postdose plasma and urine samples are shown in Fig. 2. Mean quinapril and I plasma concentration–time profiles are shown in Fig. 3. The assay method is suitable to quantify quinapril and I in plasma and urine and characterize their pharmacokinetic profiles following typical quinapril doses.

CONCLUSIONS

A GC method for the quantitative determination of quinapril and its active metabolite I in human plasma and urine has been developed for concentrations ranging from 10 to 1000 ng/ml in plasma and 50 to 2000 ng/ml in urine. This method is currently being used for analysis of plasma and urine samples collected in clinical pharmacokinetic studies.

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