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# Determination of eprosartan in human plasma and urine by LC/MS/MS

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## Abstract

A protein precipitation, liquid chromatography/tandem mass spectrometry (LC/MS/MS) method has been developed and validated for the determination of eprosartan in human plasma and urine. The solvent system also served as a protein precipitation reagent. The chromatographic separation was achieved on a CAPCELL PAK C18 column (50 mm × 2.0 mm, 5 μm, Shiseido). A mobile phase was consisted of 0.5% formic acid in water and 0.5% formic acid in acetonitrile (72:28). Detection was by positive ion electrospray tandem mass spectrometry on a Sciex API3000. The standard curves, which ranged from 5 to 2000 ng/mL in human plasma and from 0.25 to 50 μg/mL in urine, were fitted to a $1/x$ weighted quadratic regression model. The method proved to be accurate, specific and sensitive enough to be successfully applied to a pharmacokinetic study.
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*Keywords:* Eprosartan; LC/MS/MS; Protein precipitation; Plasma; Urine

## 1. Introduction

Eprosartan (eprosartan; Fig. 1) is a highly selected, non-peptide angiotensis-II antagonist. The compound has been shown to inhibit angiotensin-II included vasoconstriction in pre-clinical species and cause reductions in systolic and diastolic blood pressure at peak effect after dosing in clinical patients. It is currently being developed for the treatment of hypertension as other compounds of the angiotensin-II receptor antagonists (ARA-II) family. These are safe and effective agents for the treatment of hypertension and heart failure, either alone or in combination with diuretics. Therefore, they have been proposed as an alternative to the traditional angiotensin-converting enzyme (ACE) inhibitors [1].

Due to the high polarity, it is difficult to seperate eprosartan from some plasmatic interferences in human plasma. Ferreiros et al. [2,3] and Lundberg et al. [4] reported the limit of quantification of plasma eprosartan using SPE-HPLC-UV method to be 150 and 10 ng/mL, respectively. Hillaert et al. [5–7] reported

the determination of eprosartan using a capillary zone electrophoretic method. In these methods, the preparation of plasma samples was complicated and the separation of eprosartan and an internal standard from clinical samples was time-consuming. Zhang et al. [8] and Li et al. [9] reported the determination of telmisartan by LC/MS/MS has a minimum detection limit of 0.5 ng/mL [9]. Iwasa et al. [10] also reported the determination of losartan and its major metabolite using an HPLC method was followed by electrospray ionization tandem mass spectrometric. But few articles have reported the determination of eprosartan by LC/MS/MS. The purpose of this study was to improve the LC/MS/MS method with simple sample preparation for the determination of eprosartan in human plasma and urine to support a pharmacokinetic study after oral administration of eprosartan.

## 2. Experimental

### 2.1. Reagents and chemicals

Eprosartan (99.5% pure, Batch number: ARS0303AA) and D6-eprosartan (internal standard) (97.6% pure, Batch number: WMH00310A) were obtained from Solvay Pharmaceuticals (Hannover, Germany). Formic acid was obtained from Fisher Scientific (Fairlawn, NJ, USA). Methanol and acetonitrile, both
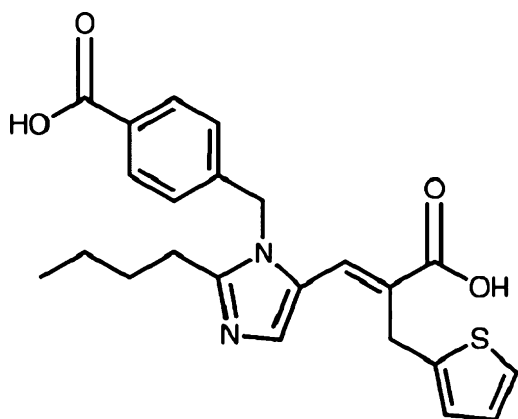
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Fig. 1. Chemical structure of eprosartan.

HPLC grade, were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Deionized/distilled (DI) water was prepared from tap water in our own department.

## 2.2. Eprosartan standard, QC and IS preparation

Primary stock solution of eprosartan (5 mg/mL) and D6-eprosartan (100 μg/mL) were prepared in DI water. Working standard solutions of eprosartan were prepared by combining aliquots of primary stock solution and diluting with DI water. The working solutions of D6-eprosartan for internal standard (I for 1 μg/mL and II for 50 ng/mL) were prepared by diluting aliquots of stock solution with DI water. All standard solutions were stored at 4 °C in the dark when not in use. Calibration standards of eprosartan in human plasma (5, 10, 50, 100, 500, 1000, 2000 ng/mL) and that in human urine (0.25, 0.5, 2.5, 5, 12.5, 25, 50 μg/mL) were prepared by spiking the working standard solutions into a pool of drug-free samples.

QC stock solution of eprosartan (5 mg/mL) was prepared from a separate weighing and was also dissolved in DI water. Dilutions were used to prepare four levels of QCs: high QC (1600 ng/mL eprosartan in plasma and 40 μg/mL eprosartan in urine), medium QC (1000 ng/mL eprosartan in plasma and 20 μg/mL eprosartan in urine), low QC (5, 20 ng/mL eprosartan in plasma and 0.25, 1 μg/mL eprosartan in urine) and LLOQ QC (5 ng/mL eprosartan in plasma and 0.25 μg/mL eprosartan in urine). The QC standards were prepared in plasma or urine like the calibration standards. QCs were stored at −20 °C.

## 2.3. Sample preparation

### 2.3.1. Plasma sample preparation

Fifty microliters of plasma samples or QC was added into 2.0 mL labeled microcentrifuge tubes. To each sample, 5 μL of the working internal standard solution (1 μg/mL) was added. The tubes were capped and vortexed for 5 s. Then 50 μL acetonitrile was added into microcentrifuge tubes and the samples were centrifuged in Heraeus Biofuge 28RS at 15,000 rpm and 20 °C for 2 min. The supernatant layer was injected into the LC/MS/MS system.

### 2.3.2. Urine sample preparation

Twenty microliters of urine samples or QC was added into 2.0 mL labeled microcentrifuge tubes. To each sample, 1 mL of the DI water was added to dilute the urine sample. The tubes were capped and vortexed for 30 s. Twenty microliters of the above-diluted sample was added into 2.0 mL microcentrifuge tubes and 1 mL of the working internal standard solution (50 ng/mL) was added. The tubes were capped and vortexed for 30 s. All samples were then centrifuged in Heraeus Biofuge 28RS at 15,000 rpm and 20 °C for 2 min. The supernatant layer was injected into the LC/MS/MS system.

## 2.4. LC/MS/MS analysis

The liquid chromatography separation system consisted of LC-10AD VP (pump), DGU-14AM and SIL-HTc (autosampler) (Shimadzu Corporation, Kyoto, Japan). The separation column was a CAPCELL PAK C18 column (50 mm × 2.0 mm, 5 μm) (Shiseido Corporation, Tokyo, Japan). An XW-80 vortex was obtained from Shanghai Medical University Apparatus Co. (Shanghai, China). The Biofuge 28RS centrifuge was obtained from Heraeus Sepatech Co. (Osterode, Germany).

The isocratic mobile phase consisted of 72% solvent A (0.5% formic acid in water) and 28% solvent B (0.5% formic acid in acetonitrile). The flow rate was set to 0.30 mL/min. The injection volume was 3 μL and the run time was 2 min.

A Sciex API3000 LC/MS/MS system (Foster City, CA, USA) operating under Analyst 1.3.1 software was used. The electrospray ion source was run in a positive ionization mode for all experiments. The typical ion source parameters were: declustering potential (DP), 36 V; collision energy (CE), 35 eV; focusing potential (FP), 190 V; collision cell exit potential (CXP), 11 V; spray voltage, 5500 V and ion temperature, 400 °C. Nebulizer gas (NEB), curtain gas (CUR) and collision gas (CAD) were set to 75, 40, 120 psi, respectively. Nitrogen gas was used for CUR, CAD, NEB. The sample was analyzed via selected reaction monitoring (SRM). The monitoring ions were set as $m/z$ 425.5 → 207.3 for eprosartan and $m/z$ 431.5 → 207.3 for D6-eprosartan. The scan dwell time was set at 0.2 s for every channel.

## 3. Results

### 3.1. Method validation

#### 3.1.1. Standard curve

After the protein precipitation and LC/MS/MS conditions were defined, a full validation was performed to assess the performance of the method. Seven-point calibration standard curves of eprosartan ranging from 5 to 2000 ng/mL in human plasma and from 0.25 to 50 μg/mL in human urine were used in duplicate in each analytical run. Peak area ratios of eprosartan to IS were used for regression analysis. A weighted ($1/x$) quadratic regression model, where $x$ is the concentration of eprosartan, was fitted to each standard curve. The %CV at each level of eprosartan varied from 0.00 to 9.00 in plasma and from 0.00 to 13.00 in urine. The mean of the absolute value of percent deviation from the theoretical value of eprosartan in plasma and that

Table 1
Mean inter-batch back-calculated standard and standard curve results of eprosartan

| | Mean inter-batch back-calculated standard results | | | | | | | Standard curve reasults | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Standard | | | | | | | Slope | Y-intercept | γ |
| | STD1 (5 ng/mL) | STD2 (10 ng/mL) | STD3 (50 ng/mL) | STD4 (100 ng/mL) | STD5 (500 ng/mL) | STD6 (1000 ng/mL) | STD7 (2000 ng/mL) | | | |
| Plasma | | | | | | | | | | |
| Run ID I | 5.45 | 10.1 | 48.6 | 93.5 | 491 | 1010 | 2000 | 0.0121 | −0.00917 | 0.9999 |
| Run ID II | 5.28 | 10.2 | 49.0 | 93.6 | 498 | 1010 | 2000 | 0.0119 | −0.00414 | 0.9999 |
| Run ID III | 5.03 | 10.2 | 50.3 | 97.7 | 488 | 1020 | 2000 | 0.0116 | −0.00135 | 0.9999 |
| Run ID IV | 5.18 | 10.3 | 49.6 | 96.2 | 488 | 998 | 2020 | 0.0116 | −0.00310 | 0.9999 |
| Run ID V | 5.30 | 10.2 | 46.8 | 101.0 | 488 | 976 | 2040 | 0.0119 | −0.00882 | 0.9997 |
| Mean | 5.25 | 10.2 | 48.9 | 96.4 | 491 | 1003 | 2012 | 0.0118 | −0.0053 | 0.9999 |
| S.D. | 0.16 | 0.07 | 1.32 | 3.13 | 4.34 | 16.9 | 17.9 | 0.0002 | 0.0035 | 0.0001 |
| %CV | 2.96 | 0.69 | 2.70 | 3.24 | 0.88 | 1.68 | 0.89 | 1.83 | NA | 0.01 |
| %Deviation (absolute value) | 4.96 | 2.00 | −2.28 | −3.60 | −1.88 | 0.28 | 0.60 | | | |
| n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

| | Mean inter-batch back-calculated standard results | | | | | | | Standard curve reasults | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Standard | | | | | | | Slope | Y-intercept | γ |
| | STD1 (0.25 µg/mL) | STD2 (0.5 µg/mL) | STD3 (2.5 µg/mL) | STD4 (5 µg/mL) | STD5 (12.5 µg/mL) | STD6 (25 µg/mL) | STD7 (50 µg/mL) | | | |
| Urine | | | | | | | | | | |
| Run ID I | 0.282 | 0.480 | 2.42 | 4.90 | 12.4 | 23.7 | 51.5 | 0.460 | −0.00957 | 0.9992 |
| Run ID II | 0.253 | 0.515 | 2.41 | 4.94 | 12.5 | 25.0 | 50.0 | 0.440 | 0.00845 | 1.0000 |
| Run ID III | 0.251 | 0.473 | 2.57 | 4.98 | 13.1 | 24.6 | 49.8 | 0.448 | 0.00334 | 0.9998 |
| Run ID IV | 0.245 | 0.505 | 2.36 | 5.25 | 13.1 | 24.9 | 49.5 | 0.436 | 0.02340 | 0.9997 |
| Run ID V | 0.261 | 0.482 | 2.54 | 4.87 | 12.5 | 25.1 | 50.0 | 0.464 | 0.00448 | 1.0000 |
| Mean | 0.258 | 0.491 | 2.46 | 4.99 | 12.7 | 24.6 | 50.2 | 0.450 | 0.0060 | 0.9997 |
| S.D. | 0.014 | 0.018 | 0.092 | 0.153 | 0.345 | 0.580 | 0.787 | 0.012 | 0.0118 | 0.0003 |
| %CV | 5.53 | 3.69 | 3.75 | 3.07 | 2.71 | 2.36 | 1.57 | 2.71 | NA | 0.03 |
| %Deviation (absolute value) | 3.24 | −1.82 | −1.68 | −0.28 | 1.60 | −1.44 | 0.30 | | | |
| n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

Note: Calibration curves were weighed 1/conc. NA, Not applicable; conc, concentration; STD, standard.

in urine varied from 0.64 to 5.20 and from 0.90 to 4.06, respectively. The %CV of the five slopes of eprosartan in plasma was 1.83 and that in urine was 2.71. The lowest coefficient of determination ($\gamma$) among the five calibration curves of eprosartan in plasma was 0.9997 (mean = 0.9998) and that in urine was 0.9992 (mean = 0.9997) (Table 1). Thus, the calibration curves did not exhibit any non-linearity within the chosen range. Based on the standard data presented here, it was concluded that the calibration curves used in this method were precise and accurate for the measurement of eprosartan in human plasma and urine.

### 3.1.2. Accuracy and precision

Intra- and inter-day accuracy and precision for assays were characterized by the four levels of QCs run in six replicates. Four levels of QCs were: high QC (1600 ng/mL eprosartan in plasma and 40 µg/mL eprosartan in urine), medium QC (1000 ng/mL eprosartan in plasma and 20 µg/mL eprosartan in urine), low QC (5, 20 ng/mL eprosartan in plasma and 0.25, 1 µg/mL eprosartan in urine) and LLOQ QC (5 ng/mL eprosartan in plasma

and 0.25 µg/mL eprosartan in urine). Accuracy was assessed by calculating the percent deviation from the theoretical concentration. Precision was determined by calculating the coefficient of variation for intra- and inter-day replicates.

Table 2 shows a summary of the individual QC data obtained in the four levels for the validation. As can be seen, the assay for eprosartan in plasma and urine were both accurate and precise on intra- and inter-day for each level. The greatest mean inter-day percent deviations of eprosartan were 9.20% in plasma and 9.60% in urine for the LLOQ, respectively. All QC levels for eprosartan had intra- and inter-day percent deviations less than 10% and the LLOQ accuracy and precision results were within ±10%.

Table 3 shows a summary of the recovery data obtained in the four levels for the validation. The extractive recovery the four levels in plasma were (48.90 ± 7.172), (50.92 ± 8.016), (56.05 ± 2.81) and (58.69 ± 12.13)% and relative recovery were (104.80 ± 10.87), (96.30 ± 3.48), (99.35 ± 1.519) and (101.10 ± 3.59)%, respectively. The extractive recovery the

Table 2
Intra- and inter-day accuracy and precision of eprosartan in human plasma and urine

| | Eprosartan in human plasma | | | | Eprosartan in human urine | | | |
|---|---|---|---|---|---|---|---|---|
| | Low (5 ng/mL) | Low (20 ng/mL) | Middle (1000 ng/mL) | High (1600 ng/mL) | Low (0.25 µg/mL) | Low (1 µg/mL) | Middle (20 µg/mL) | High (40 µg/mL) |
| Intra-day accuracy and precision | | | | | | | | |
| Mean | 5.07 | 19.37 | 993.50 | 1615.00 | 0.26 | 1.03 | 20.11 | 40.73 |
| S.D. | 0.31 | 0.70 | 15.19 | 53.20 | 0.013 | 0.035 | 0.80 | 1.50 |
| %CV | 6.11 | 3.63 | 1.53 | 3.29 | 4.76 | 3.35 | 3.97 | 3.67 |
| %Deviation | 1.47 | −3.17 | −0.65 | 0.94 | 5.33 | 2.75 | 0.58 | 1.81 |
| $n$ | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Inter-day accuracy and precision | | | | | | | | |
| Mean | 5.29 | 19.60 | 1018.20 | 1634.00 | 0.26 | 0.97 | 19.76 | 39.72 |
| S.D. | 0.34 | 1.00 | 24.52 | 53.67 | 0.018 | 0.062 | 0.48 | 1.61 |
| %CV | 6.44 | 5.09 | 2.41 | 3.28 | 6.60 | 6.40 | 2.44 | 4.04 |
| %Deviation | 5.72 | −2.00 | 1.82 | 2.13 | 4.76 | −2.90 | −1.20 | −0.70 |
| $n$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

four levels in urine were (99.05 ± 4.703), (102.20 ± 5.801), (98.32 ± 2.011) and (100.50 ± 1.378)% and relative recovery were (107.70 ± 5.771), (102.70 ± 3.316), (99.58 ± 5.863) and (101.40 ± 4.674)%, respectively. The sensitivity is good enough for the detection of the drug concentration.

### 3.1.3. Specificity and matrix effects

Six different lots of control human plasma or urine samples were analyzed with and without standards to determine whether any endogenous plasma or urine constituents interfered with the analyte or the IS. The degree of interference was assessed by inspection of SRM chromatograms. No significant interfering peaks from the plasma or urine were found at the retention time or in the ion channel of analyte or the IS (Figs. 2 and 3).

Fifty microliters of blank plasma was added into a 2.0 mL labeled microcentrifuge tube, and then 50 µL acetonitrile was added. The tube was capped and vortexed for 1 min. The sample was centrifuged in the Heraeus Biofuge 28RS at 15,000 rpm and 20 °C for 2 min. Fifty microliters of the supernatant layer was extracted into a 2.0 mL microcentrifuge tube, and 2.5 µL suitable concentration of the working standard solution for

eprosartan or IS were added. The concentration was equal to the LLOQ for eprosartan and IS, respectively. Six replicates for eprosartan and IS were prepared, respectively. We replaced blank plasma with distilled water and prepared standard solution in the same way. The matrix effect was assessed by comparing the peak area of the former with the peak area of the corresponding standard solution. The means of the ratios for eprosartan and IS were 0.78 and 0.73, respectively. Combined with the fact that there was no significant lot-to-lot variation in LLOQ, it was concluded that the low matrix effect in human plasma did not compromise the performance of the method.

Twenty microliters of 0.25 µg/mL eprosartan in urine was added into a 2.0 mL labeled microcentrifuge tube and 1 mL of the DI water was added. The tubes were capped and vortexed for 30 s. Twenty microliters of the above-mentioned solution was added into 2.0 mL microcentrifuge tubes and 1 mL of the working internal standard solution (50 ng/mL) was added. The tubes were capped and vortexed for 30 s. The samples were then centrifuged in Heraeus Biofuge 28RS at 15,000 rpm and 20 °C for 2 min. The concentration was equal to the LLOQ for eprosar-

Table 3
Recovery of eprosartan in human plasma and urine

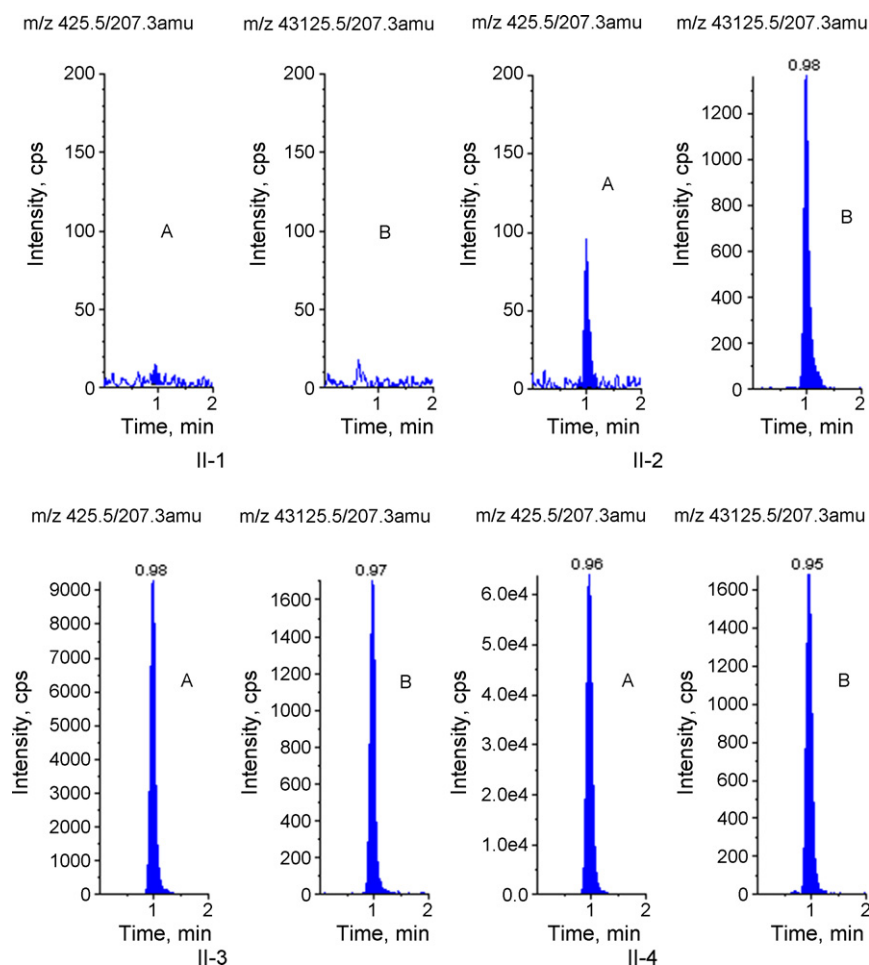| | Eprosartan in human plasma | | | | Eprosartan in human urine | | | |
|---|---|---|---|---|---|---|---|---|
| | Low (5 ng/mL) | Low (20 ng/mL) | Middle (1000 ng/mL) | High (1600 ng/mL) | Low (0.25 µg/mL) | Low (1 µg/mL) | Middle (20 µg/mL) | High (40 µg/mL) |
| Relative recovery | | | | | | | | |
| Mean | 104.80 | 96.30 | 99.35 | 101.10 | 107.70 | 102.70 | 99.58 | 101.40 |
| S.D. | 10.87 | 3.48 | 1.519 | 3.59 | 5.771 | 3.316 | 5.863 | 4.674 |
| %CV | 10.37 | 6.61 | 1.529 | 3.55 | 5.357 | 3.228 | 5.888 | 4.608 |
| %Deviation | 4.80 | −3.70 | −0.65 | 1.10 | 7.70 | 2.70 | −0.42 | 1.40 |
| $n$ | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Extractive recovery | | | | | | | | |
| Mean | 48.98 | 50.92 | 56.05 | 58.69 | 99.05 | 102.20 | 98.32 | 100.50 |
| S.D. | 7.172 | 8.016 | 2.81 | 12.13 | 4.703 | 5.801 | 2.011 | 1.378 |
| %CV | 14.64 | 15.74 | 5.013 | 20.67 | 4.748 | 5.677 | 2.046 | 1.372 |
| %Deviation | −51.02 | −49.08 | −43.95 | −41.31 | −0.95 | 2.20 | −1.68 | 0.50 |
| $n$ | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

Fig. 2. Chrornatography of eprosartan in plasma. (A) Eprosartan; (B) D6-eprosartan (Internal Standard). (II-1) Blank plasma; (II-2) 5 ng/mL eprosartan added into blank plasma (LLOQ); (II-3) 500 ng/mL eprosartan added into blank plasma; (II-4) a human plasma collected 2.5 h after a single dose of 600 mg eprosartan.

tan and IS, respectively. Six replicates for eprosartan and IS were prepared, respectively. We replaced blank urine with distilled water and prepared standard solution in the same way. The matrix effect was assessed by comparing the peak area of the former with the peak area of the corresponding standard solution. The means of the ratios for eprosartan and IS were 0.99 and 0.98, respectively. Combined with the fact that there was no significant lot-to-lot variation in LLOQ, it was concluded that the matrix effect in human urine did not compromise the performance of the method.

### 3.1.4. Stability

The stability of eprosartan in human plasma and urine was investigated using the LLOQ (5 ng/mL in plasma and 0.25 μg/mL in urine), low (20 ng/mL in plasma and 1 μg/mL in urine), medium (1000 ng/mL in plasma and 20 μg/mL in urine) and high (1600 ng/mL in plasma and 40 μg/mL in urine) QCs. Test conditions included three freeze–thaw cycles and room temperature (4 h). Stability was also checked by extracting the appropriate QC which had been maintained at specific temperature (4 °C) for the specified time and analyzing the extracts for eprosartan. Extracted sample stability in the autosampler (4 °C) was tested by comparing the initial results from the QC

extract with that determined after 24 h of autosampler storage. Deterioration of eprosartan was defined as greater than a 15% difference of tested sample versus control at the sample nominal concentration.

There was no deterioration for eprosartan in plasma and urine at any QC level for the three freeze–thaw cycles, suggesting that drug concentrations can be confidently determined in samples that had been thawed up to three times prior to analysis or that have been thawed and kept at ambient temperature for up to 4 h. The 4 h stability test at ambient temperature was performed since the plasma sample could stably stand on the bench for up to 4 h after thawing or before freezing.

The stability of eprosartan in the extract was also tested after 24 h storage at 4 °C to allow for sample waiting or re-injection. Eprosartan in plasma and urine was considered stable to storage at 4 °C for 24 h if the relative error (RE) was less than 15% at all QC concentrations after the treatment. The results showed that eprosartan in plasma and urine were stable following storage at 4 °C for 24 h.

### 3.1.5. Diluting study

Ten thousand nanograms per milliliter plasma samples were prepared and then diluted to 10 times with blank plasma.
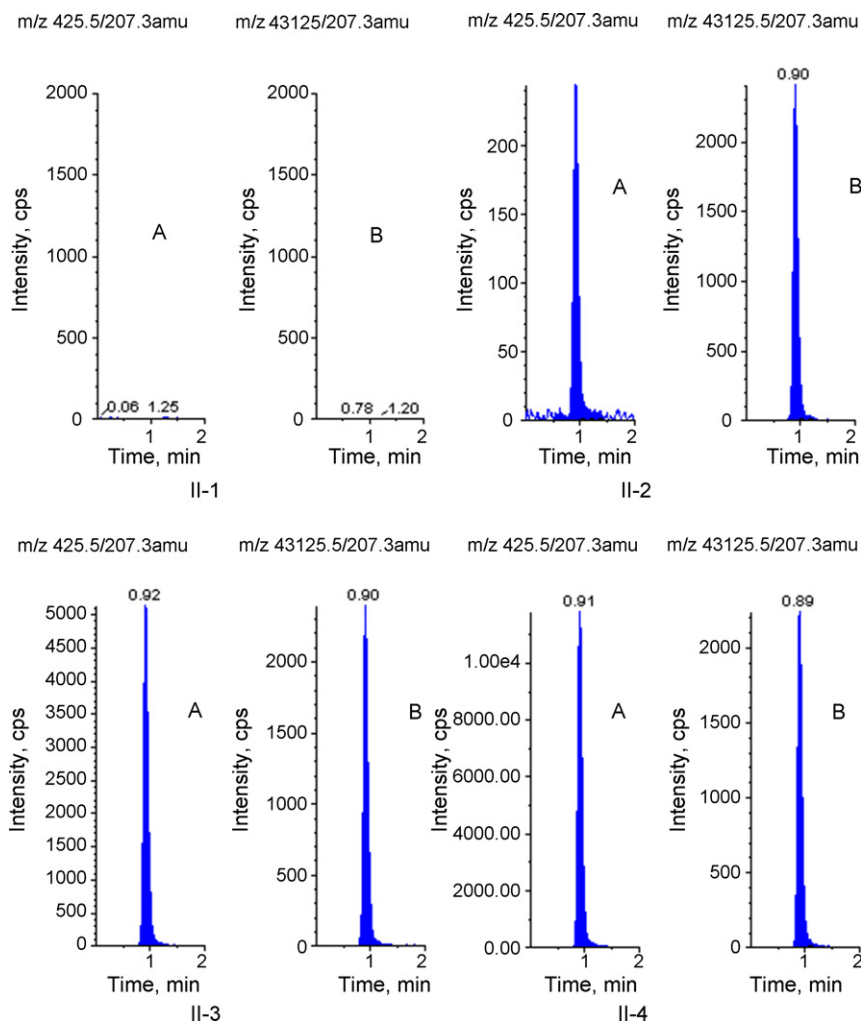
Fig. 3. Chrornatography of eprosartan in urine. (A) Eprosartan; (B) D6-eprosartan (Internal Standard). (II-1) Blank urine; (II-2) 0.25 μg/mL eprosartan added into blank urine (LLOQ); (II-3) 2.5 μg/mL eprosartan added into blank urine; (II-4) a human urine collected 4–8 h after a single dose of 600 mg eprosartan.

The plasma samples were extracted following Section 2.3.1. The percent deviations of accuracy and precision were within ±10%.

One hundred micrograms per milliliter urine samples were prepared and then diluted to 10 times with blank urine. The urine samples were extracted following Section 2.3.2. The percent deviations of accuracy and precision were within ±10%.

If the concentration of plasma or urine samples exceeded the range of standard calibration curve, we would dilute the samples following the diluting study.

### 3.2. Clinical application

Plasma and urine samples from 20 healthy volunteers receiving a 600 mg eprosartan tablet were analyzed as described above. The plasma sample time was 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 16, 24 and 32 h after administration. Five milliliters blood samples were collected by venous puncture or indwelling venous catheter into lithium-heparin containing vacutainers. Within 10 min of collection, the blood samples were centrifuged at $1500 \times g$ and 4 °C for 10 min. Blood samples were handled on ice between sample collection and centrifugation. The plasma were separated and transferred into screw cap polypropylene tubes. Plasma samples were stored at the study center at −20 °C until shipment to the laboratory where the analyses of eprosartan plasma concentrations were performed.

The urine sample time was 0–4, 4–8, 8–12, 12–24 and 24–32 h. In every collection interval, urine samples was stored in refrigerator at 4 °C. The volume and pH of each sample were measured after homogenization and documented. Two aliquots of 10 mL each were saved and stored at the study center at −20 °C until analysed.

The maximum plasma concentrations of eprosartan ranged from 1755.00 to 7550.00 ng/mL ($n = 20$) and the mean of the maximum concentration was $3664.25 \pm 1653.94$ ng/mL. As can be seen from the mean eprosartan pharmacokinetic curve (Fig. 4), the time to maximum plasma concentration ($T_{max}$) was $1.63 \pm 0.46$ h. The areas under the concentration–time curve ($AUC_{0-t}$) was $14818.75 \pm 7312.11$ ng h/mL and $AUC_{0-\infty}$ was $15092.07 \pm 7374.53$ ng h/mL. The mean terminal elimination half-life for eprosartan was $8.34 \pm 3.90$ h. The amount of unchanged eprosartan excreted into urine (Ae) was
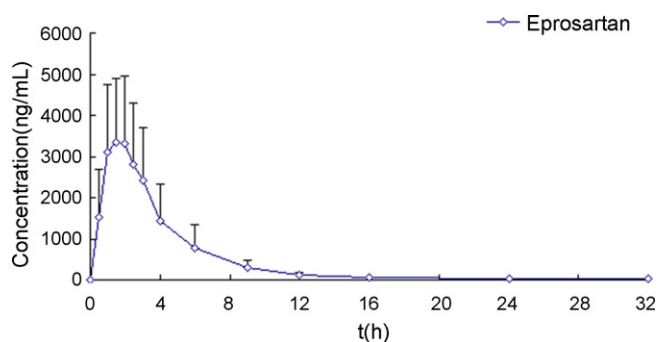
Fig. 4. Mean plasma concentration–time curve of eprosartan for 20 volunteers after a single oral administration of 600 mg eprosartan.

$18.44 \pm 6.43$ mg, and the fraction of unchanged eprosartan excreted into urine was $3.07 \pm 1.07\%$.

## 4. Conclusion

Inhibition of the RAAS using orally active A-II receptor antagonists offers a novel approach to the treatment of hypertension and congestive heart failure and may give therapeutic advantages over ACE inhibitors based on their specificity of action. Eprosartan is A-II which has the primary effector hormone of the RAAS and exhibits a wide range of activities.

Due to the similar polarity of eprosartan and plasma endogenous compounds, the use of an isocratic elution mode did not allow the optimum resolution of the studied drug and a gradient elution mode was used [2], which take 14 min. Moreover, to improve chromatographic peaks shape and avoid tails, a little percentage of TFA was added to the mobile phase [2], which would reduce the efficiency of column. So it needs to establish a highly sensitive and specific method to determine the drug concentration. Ferreiros et al. [2,3] and Lundberg et al. [4] use SPE to separate eprosartan from plasmatic interferences, which was time-consuming and complicated. Because of the sensitivity and specificity of LC/MS/MS, by the means of protein precipitation, the LLOQ of eprosartan in plasma was 5 ng/mL, which was enough for the determination of eprosartan in plasma and urine. D6-eprosartan which is the most similar to eprosartan in the structure was used as the internal standard. So the analytic results were more accurate and stable. This method has been applied to measure thousands of plasma samples related the pharmacokinetics trials of eprosartan in healthy volunteers.

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