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Short communication

Simple high-performance liquid chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate

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Abstract

A simple high-performance liquid chromatographic (HPLC) method was developed for the determination of losartan and its E-3174 metabolite in human plasma, urine and dialysate. For plasma, a gradient mobile phase consisting of 25 m*M* potassium phosphate and acetonitrile pH 2.2 was used with a phenyl analytical column and fluorescence detection. For urine and dialysate, an isocratic mobile phase consisting of 25 m*M* potassium phosphate and acetonitrile (60:40, v/v) pH 2.2 was used. The method demonstrated linearity from 10 to 1000 ng/ml with a detection limit of 1 ng/ml for losartan and E-3174 using 10 μl of prepared plasma, urine or dialysate. The method was utilized in a study evaluating the pharmacokinetic and pharmacodynamic effects of losartan in patients with kidney failure undergoing continuous ambulatory peritoneal dialysis (CAPD). © 1997 Elsevier Science B.V.

Keywords: Losartan; E-3174

1. Introduction

Losartan (2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole, potassium salt) is an orally active selective AT_1-receptor antagonist employed in the management of essential hypertension [1–3]. The metabolite E-3174 (2-*n*-butyl-4-chloro-1-[(2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid) is a pharmacologically active metabolite of losartan [4,5]. One HPLC method published to date for measurement of losartan and E-3174 utilized a conventional analytical column (e.g., 4.6 mm internal diameter), an internal standard, and tedious liquid–liquid extraction [6]. The method detailed in the present communication utilizes a simple sample preparation step for each matrice and does not require an internal standard. In addition, this method employs current HPLC midbore column technology which provides not only excellent sensitivity but also reduces mobile phase requirements.

2. Experimental

2.1. Chemicals

Losartan (CAS 124750-99-8) and E-3174 were

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kindly provided by Dupont Merck Pharmaceutical Company (Wilmington, DE, USA). *o*-Phosphoric acid (85%) was HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Acetonitrile was Optima HPLC grade and purchased from Fisher Scientific. Ultrapure distilled and deionized water was prepared in-house and filtered prior to use. Peritoneal dialysis solution consisted of 2.5% dextrose (w/v), 0.5% NaCl (w/v), 0.005% $MgCl_2$ (w/v), 0.018% $CaCl_2$ (w/v), 0.44% sodium lactate (w/v) pH 5.2 and was purchased from Baxter Scientific (Charlotte, NC, USA).

2.2. HPLC equipment and mobile phase

The HPLC equipment consisted of a Varian pump Model 9010 solvent delivery system (Walnut Creek, CA, USA). The analytical column was a Hypersil Phenyl, 150 mm×3.2 mm I.D., 3 μm packing (Phenomenex, Torrance, CA, USA). The C_{18} guard column, 30 mm×4.6 mm I.D., 40–50 μm pellicular packing (Alltech, Deerfield, IL, USA) was replaced prior to each analytical run which typically consisted of approximately 50 samples. For the urine and dialysate analysis, the mobile phase consisted of 25 m*M* potassium phosphate–acetonitrile (60:40, v/v) with pH 2.2. For plasma analysis, the gradient mobile phase consisted of 25 m*M* potassium phosphate–acetonitrile with pH 2.2 and is listed in Table 1. The mobile phase was degassed daily using helium sparging and the flow rate was maintained at 0.75 ml/min. Typical operating pressure was 14.2 MPa at ambient temperature. An injection volume of 10 μl of the prepared sample was accomplished using a WISP Model 712 (Waters, Milford, MA, USA) autosampler. Compound detection was achieved using a Shimadzu RF-535 Fluorescence Detector (Tokyo, Japan) with excitation and emission wavelengths of 250 and 375 nm, respectively. The detector operated at high sensitivity setting with a 1 s response time. A 345 kPa back-pressure regulator (SSI, State College, PA, USA) was coupled to the detector outlet to prevent outgassing. Data acquisition and component computations were performed using Turbochrom (PE Nelson, Norwalk, CT, USA) chromatography software on a Hewlett Packard (Palo Alto, CA, USA) 486 DX-33 personal computer.

Table 1
Gradient conditions for plasma analysis

Time (min)	Acetonitrile (%)
0	35
5	35
9	60
12	60
13	35

2.3. Standard and control preparation

Stock standards of losartan and E-3174 (1 mg/ml) were prepared in methanol–deionized water (50/50, v/v) and stored at 4°C. Working plasma standards of 10, 50, 100, 250, 500 and 1000 ng/ml losartan and E-3174 were prepared using blank plasma as the diluent. Working urine standards of 50, 100, 250, 500 and 1000 ng/ml losartan and E-3174 were prepared using blank urine as the diluent. Working dialysate standards of 10, 20, 40 and 100 ng/ml were prepared using blank dialysate as the diluent. Control plasma samples of 25, 200 and 750 ng/ml, control urine samples of 75, 400 and 750 ng/ml, and control dialysate samples of 25 ng/ml were spiked with losartan and E-3174 and were prepared using blank plasma, urine, or dialysate as the diluent, respectively. All working standards and controls were stored and maintained at −20°C with the patient samples.

2.4. Sample conditions

Eight end-stage renal disease subjects undergoing maintenance CAPD received losartan once daily for eight successive days at a dose of 100 mg. Blood samples were obtained at specified time intervals for 24 h following the final dose of losartan. Blood samples were centrifuged at 1600 *g* for 15 min, plasma separated and stored at −20°C. In those patients who were not anuric, urine was collected at each void and pooled at the end of 24 h. Each subject underwent 4 dialysate exchanges during the 24 h following the last dose of losartan. Varying dialysate dextrose concentrations (1.5–4.25%, w/v) were utilized over this time span. Aliquots from each dialysate exchange and the available urine were obtained and stored at −20°C pending analysis. Prior

to analysis, urine and dialysate samples were thawed to ambient temperature, mixed thoroughly by inversion, and allowed to sit 15 min for particulates to settle out. Plasma samples were thawed to ambient temperature, mixed thoroughly by inversion, and centrifuged at 1200 *g* for 10 min to eliminate fibrinous material.

2.5. Sample preparations

Plasma samples were prepared by pipetting 150 μl of plasma and 150 μl acetonitrile into a polypropylene bullet centrifuge tube. Plasma proteins were precipitated by vortexing for 15 s. The samples were centrifuged at 13 000 *g* for 10 min. The clear supernatant was transferred to polypropylene autosampler microvials. Urine samples were prepared by pipetting 50 μl of urine and 450 μl of deionized water into a 12×75 mm polypropylene culture tube and vortex mixing for 10 s. The diluted urine samples were transferred to polypropylene autosampler microvials. Dialysate samples were injected neat. For plasma, urine and dialysate, 10 μl was injected into the HPLC system.

3. Results and discussion

3.1. Chromatography

The method demonstrated excellent chromatographic selectivity with no endogenous plasma interferences at the retention times for losartan and E-3174 (7.3 and 9.3 min, respectively) (Fig. 1A). Chromatograms of prepared blank human plasma containing low (10 ng/ml) and high (1000 ng/ml) concentrations of losartan and E-3174 (Fig. 1B,C, respectively) indicated good detector response and baseline resolution from endogenous substances with an analytical run time of 20 min. A typical chromatogram for plasma from one subject dosed with losartan is shown in Fig. 1D. Chromatograms demonstrating the excellent selectivity and sensitivity of the method for urine and dialysate samples are in Figs. 2 and 3, respectively. To extend column lifetime, the analytical phenyl column was flushed after each analytical run (approximately 50 samples) for 2 h at 0.75 ml/min with acetonitrile–deionized water (85:15, v/v) to eliminate retained substances from the column.

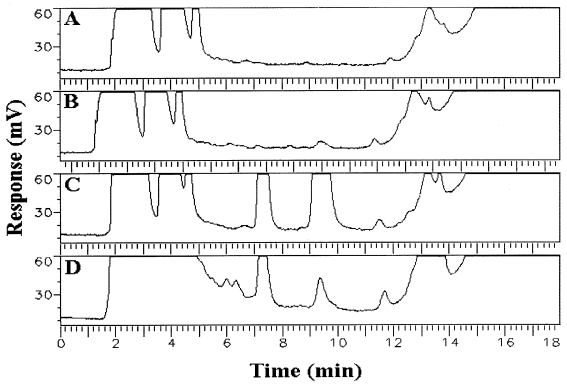

Fig. 1. Chromatograms of (A) prepared blank human plasma, (B) prepared blank human plasma spiked with 10 ng/ml losartan and E-3174, (C) prepared blank human plasma spiked with 1000 ng/ml losartan and E-3174, (D) subject dosed with 100 mg losartan tablet (4 h sample – losartan concentration 478 ng/ml and E-3174 concentration 55 ng/ml). Peak at 7.3 min=losartan, 9.3 min=E-3174.

3.2. Linearity, limit of detection and computations

The plasma method was linear throughout the tested concentration range of 10 to 1000 ng/ml with a mean correlation coefficient (n=5 analytical runs)

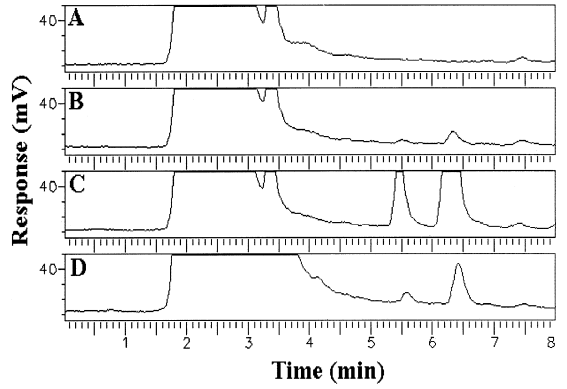

Fig. 2. Chromatograms of (A) prepared blank human urine, (B) prepared blank human urine spiked with 50 ng/ml losartan and E-3174, (C) prepared blank human urine spiked with 1000 ng/ml losartan and E-3174, (D) subject dosed with 100 mg losartan tablet (24 h sample – losartan concentration 135 ng/ml and E-3174 concentration 166 ng/ml). Peak at 5.5 min=losartan, 6.3 min=E-3174.

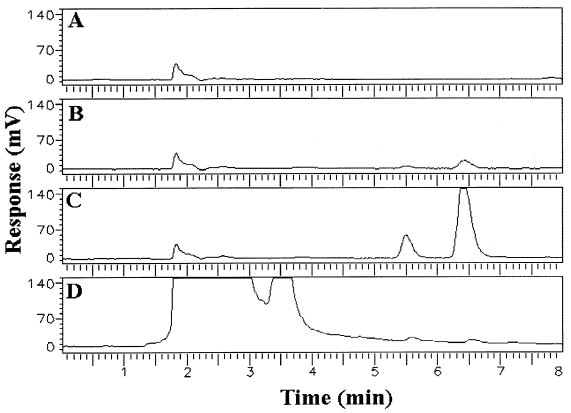

Fig. 3. Chromatograms of (A) blank dialysate, (B) prepared blank dialysate spiked with 10 ng/ml losartan and E-3174, (C) prepared blank dialysate urine spiked with 100 ng/ml losartan and E-3174, (D) subject dosed with 100 mg losartan tablet (4 h sample – losartan concentration 16 ng/ml and E-3174 concentration 3 ng/ml). Peak at 5.5 min=losartan, 6.5 min=E-3174.

of 0.99975 and 0.99979 for losartan and E-3174, respectively. The urine method was linear throughout the concentration range of 50 to 1000 ng/ml with a mean correlation coefficient ($n=2$ analytical runs) of 0.99981 and 0.99977 for losartan and E-3174, respectively. The dialysate method was linear throughout the concentration range of 10 to 100 ng/ml with a correlation coefficient ($n=1$ analytical run) of 0.99997 and 0.99978 for losartan and E-3174, respectively. The limit of detection for the method (1 ng/ml) was determined by evaluation of a spiked standard in each matrice at 1 ng/ml ($n=3$). For plasma component calculations, linear regression utilizing weighting ($1/x$) with external standardization and peak height was used for losartan and E-3174. For urine and dialysate calculations, normal linear regression with external standardization and peak height was used for losartan and E-3174. The lowest standard calibrator for plasma, urine and dialysate was used as the limit of quantitation for reporting calculated patient results.

3.3. Accuracy, precision and recovery

The accuracy and precision for the method was determined by evaluation of replicate control samples over the course of all analytical runs at losartan and E-3174 concentrations of 25, 200 and 750 ng/ml for plasma, 75, 400 and 750 ng/ml for urine, and 25 ng/ml for dialysate. The accuracy of the method was reported as the percentage error of theoretical versus measured losartan and E-3174 concentrations and was less than 4.8% for all losartan plasma control samples and less than 11.2% for all E-3174 plasma control samples. The percentage error was less than 1.8% for all losartan and E-3174 urine control samples and less than 7.2% for all losartan and E-3174 dialysate control samples. The precision of the method was reported as percentage relative standard deviation and was less than 10.7% for all plasma losartan control samples and less than 8.4% for all plasma E-3174 control samples. The percentage relative standard deviation was less than 5.9% for all urine losartan and E3174 urine control samples and less than 4.2% for all dialysate losartan and E-3174 control samples. Absolute recovery for the plasma method was evaluated by comparing the standards which were prepared in blank plasma versus standards prepared in deionized water. The average absolute recovery for the plasma method was determined to be 99%. Absolute recovery for the urine and dialysate methods was not performed as the methods do not employ formal extractions (e.g., liquid–liquid, solid phase). In addition, the standards and controls used for analysis were treated identical to the patient plasma, urine and dialysate samples thus controlling for potential errors in micropipetting.

3.4. Pharmacokinetic study

Fig. 4 provides plasma concentration versus time

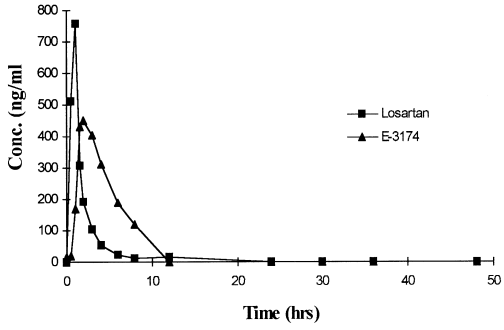

Fig. 4. Pharmacokinetic profile for losartan and E-3174 in plasma in one subject dosed with 100 mg losartan.

profiles for losartan and E-3174 in an individual patient over the 50 h time span following the final 100 mg dose of losartan. C_{max} for losartan was 757 ng/ml and was achieved at the 1 h time point whereas the C_{max} for E-3174 of 448 ng/ml was reached at the 2 h time point. Both losartan and E-3174 were undetectable beyond the 12 h sampling time point.

4. Conclusion

A simple method was developed for evaluating losartan and its E-3174 metabolite in plasma, urine and dialysate. We employed a precipitate and shoot method for plasma samples, a dilute and shoot technique for urine samples and neat injections for dialysate samples. This eliminated both the need for sample extractions and an internal standard, thus making this method extremely cost effective. In addition, this method used current midbore HPLC column technology which afforded excellent sensitivity as well as reducing mobile phase requirements. In this regard, analytical costs were substantially reduced as it relates to the procurement and subsequent hazardous waste disposal of acetonitrile. It is noteworthy that use of the midbore column did not necessitate modifications to the HPLC system (i.e. injection volume and detector cell volume). The method had an analytical run time of 20 min and readily achieved baseline resolution between losartan, E-3174 and endogenous substances. The method was employed in the evaluation of plasma, urine and dialysate samples from a pharmacokinetic and pharmacodynamic study of losartan in CAPD without significant methodological problems.

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