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Short communication

Simultaneous and rapid quantitation of benazepril and benazeprilat in human plasma by high performance liquid chromatography with ultraviolet detection

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Abstract

A sensitive and accurate high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detector was developed and validated for simultaneous determination of benazepril (BZL) and its active metabolite, benazeprilat (BZT), in human plasma. The plasma sample, after spiked with riluzole as an internal standard (IS), was subjected to a solid-phase extraction (SPE) prior to a HPLC analysis. Chromatographic separations were achieved on a Hypersil BDS C_{18} (300 mm × 4.6 mm, 5 μm). The mobile phase consisted of phosphate buffer (pH 2.6; 10 mM) and acetonitrile mixture in a gradient mode. Detection was carried out at a wavelength of 237 nm. The retention times of BZL, BZT and IS were at about 6.2, 15.4 and 16.2 min, respectively. The calibration curve was linear in the range of 20–2000 ng/mL for both BZL and BZT ($r^2 > 0.997$). At three quality control concentrations of 100, 500, and 1500 ng/mL, the intra-day and inter-day relative standard deviation ranged from 2.8 to 8.6% for BZL and from 2.2 to 8.5% for BZT, while the mean absolute percentage error ranged from −7.5 to 6.7% for BZL and from −6.0 to 3.2% for BZT. The limit of detection (LOD) was 10 ng/mL and the limit of quantification (LOQ) was 20 ng/mL for both BZL and BZT in human plasma. The method was successfully applied to bioequivalence evaluation of benazepril hydrochloride formulations in healthy Chinese.

Keywords: Benazepril; Benazeprilat; HPLC; Human; Bioequivalence; C_{max}; AUC; Tablet

1. Introduction

Benazepril hydrochloride (3-[1-(ethoxycarbonyl)-3-phenyl-(1*S*)-propylamino]-2,3,4,5-tetrahydro- 2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid monohydrochloride, BZL) [1], is a new angiotensin-converting enzyme (ACE) inhibitor, which is shown to be effective in the treatment of essential hypertension, chronic heart failure, myocardial infarction and diabetic nephropathy [2–5]. The anti-hypertensive effect of BZL appeared to be equivalent or superior to that of captopril, enalapril, hydrochlorothiazide, nitrendipine, nifedipine, and propranolol at usual therapeutic doses [2]. BZL in combination with hydrochlorothiazide or nifedipine achieved a greater reduction of blood pressure than benazepril alone, and this combination approach may be suitable for patients with more severe hypertension. The compound, a dicarboxylic acid monoethylester, is a prodrug [6], which is hydrolyzed by hepatic enzymes in vivo upon intestinal absorption, to a pharmacologically active metabolite, the diacid benazeprilat (3-[(1-carbonyl-3-

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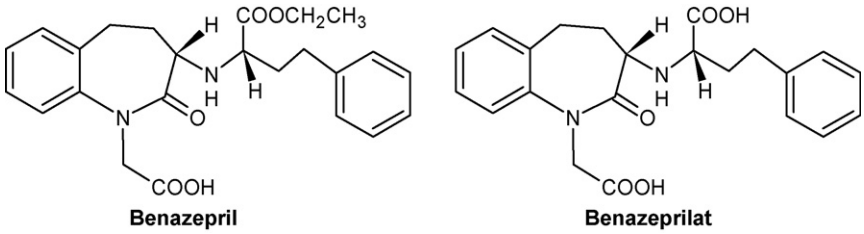

Fig. 1. Chemical structures of benazepril and benazeprilat.

phenyl-(1*S*)-propyl)-amino]-2,3,4,5-tetrahydro-2-oxo-1-(3*S*)-benazepine-1-acetic acid, BZT) [7,8]. Due to the relative long elimination half-life of the active metabolite of BZL, BZT, has a long duration of inhibitory effect on ACE. The structures of the parent drug and its active metabolite are presented in Fig. 1.

Studies have been performed to evaluate the pharmacokinetics of BZL in healthy subjects [7,9–14], the elderly [15] and patients with hypertension, major proteinuria, or liver cirrhosis [7,16,17]. Sun et al. [9] reported that after a single oral administration of 10 mg benazepril hydrochloride tablet to 12 healthy male Caucasians in South Wales, UK, the mean area under the plasma concentration–time curve ($AUC_{0-4\,h}$), maximum plasma concentration (C_{max}) and time to C_{max} (t_{max}) values for BZL were 140 ng h/mL, 149 ng/mL, and 0.6 h, respectively; while the mean $AUC_{0-24\,h}$, C_{max}, and t_{max} values for BZT were 1410 ng h/mL, 257 ng/mL, and 1.5 h, respectively. Reports on biopharmaceutical properties of benazepril hydrochloride in solid dosage forms, however, are limited. To assess the bioequivalence of benazepril formulations, the plasma concentration–time profiles of both BZL and BZT in human subjects have to be determined. A reliable quantitative method that allows a simultaneous determination of the concentration of BZL and BZT in plasma is thus needed. Several analytical methods have been developed, such as high-performance liquid chromatography (HPLC), capillary electrophoresis and an enzymatic method coupled with HPLC or liquid scintillation counting [7,9–12,15,16,18–21]. Most of these methods, however, are applicable for the purity evaluation of benazepril in bulk drugs and pharmaceutical dosage forms. To date, there are four published reports describing simultaneous determination of benazepril and benazeprilat in human plasma using mass spectrometry, including gas chromatography–mass spectrometry (GC–MS) [22,23] and liquid chromatography–mass spectrometry (LC–MS) [24,25]. These mass spectrometric methods are very specific and sensitive for the determination of BZL and BZT. GC–MS, however, needs deuterium-labeled BZL and BZT as internal standards, which are not commercially available, and sample derivatization, while LC–MS analysis often involves complicated sample preparation and analytical procedure and its use could be hampered by the high cost of the equipment.

In this paper, a simple reversed-phase HPLC with direct UV detection for simultaneous analysis of BZL and its active metabolite BZT in human plasma was developed. The method was successfully applied to study the relative bioavailability of two 10 mg benazepril hydrochloride tablet products.

2. Materials and methods

2.1. Chemicals and reagents

Benazepril hydrochloride (BZL), benazeprilat (BZT), and riluzole (used as the internal standard, IS) were kindly supplied by Ranbaxy Laboratories Limited Inc. (Gurgaon, Haryana, India). The three compounds have a purity of 99.9% as determined by HPLC. HPLC-grade acetonitrile and methanol were purchased from Dikma Technology Inc. (Markham, Ontario, Canada). Ultrapure water was prepared using a Milli-Q purification system (Barnstead International Inc., Dubuque, IO). Other reagents were of analytical-grade and purchased from Yonghua Ltd. (Shanghai, China). Blank (drug-free) plasma from healthy human blood donors was obtained from the Central Laboratory of the Blood Transfusion Service in Guangzhou (Canton, China).

2.2. Instruments and chromatographic conditions

A Waters (Milford, MA) Alliance HPLC system consisted of a 2487 dual wavelength UV detector, a 1525 Binary HPLC pump and a 717 plus autosampler equipped with a Hypersil BDS C18 analytical column (5 μm, 300 mm × 4.6 mm) (Dalian, China). Peaks were monitored by UV absorbance at 237 nm. The analysis data were acquired and processed using Breeze software (v.3.3). A mixture of acetonitrile (A) and phosphate buffer (pH 2.6, 10 mM) (B) was used as the mobile phase. The gradient elution was programmed as follows: 0–6 min, 70% B; 6–15 min, 70–50% B; 15–16 min, 50% B; 16–20 min, 50–70% B, and 20–23 min, 70% B. The column temperature was maintained at room temperature (25 °C). The flow rate was 1.0 mL/min.

2.3. Preparation of stock and working solutions

A stock solution (500 μg/mL) of BZL as well as that of riluzole (IS) was prepared in 50% methanol, i.e., a mixture of water:methanol (1:1; v/v), while that (100 μg/mL) of BZT was prepared in a mixture of water:methanol:aqueous ammonia (1:1:0.05; v/v/v). Working standard solutions were prepared by appropriately diluting the respective stock solutions with 50% methanol to yield final concentrations of 50, 10 and 2 μg/mL for BZL and BZT and that of 20 μg/mL for riluzole. All stock and working standard solutions were stored at 4 °C until used for analysis.

2.4. Plasma sample preparation

To 1.0 mL of each plasma sample, 10 μL riluzole (20 μg/mL) as an IS and 1 mL phosphate buffer (pH 2.6, 10 mM) were added in a 10 mL tube. The need for 1.0 mL plasma sample was due to the efficient concentration (about 1:5, i.e., 1.0 → 0.2 mL) by the solid-phase extraction (SPE) step. The mixtures were vortex-mixed for 30 s and then subjected to SPE using Bond-Elut C8 column (130 mg/3 mL, Analytic Chem. Pte Ltd., New Delhi, India). The column was preconditioned by passing 2 mL of methanol followed by 2 mL of water. After the plasma sample loading, the column was washed with 2 mL each of acetic acid, water, and ethanol sequentially. Finally, the analytes of interest were eluted out of the column by passing a mixture (2 mL) of aqueous ammonia:methanol (1:49; v/v) and the final methanol elute collected was subjected to evaporation under vacuum drying. After dried, the residues were reconstituted in 200 μL of mobile phase, i.e., acetonitrile:phosphate buffer (pH 2.6, 1 mM, 1:1), and 50 μL of the reconstituted solution was injected into the chromatographic system for analysis.

2.5. Standard preparation

The known amounts of both working solutions of BZL and BZT were added to pooled drug-free plasma to achieve standard solutions containing 20, 50, 100, 250, 500, 1000, 1500 and 2000 ng/mL of each compound. These concentrations were used to construct standard calibration curves. Of which, the concentrations of 100, 500 and 1500 ng/mL were chosen for the quality control (QC) samples. No internal standard was added prior to analysis.

2.6. Validation procedures

2.6.1. Linearity, accuracy, precision and specificity

The linearity of the analytical procedure for each compound was evaluated by processing an eight-point calibration curve, and analyzed on three occasions. The calibration curves were constructed by least-squares linear regression of peak area ratios of each analyte to the IS *versus* the nominal concentrations of each compound spiked to drug-free plasma samples.

For determining the intra-day accuracy and precision, five aliquots of QC samples of BZL and BZT were analyzed on the same day. The inter-day accuracy and precision were assessed by analysis of five precision and accuracy batches on 5 different days. In order to study the specificity of the present method, six independent blank human plasma samples were subject to the same analytical procedure so that potential chromatographic interference from sample matrix was evaluated.

2.6.2. Limit of detection and limit of quantitation

Limit of detection (LOD) was determined as the lowest concentration to be detected, taking into consideration of a signal-to-baseline noise ratio of 3. Limit of quantitation (LOQ) was defined as the lowest point on the calibration curve that could be analyzed within 20% of the nominal value for both precision and accuracy. Precision was assessed by relative standard deviation (R.S.D.), while accuracy was assessed by mean absolute percentage error in measured value (calculated by taking the difference between the measured and nominal values expressed as the percentage of the nominal value). The run for LOD and LOQ determination was repeated five times for confirmation.

2.6.3. Recovery

Extraction recoveries of BZL and BZT from plasma were estimated by comparing the peak area ratios of both analytes added to blank plasma undergoing SPE to those of the same quantities added to the mobile phase without undergoing SPE.

2.6.4. Stability

To determine the room temperature stability of BZL and BZT in human plasma, five aliquots of each QC samples were kept at room temperature (25 °C) for 6 h. To determine the long-term stability of the two compounds in human plasma, five aliquots of each QC samples were kept at −20 °C for 30 days. Both of the stability samples were subject to the assay procedure and concentrations obtained were then compared to the nominal values of the QC samples. Effects of three freeze and thaw cycles on stability of frozen plasma samples containing BZL and BZT was determined to establish the ruggedness of the method. Five aliquots of QC samples were stored at −20 °C and subject to three freeze–thaw cycles. After the completion of the third cycle the samples were subjected to the assay procedure and results obtained were then compared to the respective nominal values. All values within ±15.0% were deemed to be qualified for the test.

2.7. Human pharmacokinetic study

After an overnight fasting, 24 healthy male volunteers, who had given their written informed consent, were administered a single oral dose of 40 mg benazepril hydrochloride tablet as test formulation (formulated by Ranbaxy Laboratories Limited Inc., Gurgaon, Haryana, India) or a 40 mg Lotensin® tablet as reference product (Novartis International AG, Basel, Switzerland) in a double-blind cross-over design with a 14-day washout period between each treatment. The study proposal was approved by the Research Ethics Committee of Sun Yat-sen University (Guangzhou, China). A series of blood (5 mL) were collected at 0.16, 0.33, 0.5, 0.67, 0.83, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after drug administration. The plasma samples were obtained by centrifugation at 3000 × *g* for 10 min and stored at −20 °C until analysis.

2.8. Pharmacokinetic calculations and statistical analysis

The area under the plasma concentration–time curve (AUC_{0-t}) from time zero to the last measurable time (t_z) point for BZL and BZT was calculated using the linear trapezoidal rule. The total area under the plasma concentration–time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated as the sum of AUC_{0-t} and the extrapolated area, which was estimated as the last measurable serum concentration (C_z) divided by the terminal rate constant (λ_z), where λ_z was estimated using the terminal

log-linear phase of the plasma concentration–time curve. The elimination half-life ($t_{1/2,\lambda z}$) was calculated by dividing 0.693 by (λ_z). The maximum observed plasma concentration (C_{max}) and the corresponding time of its occurrence (T_{max}) were obtained by visual inspection of the plasma concentration–time curves. The mean residence time (MRT) was determined as the ratio of area under the first moment curve divided by $AUC_{0-\infty}$. The 90% confidence intervals (CIs) for $AUC_{0-\infty}$ and C_{max} of BZL and BZT for the two formulations and the ratios of C_{max} and $AUC_{0-\infty}$ for test formulation over reference formulation were calculated using Schuirmann's two one-sided *t*-tests procedure for bioequivalence testing [26].

3. Results and discussion

3.1. Chromatographic conditions for separation of analytes

The effects of solvent composition and pH of the mobile phase on the retention time of BZL and BZT were investigated. Methanol:phosphate buffer (pH 2.6, 10 mM) (60:40; v/v) and acetonitrile:phosphate buffer (pH 2.6, 10 mM) (30:70; v/v) were the two delivery solvent systems to be compared under the isocratic mode. The use of acetonitrile appeared to be better than that of methanol with respect to the sensitivity and symmetrical peak of analytes. Because of the wide difference in polarity of the target analytes, a gradient instead of isocratic mode was employed to reduce the analytical run time by shortening the retention times of benazepril and IS without significantly affecting that of benazeprilat. The optimal percentage of acetonitrile in the mobile phase under the gradient mode was explored and found to be as follows: 0–6 min, 30%; 6–15 min, 30–50%; 15–16 min, 50%; 16–20 min, 50–30%, and 20–23 min, 30%.

A decrease of pH value from 4.0 to 2.5 caused a decrease of the retention time of BZL, likely due to an increase in the protonation of the nitrogen atom in the benzazepine ring ($pK_a = 5.02$), but led to a delay in the elution of BZT, possibly due to a reduction in the ionization of the second carboxyl group ($pK_a = 2.02$). The optimal pH of the mobile phase was found to be 2.6, at which the analyte peaks were well defined and resolved. The optimal wavelength for detection was 237 nm, at which the best detector responses for BZL and BZT were obtained.

Fig. 2 shows the chromatograms of a blank human plasma sample, a plasma sample spiked with BZL, BZT and IS, and a plasma sample from a volunteer 1 h after oral administration of benazepril hydrochloride tablet. Under the optimal chromatographic conditions, the retention times for BZL, BZT and IS obtained were 6.2, 15.4 and 16.2 min, respectively, and no endogenous interfering peaks appeared at the retention times of the target analytes. The assay specificity for BZL and BZT determination was also indicated by the absence of interfering peaks in the presence of potentially combined drugs such as propranolol, digoxin, nifedipine, hydrochlorothiazide, or verapamil. The baseline separation was noted among all the analyte peaks with a good resolution ($R_s > 2.3$).

In HPLC analysis, the use and proper choice of internal standard is the guarantee for good precision and accuracy. However, few previously reported HPLC methods for BZL and BZT determination utilized an internal standard. In our study, enalapril, captopril, hydrochlorothiazide and riluzole were tested as the internal standard. Since the peak of hydrochlorothiazide overlaid with that of BZT, and the peak of enalapril or captopril overlaid with that of BZL, only riluzole could be well separated with the two target compounds.

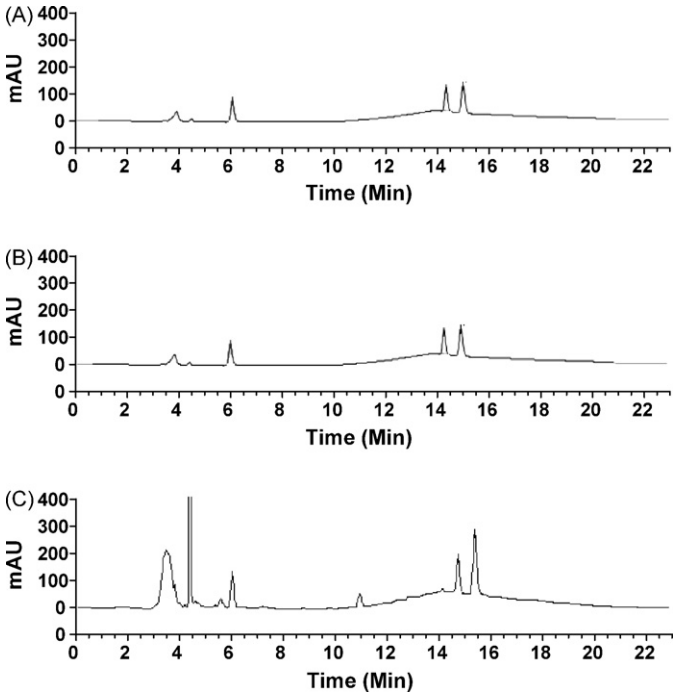

Fig. 2. Chromatograms of (A) blank plasma; (B) plasma spiked with riluzole (used as IS), benazepril at 250 ng/mL and benazeprilat at 500 ng/mL (retention times; benazeprilat: 6.2 min, benazepril: 15.4 min, and IS: 16.2 min); and (C) plasma from a healthy volunteer 1 h after oral administration of 40 mg benazepril hydrochloride tables. The measured concentration of benazepril and benazeprilat was 331.5 and 694.2 ng/mL, respectively.

During the HPLC assay development, several types of SPE cartridges were evaluated. The chromatographic properties of both BZL and BZT were more consistent and reproducible when using a C_8 column compared to a HLB Oasis cartridge or a C_{18} column, making the former suitable for the extraction of analytes from human plasma sample. In order to reduce the ionization of both analytes and increase their adsorption to SPE column, phosphate buffer (pH 2.6) was added to acidify plasma samples.

3.2. Calibration standards, limit of detection and limit of quantification

For both analytes, the calibration curve was linear over the range of 20–2000 ng/mL. The corresponding regression equations were $Y = 0.0012X - 0.0024$ ($r^2 = 0.9988$) and $Y = 0.0013X - 0.0017$ ($r^2 = 0.9973$), respectively, where Y represents the ratio of peak area ratio of analyte to IS, and X represents analyte concentration.

For both BZL and BZT, the LOD and the LOQ were 10 and 20 ng/ml, respectively. The LOD value obtained for benazepril in the present study is significantly improved compared to those reported in the literature (about 300 ng/mL [18] or 550 ng/mL

Table 1
Intra-day precision and accuracy of the assay of benazepril and benazeprilat in human plasma ($n = 5$)

Measured concentration of analyte (ng/mL) and variation	Nominal concentration of benazepril (BZL, ng/mL)			Nominal concentration of benazeprilat (BZT, ng/mL)		
	100	500	1500	100	500	1500
Mean	92.5	483.1	1510.1	94.0	507.8	1463.7
S.D.	3.2	41.7	72.3	2.0	29.0	76.3
R.S.D. (%)	3.4	8.6	4.8	2.2	5.7	5.2
Mean absolute percentage error (%)	−7.5	−3.4	0.7	−6.0	1.6	−2.4

Abbreviations: S.D: standard deviation; R.S.D.: relative standard deviation; BZL: benazepril; BZT: benazeprilat.

Table 2
Inter-day precision and accuracy of the assay of benazepril and benazeprilat in human plasma ($n = 5$)

Measured concentration of analyte (ng/mL) and variation	Nominal concentration of benazepril (BZL, ng/mL)			Nominal concentration of benazeprilat (BZT, ng/mL)		
	100	500	1500	100	500	1500
Mean	93.7	533.4	1414.1	99.4	516.0	1471.7
S.D.	6.0	15.1	105.4	8.0	43.7	76.6
R.S.D. (%)	6.4	2.8	7.5	8.1	8.5	5.2
Mean absolute percentage error (%)	−6.3	6.7	−5.7	−0.6	3.2	−1.9

Abbreviations: S.D: standard deviation; R.S.D.: relative standard deviation; BZL: benazepril; BZT: benazeprilat.

[20]) using the HPLC–UV method. This significant improvement of sensitivity is due partly to the sample preparation using the SPE method in which the analytes extracted from 1.0 mL plasma sample are reconstituted in a much smaller volume (0.2 mL) of the final solution prior to its injection into the HPLC system, and partly due to the gradient mode system employed.

3.3. Precision, accuracy and recovery

Table 1 shows the intra-day precision and accuracy of the assay of BZL and BZT in human plasma. The coefficients of variation were lower than 8.6% and the percent error values were within the range of −6.5–1.6% for all the three QC samples. Table 2 summarizes the inter-day precision and accuracy of the assay of BZL and BZT in human plasma. The coefficients of variation were lower than 8.56% and the percent error values were within the range of −6.3–6.7% for all the three QC samples tested.

The mean extraction recovery of BZL in human plasma was 67.3–75.3%, while that of BZT in human plasma was 63.9–71.4%, for all the three QC samples tested with the coefficient of variation being less than 9.5%.

3.4. Stability

No significant degradation of BZL and BZT in plasma was observed under the following storage conditions: at room temperature for 6 h, frozen-thawed three times, and frozen at −20 °C for 1 month. Table 3 summarizes the respective plasma concentrations of both analytes obtained for the three QC samples.

Table 3
Recovery of benazepril and benazeprilat in plasma under various storage conditions ($n = 5$)

Condition/measured concentration of analyte (ng/mL) and variation	Nominal concentration of benazepril (BZL, ng/mL)			Nominal concentration of benazepril (BZT, ng/mL)		
	100	500	1500	100	500	1500
Three freeze–thaw cycle						
Mean	93.0	478.4	1474.5	92.7	490.2	1439.5
S.D.	5.6	13.2	113.6	8.4	41.6	83.8
R.S.D. (%)	6.0	2.8	7.7	9.1	8.5	5.8
6 h at 25 °C						
Mean	95.9	484.5	1452.6	92.3	477.3	1374.1
S.D.	6.2	15.3	114.8	8.1	44.8	87.2
R.S.D. (%)	6.5	3.2	7.9	8.8	2.1	6.3
30 day frozen at −20 °C						
Mean	90.4	479.3	1399.6	89.7	453.4	1391.6
S.D.	7.2	18.3	122.7	7.3	47.2	82.3
R.S.D. (%)	8.0	3.8	8.8	8.1	10.4	5.9

Abbreviations: S.D.: standard deviation; R.S.D.: relative standard deviation; BZL: benazepril; BZT: benazeprilat.

The difference between the observed and nominal values for BZL were −6.2 to −6.8%, −1.7 to 7.0% and −4.1 to −10.0%, respectively, while those for BZT were −5.1 to −7.2%, −3.6 to −8.2% and −4.1 to −6.8%, respectively. All the differences were lower than 11%. The R.S.D. values ranged from 2.1 to 10.4%.

3.5. Application to pharmacokinetic study in humans

This method was successfully applied to study the pharmacokinetics and bioequivalence of two benazepril hydrochloride formulations. Fig. 3 shows the mean plasma concentration–time profiles of for BZL and BZT after administration of a single dose of 40 mg test and reference benazepril hydrochloride tablets in healthy Chinese volunteers. The plasma concentrations of BZL at 6–48 h and those of BZT at 36–48 h were below the LOQ (20 ng/mL). The calculated pharmacokinetic parameters for both BZL and BZT are shown in Table 4. After single oral administration of 4 × 10 mg benazepril hydrochloride tablet to healthy volunteers, the mean $AUC_{0–4\,h}$, C_{max}, and t_{max} values for BZL of the test formulation were 919.8 ng h/mL, 1302.5 ng/mL, and 0.4 h, respectively; while the mean $AUC_{0–24\,h}$, C_{max}, and t_{max} values for BZT of the test formulation were 3846.1 ng h/mL, 783.7 ng/mL, and 1.2 h, respectively. The 90% CIs for C_{max} and $AUC_{0\text{-}\infty}$ of BZL in the test formulation were 87.5–106.0% and 88.0–105.8% of those values in reference formulation, respectively; and the 90% confidence limits for C_{max} and $AUC_{0\text{-}\infty}$ of BZT in the test formulation were 86.2–108.6% and 85.2.0–103.9% of those values in reference formulation, respectively. The results clearly indicated that the 90% CIs of the ratios for C_{max} and $AUC_{0\text{-}\infty}$ for both BZL and BZT laid within the

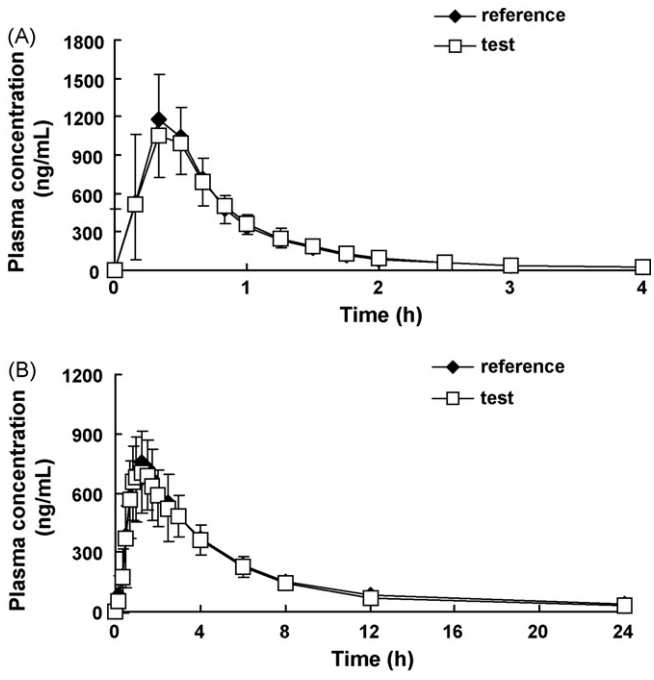

Fig. 3. Mean plasma concentration–time profiles of benazepril (A) and benazeprilat (B) after a single oral administration of 4 × 10 mg of benazepril hydrochloride test and reference tablets in 24 healthy volunteers. Data are the mean ± S.D.

Table 4
Pharmacokinetic parameters of benazepril and benazeprilat in healthy volunteers ($n = 23$) after the administration of both reference and test formulations containing 40 mg benazepril hydrochloride

Parameter	Benazepril (BZL)			Benazepril (BZT)		
	Reference formulation	Test formulation (90% CI)	Ratio (90% CI)	Reference formulation	Test formulation	Ratio ± S.D. (90% CI)
C_{max} (ng/ml)	1346.9 ± 442.9	1302.5 ± 340.6 (1179.1–1427.3)	0.97 ± 0.42 (0.88–1.06)	799.5 ± 181.6	783.7 ± 240.5 (689.7–868.3)	0.98 ± 0.38 (0.86–1.09)
T_{max} (h)	0.4 ± 0.1	0.4 ± 0.2 (0.2–0.6)	1.00 ± 0.56 (0.86–1.09)	1.3 ± 0.3	1.2 ± 0.4 (1.1–1.4)	0.92 ± 0.40 (0.88–1.05)
$t_{1/2,\lambda z}$ (h)	1.6 ± 1.4	1.6 ± 1.8 (1.2–2.0)	1.00 ± 1.43 (0.87–1.09)	4.1 ± 1.6	4.2 ± 1.3 (3.9–4.5)	1.02 ± 0.50 (0.87–1.07)
MRT (h)	0.8 ± 0.1	0.8 ± 0.2 (0.6–1.0)	1.00 ± 0.28 (0.89–1.02)	4.3 ± 0.9	4.3 ± 1.1 (4.1–4.5)	1.00 ± 0.33 (0.88–1.05)
$AUC_{0–4\,h}$ (for BZL) $AUC_{0–24\,h}$ (for BZT, ng h/ml)	928.7 ± 235.4	919.8 ± 220.7 (834.5–1007.9)	0.99 ± 0.35 (0.87–1.05)	3621.8 ± 940.0	3503.7 ± 1055.0 (3102.2–3837.6)	0.97 ± 0.40 (0.85–1.04)
$AUC_{0\text{-}\infty}$ (ng h/ml)	1061.6 ± 281.0	1028.4 ± 187.3 (934.5–1123.5)	0.97 ± 0.21 (0.88–1.06)	4034.6 ± 1059.6	3846.1 ± 1111.1 (3423.4–4190.3)	0.95 ± 0.39 (0.85–1.04)

Data are the mean ± S.D.
Abbreviations: $AUC_{0\text{-}tz}$: area under the plasma concentration–time curve ($AUC_{0\text{-}t}$) from time zero to the last measurable time (t_z) point; $AUC_{0\text{-}\infty}$: total area under the plasma concentration–time curve from time zero to infinity; BZL, benazepril; BZT, benazepril; C_{max}: maximum plasma concentration; CI, confidence interval; MRT: mean residual time; T_{max}: maximum time to reach C_{max}; $t_{1/2,\lambda z}$: elimination half-life.

equivalent limits (80–125% of the reference), indicating that the test and reference formulations are deemed to be bioequivalent with respect to the rate and extent of absorption. By normalizing our presented data with the administered dose to a 10 mg dose of benazepril, the plasma concentrations of BZL in Chinese are higher than those in Caucasian; whereas those for BZT in Chinese are lower than Caucasian as reported by Sun et al. [9]; suggesting an ethnic difference in absorption and disposition of BZL.

4. Conclusions

In conclusion, a simple reversed-phase HPLC method with UV detection for simultaneous determination of BZL and BZT in plasma was developed and validated with respect to intra- and inter-day precision and accuracy, stability, and recovery. The method was successfully applied to bioequivalence evaluation of benazepril hydrochloride formulations in healthy Chinese.

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