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Simultaneous determination of lercanidipine, benazepril and benazeprilat in plasma by LC–MS/MS and its application to a toxicokinetics study

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ABSTRACT

We aim to develop a rapid, simple, sensitive and specific LC–MS/MS method for the simultaneous quantification of lercanidipine, benazepril and benazeprilat in plasma. It is performed on the Agilent 6410 LC–MS/MS under the multiple-reaction monitoring (MRM) mode with electrospray ionization. Gliclazide was used as the internal standard (IS). Analytes and IS were extracted from plasma by solid-phase extraction. The reconstituted samples were chromatographed on a Diamond C_{18} (150 mm × 4.6 mm, 5 μm) column. The mobile phase was composed of 0.1% acetic acid–acetonitrile (50:50, v/v), with gradient flow rates: 0.6 mL/min (0–4.55 min); 4.55–4.65 min, 1 mL/min; 1 mL/min (4.65–9.5 min); 9.5–9.6 min, 0.6 mL/min; 0.6 mL/min (9.6–10 min). Method validation demonstrated that the method was of satisfactory specificity, sensitivity, precision and accuracy in linear ranges of 1–2000 ng/mL for lercanidipine, 1–2000 ng/mL for benazepril and 1–1600 ng/mL for benazeprilat, respectively. The precision (RSD%) was better than 15, and the lower limit of quantitation was identifiable and reproducible at 1 ng/mL for the three analytes. The plasma samples were stable after being stored for more than 60 days and after two freeze–thaw cycles (−20 to −25 °C). It is demonstrated that this method was successfully applied to samples from a toxicokinetics study of a compound of lercanidipine and benazepril in beagle dogs.

1. Introduction

Lercanidipine, [2-[(3,3-diphenylpropyl) methylamine]-1, 1-dimethylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5 pyridine carboxylic ester] (Fig. 1A), is a new third generation dihydropyridine calcium channel blocker (CCB) that is used in treatment of hypertension. It is categorized as once-a-day calcium antagonist with a short plasma half-life of 3 h [1]. The presence of a 3,3-diphenyl propyl methylamino-2-methyl-2-propyl chain at the 1,4-dihydropydine in lercanidipine can significantly improve its lipophilicity and action time [2], resulting in membrane-controlled pharmacokinetic characteristics with prolonged pharmacologic effect on blood pressure.

Benazepril, [2-(3S)-3-[((2S)-1-ethoxycarbonyl -3-phenyl-(1S) -propyl)-amino]-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benazepine-1-acetic acid], (Fig. 1B) is a prodrug of angiotensin-converting enzyme inhibitor (ACEI), and is demonstrated to be effective for treating congestive heart failure and hypertension. In vivo, benazepril is hydrolyzed to a pharmacologically active metabolite, the diacid benazeprilat, (3-[(1-carbonyl-3-phenyl-(1S)-propyl)-amino]-2,3,4,-5-tetrahydro-2-oxo-1-(3S)-benazepine-1-acetic acid) (Fig. 1C).

The combination therapy of CCB and ACEI was shown to be superior in lowering systolic and diastolic blood pressures when compared with either of the monotherapy regimens [3–5]. Combination therapy also has significantly fewer dose-dependent adverse experiences as against high-dose CCB monotherapy [6–8].

It is reported that analytes of the lercanidipine, benazepril and benazeprilat were identified by HPLC [9–12,2,13,14], derivative spectrophotometry [15–19], capillary electrophoresis (CE) [20], high performance thin layer chromatography (HPTLC) [21], gas chromatography–mass spectrometry [22] and liquid chromatography–mass spectrometry [1,20,23]. Few methods are reported for the assay of combination drug products containing two or more active drug substances [12]. Most of published methods only monitored the quality of the pharmaceutical dosage forms [10–12,2,13–19,21]. However, it is really important to analyze the compounds in body fluids. The bioavailability and pharmacokinetics study is dependent on the determination of the compounds in the body fluids, especially in plasma.

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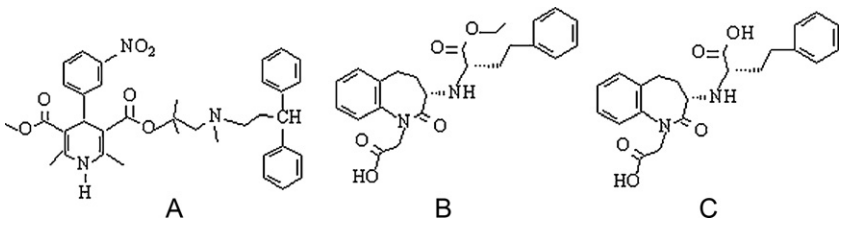

Fig. 1. Chemical structures of lercanidipine (LER), benazepril (BEN) and benazeprilat (A: LER; B: BEN; C: benazeprilat).

Until now, there is no method reported for the simultaneous quantification of lercanidipine, benazepril and benazeprilat in any of the matrices. In the present study, we aim to establish a simple, rapid and reproducible method to simultaneously estimate concentrations of lercanidipine, benazepril and benazeprilat in plasma. It will provide useful information for further pharmacokinetic studies of various compounds and is expected to be efficient in analyzing large numbers of plasma samples in clinical studies (e.g. bioequivalence). The application of this assay in a toxicokinetics study following oral administration of a compound of lercanidipine and benazepril in beagle dogs is described.

2. Experimental

2.1. Materials

Lercanidipine hydrochloride (Lot: 10011001S, purity: 99.3%), benazepril hydrochloride (Lot: 09081001, purity: 99.1%) and benazeprilat (Lot: 09081001, purity: 99.5%) were obtained from Shenzhen Salubris Pharmaceuticals Co., Ltd., Shenzhen, China. Gliclazide (Lot No. 100269-9701) was obtained from National Institute for the Control of Pharmaceutical and Biological Products, Beijing China. Methanol (Lot: H30E18) and acetonitrile (Lot: H31825) were HPLC grade from J.T.BAKER, USA. Acetic acid glacial (Lot: 605041, HPLC grade) was obtained from TEDIA Company Inc., USA.

2.2. Instruments

The LC–MS/MS procedure was performed by using an Agilent 1200 series HPLC and an Agilent 6410 Triple Quadrupole mass spectrometer equipped with an electrospray ionization source (Agilent Technologies, USA). All data were analyzed by software Agilent 6410 Quantitative Analysis version analyst data processing software.

2.3. LC–MS/MS conditions

The chromatographic separation was achieved on a Diamond C_{18} column (150 mm × 4.6 mm, 5 μm, Dikma Technologies, Beijing, China) at 30 °C with a thermostated column oven. The mobile phase was a mixture of 0.1% acetic acid–acetonitrile (50:50, v/v), with a gradient flow rate of 0.6 mL/min (0–4.55 min); 4.55–4.65 min, 1 mL/min; 1 mL/min (4.65–9.5 min); 9.5–9.6 min, 0.6 mL/min; 0.6 mL/min (9.6–10 min). The injection volume was 2 μL.

Mass spectrometric analysis was performed in the positive ion MRM mode, with spray gas pressure (350 Pa), protective air of nitrogen gas (11 L/min), dwell times (200 ms), and capillary voltage (3500 V). The fragment electric voltage, collision energy and quantification of lercanidipine, benazepril, benazeprilat and IS were achieved by monitoring the *m/z* of precursor/product ions (Table 1). All data were acquired employing Agilent 6410 Quantitative Analysis version analyst data processing software.

2.4. Preparation of standard solutions, calibration standard and quality control samples

Primary stock solutions of lercanidipine, benazepril, IS (all at 1 mg/mL) and benazeprilat (200 μg/mL) were separately prepared in acetonitrile. Primary stock solutions were diluted with the mobile phase for standard working solutions of lercanidipine (0.01, 0.1, 1, 10 μg/mL), benazepril (0.01, 0.1, 1, 10 μg/mL) and benazeprilat (0.01, 0.1, 1, 10 μg/mL). The IS was dissolved in the mobile phase to get a 20 μg/mL stock solution. All solutions were stored at 4 °C, and equilibrated to room temperature before use (approximately 15 min).

The calibration curve standard and quality control (QC) samples were freshly prepared in blank plasma by spiking with different working solutions. The calibration samples consist of seven non-zero concentrations of lercanidipine (1–2000 ng/mL), benazepril (1–2000 ng/mL) and benazeprilat (1–1600 ng/mL). QC samples of analytes were: 1 (LLOQ), 3 (LQC), 30 (MQC), 1600 ng/mL (HQC) for lercanidipine, 1 (LLOQ), 3 (LQC), 30 (MQC), 1800 ng/mL (HQC) for benazepril; and 1 (LLOQ), 3 (LQC), 30 (MQC), 1500 ng/mL (HQC) for benazeprilat.

2.5. Plasma pre-treatment

A solid phase extraction method was followed for extraction of lercanidipine, benazepril, benazeprilat and IS from plasma. Solid-phase extraction columns (OASIS HLB C_{18}, 1 cm^3/30 mg) (Part No: WAT094225; Lot No: 105A31150A; Waters Corporation, MA, USA) were preconditioned with 2 mL methanol and 2 mL water. Plasma samples (0.2 mL) were mixed with IS (20 μL, 20 μg/mL) after NaOH (20 μL, 1 M) being added. After full vortex mixing, the mixed plasma samples were applied to the column. The columns were washed with water (1 mL) and washing water was discarded. Then, the analytes and IS were eluted with methanol (1 mL). The organic phase solution was transferred to a clean tube and evaporated to dryness under a gentle stream of nitrogen gas at 40 °C. The residue was reconstituted with 100 μL mobile phase, and injected (2 μL) for LC–MS/MS analysis.

2.6. Method validation

The assays in human plasma were validated in compliance with the FDA guidelines, including selectivity, matrix effects, linearity, recovery, precision, accuracy and stability.

Table 1
Optimized mass spectrometry parameters for analytes and IS.

Analyte	Precursor/product ions	Fragment electric voltage (V)	Collision energy (eV)
Lercanidipine	612.3/280.2	90	25
Benazepril	425.2/351.2	90	20
Benazeprilat	397.2/351.2	120	20
IS	324.2/127.2	100	20

2.6.1. Selectivity

Chromatograms of six different lots of blank human plasma were identified for inspecting analytes from the potential interference of endogenous substances at the LC peak region (lercanidipine, benazepril, benazeprilat and IS).

2.6.2. Matrix effect

Matrix effect was investigated to ensure precision, selectivity and sensitivity that were not compromised by the matrix screened. Blank biological samples were extracted and then spiked with the analytes at three QC levels and IS in five replicates. The corresponding peak areas were compared to those of standard solutions, and the peak area ratio was defined as the matrix effect.

2.6.3. Recovery

The mean overall recoveries of the analytes and IS were determined by the ratio of the peak areas (extracted plasma standards/post extraction plasma samples). Analytes were determined by samples at three QC levels with five replicates for each QC level and extracted as above (see Section 2.4). Recovery of the IS was determined at 2 ng/mL.

2.6.4. Calibration curves

Calibration curves were prepared at seven different concentrations. Every calibration standard was injected in five replicates. Calibration curves were typically described by equation $y = ax + b$, where y represents the peak–area ratio of analyte to IS, and x represents the plasma concentration of analyte. The linearity of the calibration curve was assessed by linear regression with a weighting factor of the reciprocal of the concentration squared ($1/x^2$). The acceptance criterion for each back-calculated standard concentration was ±15% deviation from the nominal value, while that of LLOQ was ±20% (US DHHS, FDA, CDER, 2001). The calibrators for analytes were: lercanidipine: 1, 3, 10, 30, 100, 500 and 2000 ng/mL; benazepril: 1, 3, 10, 30, 100, 600 and 2000 ng/mL; benazeprilat: 1, 3, 10, 30, 100, 500 and 1600 ng/mL.

2.6.5. Precision and accuracy

The intra-assay precision and accuracy were estimated by analyzing five replicates of lercanidipine, benazepril and benazeprilat at three different QC levels in human plasma. The inter-assay precision was determined by analyzing the three-level QC samples on three consecutive days. The criteria for acceptability of data were accuracy within ±15% from the nominal values and a precision of within ±15% relative standard deviation (RSD) or CV%, but that of LQC is not supposed to exceed ±20% (US DHHS, FDA, CDER, 2001).

2.6.6. Stability

The stability of each analyte in plasma was determined by three QC levels in five replicates for 8 h (bench-top) at ambient temperature (24 ± 2 °C). Stability of lercanidipine, benazepril and benazeprilat in the injection solvent was determined periodically by injecting replicate and processed plasma samples after the sample loading (in the autosampler) for up to 10 h at 24 °C. Freezed stability of analytes in plasma was assessed by analyzing samples with three QC levels that were stored at −20 °C for 60 days. The stability of analytes in plasma following two freeze–thaw cycles was also assessed. The samples were processed as described above. The criterion for acceptability of the data is the same with that for the precision.

2.7. Toxicokinetics study

Twenty-four healthy beagle dogs (male, 12; female, 12) were used for the toxicokinetics study of the compound preparation of lercanidipine and benazepril. Beagle dogs were divided into four groups: the control, lercanidipine (15 mg/kg), benazepril (45 mg/kg) and lercanidipine + benazepril (15 + 45 mg/kg) groups, each group were orally administered after overnight fasting. Blood samples were collected before and at 5, 10, 15, 20, 30, 45 min, 1, 2, 3, 4, 6, 8, 12, and 24 h after drug administration. Plasma samples were heparinized, centrifuged, separated, transferred into Eppendorf tubes and then stored at −20 °C. The samples were determined by the chromatographic conditions described above.

3. Results and discussion

3.1. Method development

3.1.1. Mass spectrometry

MS parameters were tuned in both positive and negative ionization modes for lercanidipine, benazepril, benazeprilat and IS. However, a good response was found in positive ionization mode. The mass spectrum of positively charged lercanidipine (m/z 612.3) showed the formation of characteristic productions at m/z 280.2, 298.2, 315.0 and 387.1 (data not shown). The most sensitive mass transition was monitored from m/z 612.3–280.2. The production mass spectrum of benazepril (m/z 425.1) showed the formation of characteristic production at m/z 117.1, 146.0, 190.1 and 351.2 (data not shown) and the most sensitive mass transition was observed from m/z 425.1 to 351.2. The production mass spectrum of benazeprilat (m/z 397.2) showed the formation of characteristic production at m/z 146.0, 190.1 and 351.2 (data not shown) and the most sensitive mass transition was observed from m/z 397.2–351.2. Similarly, production mass spectrum of IS (gliclazide, m/z 324.2) shows the formation of characteristic productions at m/z 125.1, 127.2, 151.1, 153.1, 155.1 and 168.1. The most sensitive mass transition was from m/z 324.2–127.2 (Fig. 2).

3.1.2. Liquid chromatography

A simple chromatographic separation was developed for acquisition of good separation with a short run time. Following various combinations of acetonitrile and buffer on a variety of columns, MS detection received good responses with acetic acid buffer (0.1%) in the positive ionization mode. Finally, chromatographic separation was operated by using isocratic mobile phase system consisting of 0.1% acetic acid and acetonitrile (50:50, v/v), on a Diamonsil C_{18} column. The large-volume injection of highly organic supernatant sample increased matrix and eluotropic effects [24]. After repeated injection the post-preparation samples confirmed that the injection volume of 2 μL is stable. Therefore, the injection volume was 2 μL. With the chromatographic conditions as described above, lercanidipine, benazepril, and benazeprilat were eluted at retention times of 3.22, 4.39 and 2.93 min, respectively.

3.1.3. Sample pre-treatment

A sample pre-treatment method should remove interferences from the biological sample and also be reproducible with a high recovery involving a minimum number of working steps. Solid-phase extraction (SPE) was used for producing chromatographia clean samples in the study, which are essential for minimizing ion suppression and matrix effects in LC–MS/MS. Various solvents, including chloroform, ethyl acetate, dichloromethane, *n*-hexane–dichloromethane–isopropanol (20:10:1, v/v/v) and methanol were investigated and evaluated for acceptable extraction recoveries and matrix effect. Methanol, with the weakest matrix effect and no-concentration-dependent extraction recovery, was selected as the extraction solvent in this study.

3.1.4. Selection of IS

A good IS should mimic the analytes during extraction and compensate for analytes on the column, especially with

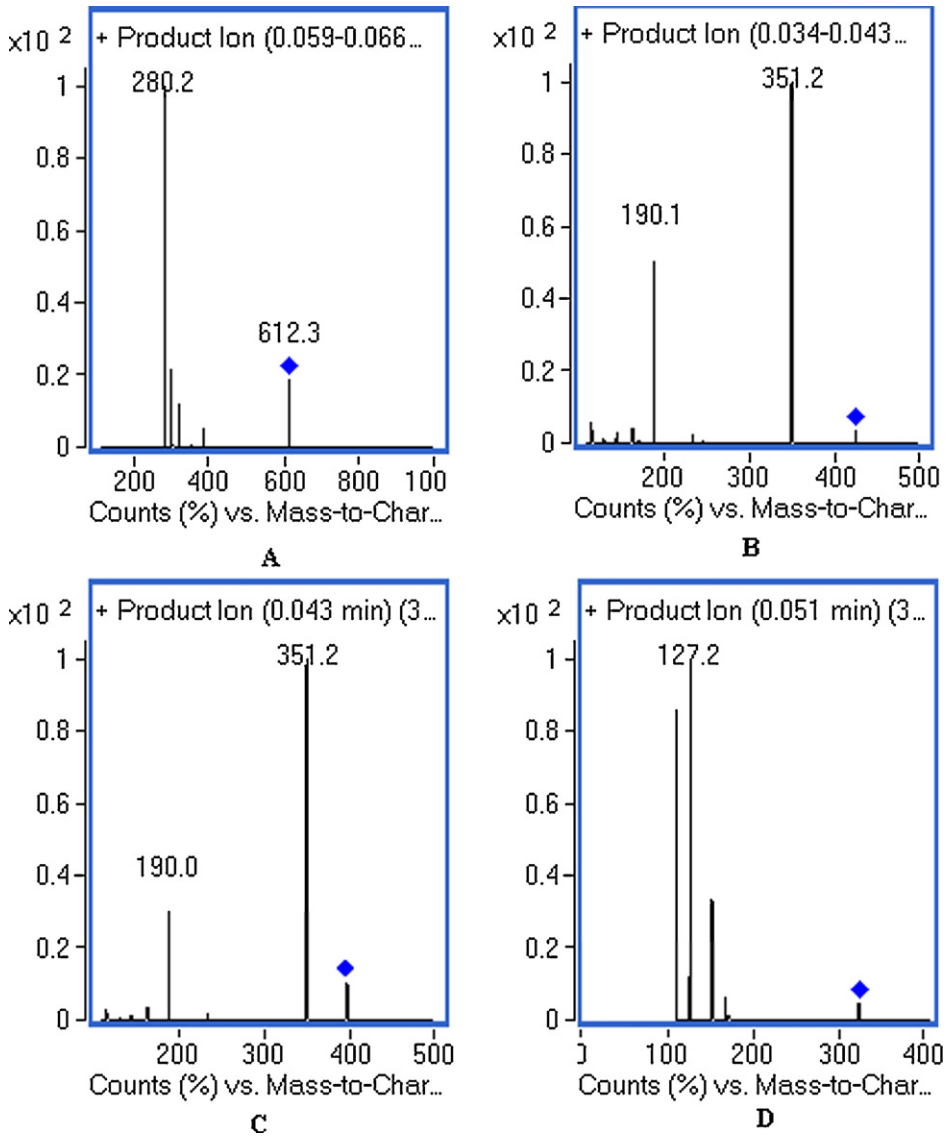

Fig. 2. MS Spectrum of lercainidipine, benazepril, benazeprilat and IS (gliclazide) (A: lercanidipine 612.3→280.2; B: benazepril 425.1→351.2; C: benazeprilat 397.2→351.2; D: IS 324.2→127.2).

LC–MS/MS, because matrix effects could induce poor analytical results. In the initial phase, several compounds, such as diazepam, acetaminophen and gliclazide, were investigated and finally gliclazide was the best choice of IS. When the flow rate was 0.6 mL/min, the retention time of gliclazide was >18 min. Although mobile phase gradient elution provides faster separation, it often causes baseline shifts and requires additional equilibrium time between runs [25]. Increasing flow rate during the run can reduce the elution time of the IS. To shorten the analysis period, a gradient flow method was conducted. With the chromatographic conditions as described above, the IS was eluted at 9.31 min.

3.2. Method validation

3.2.1. Selectivity

The interference by endogenous plasma constituents with analytes and IS was assessed by inspection of chromatograms that were derived from processed blank plasma sample. No significant interferences were found in the endogenous blank human plasma at the retention times of the analytes and IS (Fig. 3).

3.2.2. Lower limit of the quantitation (LLOQ)

The LLOQ was defined as the lowest concentration on the standard calibration curves with acceptable repeatability and recovery. The analyte response at the LLOQ should be at least five times the response of blank baseline. The LLOQ was evaluated by analyzing five replicates of spiked plasma sample at the concentration of 1 ng/mL for each analyte. The precision and accuracy were found to be 5.09% and 100% for lercanidipine; 0.97% and 114% for benazepril and 4.29% and 114% for benazeprilat, respectively.

3.2.3. Recovery and matrix effect

A simple SPE with methanol proved to be robust and provided the clean samples. The recoveries of analytes and IS were good and reproducible. No significant matrix effect was observed in human plasma for the analytes at three different QC levels and IS in 2 ng/mL. The result was shown in Table 2. It is indicated that the analytical method could be kept free from endogenous substance in human plasma.

3.2.4. Linearity

Correlation coefficient (r^2), which indicates linearity over the calibration range of all analytes, was calculated by using the $1/x^2$

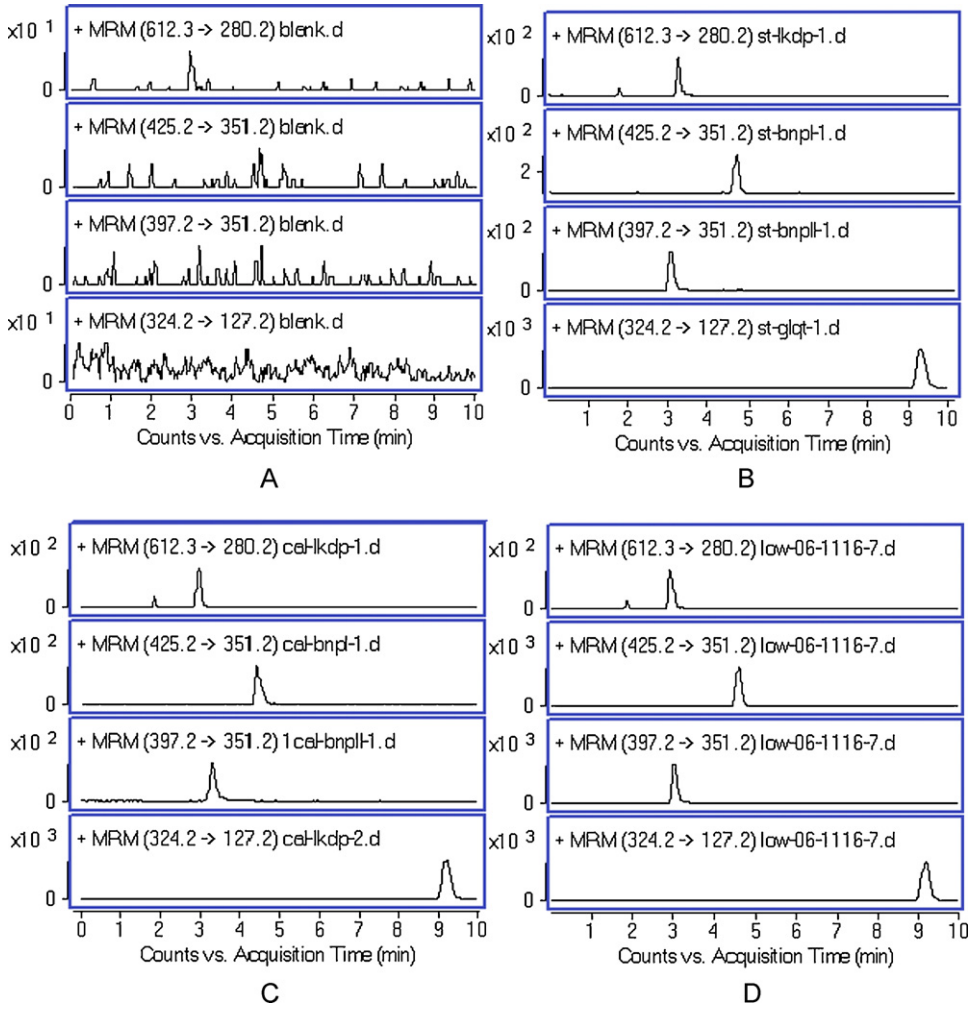

Fig. 3. Typical MRM chromatograms of lercanidipine, benazepril, benazeprilat and IS. (A: blank human plasma; B: lercanidipine, benazepril, benazeprilat and IS standard (all in 1 ng/mL); C: blank human plasma spiked with lercanidipine (1 ng/mL), benazepril (1 ng/mL), benazeprilat (1 ng/mL) and IS (2 ng/mL); D: plasma spiked with IS from dogs No 1116, 1h after oral compound lercanidipine and benazepril (15 + 45 mg/kg)).

model. The method exhibited a good linear response for the three analytes. Correlation coefficients (r^2) were all >0.99. The equation of the calibration curve was: $y = 0.016x - 0.0049$ ($r^2 = 0.9901$, $n = 5$) for lercanidipine, $y = 0.0129x - 0.0012$ ($r^2 = 0.9902$, $n = 5$) for benazepril and $y = 0.0093x + 0.0020$ ($r^2 = 0.9902$, $n = 5$) for benazeprilat.

3.2.5. Precision and accuracy

Precision and accuracy data for intra- and inter-day plasma samples of lercanidipine, benazepril and benazeprilat are shown in Table 3. The assay values on both occasions (intra- and inter-day) conform to the accepted variable limits.

Table 2
The recovery and matrix effect of LER,BEN, benazepeilat and IS ($n = 5$).

	C(ng/mL)	Recovery (%)		Matrix effect (%)	
		$\bar{x} \pm$ SD	RSD%	$\bar{x} \pm$ SD	RSD%
Lercanidipine	3	73.95 ± 1.87	2.53	94.34 ± 3.28	3.48
	30	71.94 ± 4.16	5.78	97.19 ± 4.07	4.19
	1600	72.81 ± 3.89	5.34	94.85 ± 3.87	4.08
Benazepril	3	92.57 ± 3.18	3.44	99.03 ± 3.99	4.03
	30	92.42 ± 1.26	1.37	95.78 ± 4.45	4.64
	1800	92.81 ± 4.00	4.31	97.41 ± 1.75	1.79
Benazeprilat	3	94.43 ± 4.54	4.81	98.75 ± 4.40	4.46
	30	91.90 ± 8.46	9.21	95.84 ± 6.61	6.89
	1500	94.95 ± 4.55	4.79	100.98 ± 3.46	3.43
IS	2	101.34 ± 4.90	4.83	98.13 ± 4.80	4.89

Table 3
The intra-day and inter-day precisions of LER, BEN and benazeprilat in plasma ($n = 5$).

	C(ng/mL)	Intra-day			Inter-day		
		$\bar{x} \pm$ SD	Accuracy%	RSD%	$\bar{x} \pm$ SD	Accuracy%	RSD%
Lercanidipine	3	3.06 ± 0.43	102.0	14.13	3.02 ± 0.26	100.7	8.47
	30	27.67 ± 0.52	92.2	1.88	30.74 ± 2.32	102.5	7.53
	1600	1746.75 ± 66.4	109.2	3.80	1583.3 ± 126.0	99.0	7.96
Benazepril	3	3.27 ± 0.48	108.0	14.69	3.17 ± 0.40	105.7	12.57
	30	33.92 ± 0.16	113.1	0.47	32.64 ± 2.33	108.8	7.14
	1800	1907.49 ± 46.3	106.0	2.43	1834.98 ± 214.26	101.9	11.68
Benazeprilat	3	3.04 ± 0.34	101.3	11.08	3.09 ± 0.25	103.0	8.17
	30	30.96 ± 0.80	103.2	2.58	29.93 ± 2.08	99.77	6.95
	1500	1313.92 ± 38.7	87.6	2.95	1481.89 ± 160.0	98.79	11.20

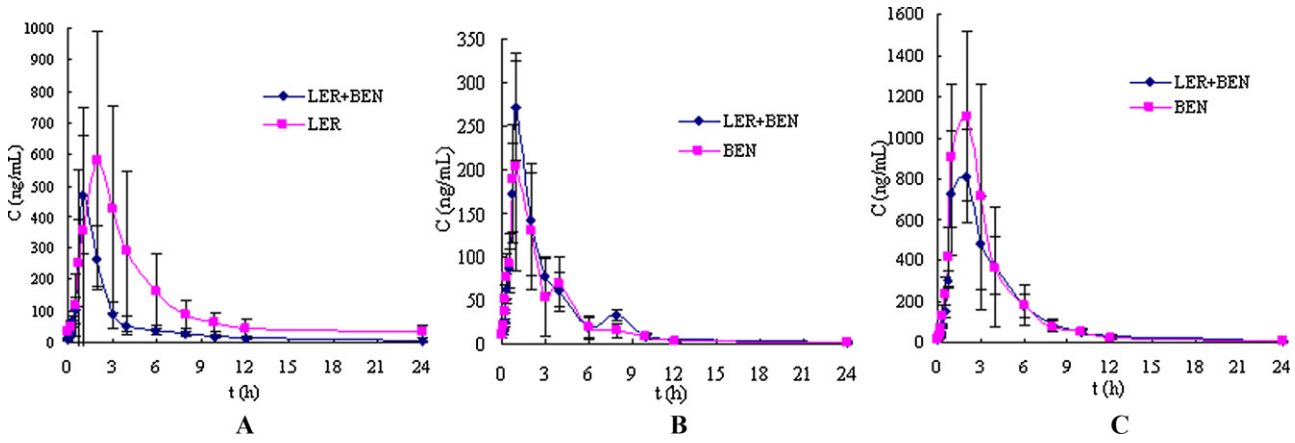

Fig. 4. Mean plasma concentration–time profile of lercanidipine, benazepril and benazeprilat in beagle dogs. (A: Mean plasma concentration–time profile of lercanidipine after administration of 15 mg/kg lercanidipine and the compound preparation of lercanidipine + benazepril (15 + 45 mg/kg); B: Mean plasma concentration–time profile of benazepril after administration of 45 mg/kg benazepril and the compound preparation of lercanidipine + benazepril (15 + 45 mg/kg); C: Mean plasma concentration-time profile of benazeprilat after administration of 45 mg/kg benazepril and the compound preparation of lercanidipine + benazepril (15 + 45 mg/kg)). (Mean ± SD, $n = 6$.)

Table 4
Stability data of LER, BEN and benazeprilat in human plasma at various conditions (n = 5).

	C(ng/mL)		Bench top (8 h)	In injector (10 h)	Two freeze-thaw	60 days at -20 °C
Lercanidipine	3	$\bar{x} \pm$ SD	3.01 ± 0.37	2.73 ± 0.33	3.12 ± 0.22	3.18 ± 0.08
		Accuracy%	100.3	91.0	104.0	106.0
	30	$\bar{x} \pm$ SD	30.48 ± 3.08	28.56 ± 1.41	31.16 ± 0.83	31.24 ± 1.79
		Accuracy%	101.6	95.1	103.9	104.1
	1600	$\bar{x} \pm$ SD	1430.55 ± 91.00	1547.20 ± 42.14	1519.33 ± 26.86	1496.28 ± 13.06
		Accuracy%	89.4	96.7	95.0	93.5
Benazepril	3	$\bar{x} \pm$ SD	2.88 ± 0.05	2.99 ± 0.25	3.32 ± 0.06	2.77 ± 0.10
		Accuracy%	96.0	99.7	110.7	92.3
	30	$\bar{x} \pm$ SD	32.19 ± 0.27	30.46 ± 4.01	30.14 ± 0.32	27.19 ± 3.84
		Accuracy%	107.3	101.5	100.5	90.6
	1800	$\bar{x} \pm$ SD	1731.87 ± 118.02	1730.31 ± 190.58	2047.09 ± 24.58	1647.28 ± 5.17
		Accuracy%	96.2	96.1	113.7	91.5
Benazeprilat	3	$\bar{x} \pm$ SD	3.42 ± 0.07	2.71 ± 0.17	2.84 ± 0.12	3.34 ± 0.17
		Accuracy%	114.0	90.3	94.7	111.3
	30	$\bar{x} \pm$ SD	27.68 ± 0.24	28.69 ± 2.61	30.17 ± 3.43	29.96 ± 1.15
		Accuracy%	92.3	95.6	100.6	99.9
	1500	$\bar{x} \pm$ SD	1352.82 ± 95.22	1313.16 ± 32.44	1498.99 ± 24.68	1579.52 ± 30.87
		Accuracy%	90.2	87.5	99.9	105.3

3.2.6. Stability studies

Stability for lercanidipine, benazepril and benazeprilat are shown in Table 4 after 8 h on bench-top, 10 h in autosampler, two freeze–thaw cycles and freezed at −20 °C for 60 days. The data conform to the acceptance criteria.

4. Toxicokinetics study

The developed assay method was successfully applied to a toxicokinetics study of the compound of lercanidipine and benazepril in beagle dogs. The mean plasma concentration–time profile of lercanidipine, benazepril and benazeprilat were shown in Fig. 4. Drug–drug interactions of lercanidipine and benazepril need further confirmation.

5. Conclusions

In summary, we developed and validated a highly sensitive, specific, reproducible and high-throughput LC–MS/MS method for simultaneous quantification of lercanidipine, benazepril and

benazeprilat with one IS. According to the validation parameters, we concluded that the developed method could be useful for bioequivalent studies and routine therapeutic drug monitoring with desired precision and accuracy. It was successfully applied to a toxicokinetics study of the compound preparation of lercanidipine and benazepril in beagle dogs.

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