A rapid, simple, and specific liquid chromatography–electrospray ionization mass spectrometry (LC–ESI-MS) method has been developed and validated for the determination of lisinopril in human plasma. Enalaprilat was used as the internal standard (IS). Sample preparation of the serum involved deproteinization with methanol twice, repeatedly. Samples were separated using a Thermo Hypersil-HyPURITY C18 reversed-phase column (150 × 2.1 mm i.d., 5 µm). Mobile phase consisted of formic acid solution (pH 2.9)—methanol—acetonitrile (58:25:17, v/v). Lisinopril and its internal standard were measured by electrospray ion source in positive selected ion monitoring mode. The method was validated with a linear range of 2.5–320 ng/mL and the lowest limits of quantitation were 2.5 ng/mL for lisinopril. The extraction efficiencies were approximately 80% and recoveries of method were in range of 94.4–98.2%. The intra-day relative standard deviation (RSD) was less than 8.8% and inter-day RSD was within 10.3%. QC samples were stable when kept at ambient temperature for 24 h, at –20°C for 30 days and after four freeze/thaw cycles. The method has been successfully applied to the evaluation of pharmacokinetics and bioequivalence of 2 lisinopril formulations in 18 healthy Chinese volunteers after an oral dose of 20 mg.

Introduction

Lisinopril, (S)-1-[N²-(1-carboxy-3-phenyl)-L-lysyl]-L-proline dehydrate (see Figure 1 for its structure), is a long-acting, non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor that is used for the treatment of hypertension and congestive heart failure in daily dosages of 10–80 mg (1–3). It reduces both angiotensin and aldosterone plasma concentrations through the inhibition of angiotensin converting enzyme.

A few analysis methods have been developed for the measurement of lisinopril in a biological matrix such as gas chromatography–mass spectrometry (GC–MS) (4,5), radioimmunoassay (RIA) (6,7), time-resolved fluoroimmunoassay (TR-FIA) (8), spectroscopy method (SPM) (9–14), high-performance liquid chromatography (HPLC) with UV/FL detection (11,14–17), electrochemistry method (ELE) (12), capillary electrophoresis method (CE) (18,19), and so on. However, limit of quantitation (LOQ) was approximately 0.02–14, 20–26, 0.4, and 0.005–8 µg/mL for SPM, CE, ELE, and HPLC, respectively. In these methods, only the LOQ of HPLC (16) could reach 0.005 µg/mL when lisinopril was derivatized, but the elution time was more than 15 min. So, these methods were not suitable for pharmacokinetic studies when several hundreds of samples require determination and the concentration of serum samples 1, 36, 48 h after administrated were less than 0.005 µg/mL in this study. LOQ of TR-FIA could reach 10 ng/mL. It had been used to study bioequivalence at the cost of large consumption of serum and the method needed a two-step reaction with difluorodinitrobenzene. But it seems not adequate to our investigation. The sensitivity of the GC–MS method was adequate for the pharmacokinetic studies of lisinopril, but the derivatization step was a complicated and time-consuming procedure (4,5). RIA was the most sensitive, with limit of detection reaching 0.2–0.4 ng/mL, but the method requires radiolabels and antilisinopril antiserum, which renders this method not readily available to all users.
investigators. In recent year, LC–MS–MS or LC–MS methods for the determination of lisinopril have been reported (20–22). Kousoulou et al. (20) extracted lisinopril from human plasma by semi-automated solid-phase extraction (SPE) using a 96-well format extraction plate after deproteinizing with acetonitrile. In this method, 0.5 mL plasma was applied and the sensitivity is 2 ng/mL. Tsakalof et al. (21) treated 1.0 mL plasma with 2 mL of 0.1M HCl and then isolated the analyte from serum by means of SPE. They achieved a linearity ranging from 6 to 150 ng/mL while the chromatographic running time was more than 5 min. Padua et al. (22) developed an LC–MS–MS method for lisinopril in which 0.5 mL plasma was used and also followed by SPE. This method was sensitive (LOQ was 2 ng/mL) and the chromatographic running time was approximately 6.5 min. Some of these LC–MS–MS methods were suitable for pharmacokinetics and bioequivalence studies. But these procedures had one or some of the following drawbacks, such as the fact that it is complicated, time-consuming, expensive, and/or great deals of plasma samples were needed.

In the present study, we developed a rapid, simple, and selective high-performance liquid chromatography–electrospray mass spectrometry (HPLC–ESI-MS) method for the determination of lisinopril in human plasma. Plasma samples were directly deproteinized with methanol. This made the pretreatment procedure simple and rapid. The method was successfully applied to the study of pharmacokinetics of lisinopril in 18 healthy Chinese volunteers after an oral dose of 20 mg.

**Experimental**

**Chemicals and reagents**

Lisinopril (purity > 99.5%) and its tablets were purchased from Wei Min Pharmaceutical Co., Ltd., (China). Enalaprilat was obtained from Huang He Pharmacy Company (Jiangsu, China). Methanol was of HPLC-grade (Tianjin Kemiou reagent factory, Tianjin, China). Acetonitrile was purchased from Caledon Company (HPLC-grade, Canada). Other reagents were all of analytical grade. Ultra-pure water prepared by a Millipore Milli-Q purification system (Millipore Corp. Bedford, MA) was used to make mobile phase and all other solutions. Drug-free and drug-containing plasma were taken from the Chinese volunteers. The plasma was stored at −20°C until further use for analysis.

**Instrumentation**

The HPLC system included a Shimadzu LC-10Advp pump, an SCL-10Advp system controller, a CTO-10Avp column oven, an FCV-10Avp low pressure gradient unit, and a DGU-14A degasser (Shimadzu, Kyoto, Japan). The mass spectrometer was an LCMS-2010 single quadrupole equipped with electrospray ionization interface (Shimadzu). The data processing was carried out using LCMSSolution software.

**Preparation of stock solutions, calibration standard solutions, and quality control samples**

A stock solution of lisinopril was prepared by dissolving the reference compound in methanol to give a final concentration of 100.0 µg/mL. A stock solution of IS was obtained by dissolving enalaprilat in methanol give a final concentration of 100.0 µg/mL. The stock solutions were stored at 4°C before use.

Stock solution of lisinopril was further diluted with blank plasma to give serial concentrations of 2.5, 5, 10, 20, 40, 80, 160, and 320 ng/mL to obtain calibration standard solutions. Stock solution of IS was diluted with methanol to 500 ng/mL to make working solution. Three concentrations levels (low, medium, and high) quality control samples were prepared in blank plasma at concentrations of 5, 40, and 160 ng/mL for lisinopril. The further procedure of both calibration standard solutions and QC samples are as described later.

**Sample preparation**

0.25 mL of plasma was pipetted into a centrifugation tube and mixed with 20 µL of IS working solution, 20 µL of 1 mol/L hydrochloric acid solution, and 600 µL of methanol. The mixture was mixed thoroughly by vortex-mixer and permitted to stand for 3 min before being centrifuged at 14000 r/min for 3 min. The supernatant was transferred and evaporated to dryness at 50°C under vacuum. The dry residue was dissolved in 200 µL of methanol. Then the supernatant was transferred after centrifuging and evaporated to dryness. The dry residue was reconstituted with 50 µL of mobile phase and centrifuged at 14000 r/min for 3 min. An aliquot of 10 µL of the supernatant was injected onto the LC–MS system for analysis.

**Mass spectrometric conditions**

An LCMS-2010 quadrupole mass spectrometer was interfaced with an ESI probe. The temperatures were maintained at 250, 250, and 200°C for the probe, CDL, and block, respectively. The voltages were set at 4.5 kV, –50 V, 25 V, 150 V, and 1.7 kV for the probe, CDL, Q-array 1, 2, 3 bias, Q-array radio frequency (RF), and detector, respectively. The flow rate of nebulizer gas was 1.5 L/min; drying N2 flow was 10 L/min; drying gas temperature was 250°C. For the quantitation of lisinopril, the analysis was performed in selection ion mode monitoring at m/z 406 (lisinopril, M+H) and 349 (Enalaprilat, IS, M+H). Tuning of mass spectrometer was accomplished with the help of autotuning function of LCMSSolution software (Version 2.02) using tuning standard solution (polypropylene glycol). Optimization and calibration of mass spectrometer were obtained with autotuning.

**Chromatographic conditions**

Chromatographic separation was accomplished using a Thermo Hypersil-HypURITY C18 (150 × 2.1 mm, 5 µm) analytical column. The column temperature was set at 40°C. The mobile phase consisted of formic acid solution (pH 2.9)–methanol–acetonitrile (58:25:17, v/v). The compounds were isocratically eluted at a flow rate of 0.20 mL/min.

**Method validation**

**Linearity**

Plasma samples were quantitated using a calibration curve. The calibration curves were constructed by plotting the ratios of the peak area of lisinopril to that of IS versus concentrations of lisinopril. Calibration standard solutions were freshly prepared and assayed in triplicate on five separate days over the range of
2.5–320 ng/mL. The unknown sample concentrations were calculated from the regression equation of the calibration curves. The LOQ for lisinopril was established on the basis of a signal-to-noise ratio (S/N) of 10 with accuracy and precision better than 20% (23).

Assay specificity and matrix effect

The matrix effects (ME) mean the possibility of suppression or enhancement of ionization. For matrix effects, four concentration level solutions of standard (2.5, 40, 160, 320 ng/mL) and internal standard (40 ng/mL) were dried and reconstituted in 50 µL mobile phase (namely, neat standard, n = 5, group A). Twenty-five blank plasmas (0.25 mL) of five different sources (five per source) were placed into 1.5 mL tubes. The plasmas were processed as described earlier, and the residues were reconstituted in 50 µL of mobile phase containing 2.5, 40, 160, and 320 ng/mL of standard and 40 ng/mL of internal standard, respectively (group B). Another 25 plasma samples from five different sources spiked with standard (2.5, 40, 160, and 320 ng/mL) and internal standard (40 ng/mL) before extraction were deproteinized. The residues were dissolved in 50 µL of mobile phase (group C). The analysis process for matrix effects (ME) and extraction efficiency (EE) of three groups of samples (A, B, and C) as follows:

\[
\text{ME} (\%) = \frac{A_3}{A_2} \cdot 100; \\
\text{EE} (\%) = \frac{A_3}{A_2} \cdot 100.
\]

Where A1 = mean peak area of each concentration in group A; A2 = mean peak area of each concentration in group B; A3 = mean peak area of each concentration in group C.

For assessment of selectivity, six batches of blank plasma from different sources were prepared as described in the “Sample preparation” section and analyzed.

Precision, accuracy, and recovery

The accuracies of the experiment were achieved by comparing the measured concentrations to the added concentrations of the analyte spiked in the blank plasma. The precision and accuracy of the method were estimated by replicating analysis (n = 5) of QC samples at three concentrations levels. Intra-day precision was evaluated by analyzing QC samples five times over 1 day, while inter-day precision was estimated by analyzing QC samples five times in three different days. The precision was defined as the intra-day and inter-day relative standard deviation (RSD) The accuracy was expressed as mean relative error [MRE% = (mean of the measured concentration – added concentration)/added concentration × 100%]. The recoveries of the method were estimated by analyzing human plasma after administration of lisinopril spiked with standard.

Stability

It is necessary for a reliable method to exploit the stability of analytes during analysis time and also upon storage for a limited time. The stability of lisinopril was assessed by placing QC samples at three concentrations levels at room temperature for 24 h. The freeze/thaw stabilities of lisinopril were also evaluated by analyzing QC samples undergoing four freeze (−20°C)/thaw (room temperature) cycles. The freeze stability of analyte was estimated by placing QC samples at −20°C for one month. The samples were brought under room temperature to thaw and measured after 30 days.

Bioequivalence study design

Subjects

The clinic study protocol was approved by the Ethical Committee of College of Pharmacy of Central South University (Chang Sha, China). Eighteen healthy Chinese volunteers (9 males and 9 females), aged 24 to 37 years, were selected for this study after clinical assessment of their health status. No subject had a history or evidence of a renal, gastrointestinal, hepatic, or hematologic abnormality or any acute or chronic disease, or allergies to any drugs. All the subjects were non-smokers and non-smokers. Subjects who had used drugs of any kind within 2 weeks before the study were excluded. No tobacco, alcohol, or drink with caffeine was allowed. Informed consent was obtained from all subjects and the nature and purpose of the study had been clearly explained.

Drug administration and blood sampling

A 2 × 2, crossover, randomized, open-label design was used. Subjects were randomly assigned to receive reference formulation followed by test formulation with a 2-week washout period between doses. After a 12-h (overnight) fast, subjects received a single, 20-mg oral dose (tablet) of lisinopril with 200 mL of water. The heparinized blood samples (4 mL) were collected immediately from a suitable forear forn vein using an indwelling catheter into heparin containing tubes before taking the drug and then at 1, 2, 4, 5, 6, 7, 8, 10, 12, 14, 24, 36, and 48 h after the administration, respectively. The blood samples were centrifuged at 4000 rpm for 10 min, and plasma samples were separated and stored at −20°C until analysis. The pharmacokinetic data were processed, and the bioequivalence of drugs was estimated using conventional methods.

Results and Discussion

Selection of LC and MS conditions

To select an appropriate ionization mode in LC–MS analysis, the mass spectra were measured in ESI and APCI positive and negative mode using injection of lisinopril and the internal standard solutions. In both ionization modes, the base peak intensities of positive ion were higher than those of negative ion, and the efficiencies of ionization in ESI were higher than APCI. So, selected ion monitoring (SIM) mode ([M+H]+ at m/z 406, 349) was used for quantitative analysis of lisinopril and the IS. Figure 1 shows the positive ion mass spectra of lisinopril (A) and the internal standard (B) by ESI scanning from m/z about 100 to 600.

The separation and ionization of lisinopril and the IS were affected by composition and pH of mobile phase. These factors were critical for achieving good chromatographic peak shape and resolution. The sensitivity of lisinopril was improved by
increasing acidity of mobile phase because of the response for lisinopril improved at lower pH. In the present study, a solution consisting of formic acid solution (pH 2.9)–methanol–acetonitrile (58:25:17, v/v/v) was chosen as an isocratic mobile phase. Under such condition, the total analytical time is less than 3.5 min. The selection of Enalaprilat as the IS was based on its chromatographic and extraction behaviors.

Method validation
Matrix effect, assay specificity and selectivity
It is important to investigate the matrix effects in order to develop a reliable and reproducible LC–MS method. The data for matrix effects and extraction efficiencies were presented in Table I. The RSD of matrix effects of analytes at four concentrations and IS in five different plasma groups were less than 8%, which strongly indicated little or no difference in ionization efficiency of analyte and IS from different plasma groups. Moreover, by comparing peak areas of standard and internal standard for samples spiked after extraction from plasma with the corresponding peak areas obtained by injecting neat standard and IS directly, the extent of the absolute matrix effect was estimated (Table I). The ME (%) > 100% indicated ionization enhancement in plasma versus neat standards, while ME (%) < 100% indicated ionization suppression. As can be seen in Table I, MEs were approximately equal to 100%. So, there was no significant difference in peak areas of the analytes prepared from five different blank plasma samples and from mobile phase. The results indicated that the matrix effects for analyte and IS were negligible.

Potential interference from endogenous substances was estimated by analyzing human plasmas of six different sources. Figure 2 showed the typical chromatograms of a blank plasma sample (A), a blank plasma sample spiked with lisinopril and its IS (B–C), and a plasma sample from one of the volunteers 6 h after an oral administration (D). The retention times of lisinopril and IS were approximately 2.1, 2.5 min, respectively. For all plasma samples, the regions of the analyte and the IS were free of interference from endogenous substances. The total run time was approximately 3.5 min. The method showed high specificity because of the analyte and its IS have no co-elution phenomenon and endogenous/ectogenous substances from plasma were not affected the elution of analytes.

Table I showed extraction efficiencies of lisinopril and enalaprilat. The extraction efficiencies observed (n = 5) were 80.0%, 79.0%, 80.5%, and 81.3% for 5, 40, 160, and 320 ng/mL, respectively and 70.8% for IS (40 ng/mL). The results were satisfactory in the analytical procedure.

Linearity and sensitivity
Calibration standards at 8 lisinopril concentrations were extracted and assayed. Non-weighted regression was used to determine the plasma concentration from peak ratios (lisinopril vs IS). Good linearity was achieved in the range of 2.5–320 ng/mL. The typical equation was $y = 0.0172x + 0.0247$ ($R^2 = 0.9978$), where y was the ratio of peak area of analyte to that of internal standard, x was the concentration of analyte (ng/mL). The limit of detection (LOD), defined at a S/N > 3, was 0.7 ng/mL. The LOQ was 2.5 ng/mL using 0.25 mL plasma with accuracy < 6% and precision < 11%.

Precision, accuracy, and recovery of the method
Both the intra- and inter-day precision and accuracy of the method were determined by analysis of replicates (n = 5) of QC samples containing known concentrations of 5, 40, and 160 ng/mL of lisinopril. The precision of the method was described as relative standard deviation (RSD) among each assay. The accuracy of the method was evaluated by analysis of human plasma after administration of analyte spiked with standard solutions. The accuracy was described as a percentage error of measured concentrations versus nominal concentrations, recovery, and the RSD, respectively. Precision and accuracy were calculated at each concentration. The mean recoveries of the method for

<table>
<thead>
<tr>
<th>Nominal conc. (ng/mL)</th>
<th>Mean peak area (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A 1</td>
</tr>
<tr>
<td>2.5</td>
<td>5148 (7.9)</td>
</tr>
<tr>
<td>40</td>
<td>82141 (5.6)</td>
</tr>
<tr>
<td>160</td>
<td>320475 (6.1)</td>
</tr>
<tr>
<td>320</td>
<td>637837 (6.8)</td>
</tr>
<tr>
<td>IS</td>
<td>52440 (5.3)</td>
</tr>
</tbody>
</table>

* Number in parentheses were RSD.

Figure 2. Selective ion chromatograms of lisinopril (peak 1) and enalaprilat (peak 2). Blank plasma (A); blank plasma spiked with lisinopril (160 ng/mL) and IS (B); blank plasma spiked with lisinopril (2.5 ng/mL) and IS (C); human plasma sample 6 h after administration of lisinopril and spiked with IS (D).
lisinopril in real plasma samples ranged from 94.4% to 98.2%. The results were shown in Tables II and III.

As can be seen from Table II, the method showed very good precision and accuracy. In this assay, the intra-day precisions (RSD) were within 8.8% and inter-day precisions were less than 10.3%, respectively. The intra-day accuracies (MRE) were within 2.0% and inter-day accuracies were less than 4.0%. This is satisfactory for the pharmacokinetic study.

**Stability study**

Lisinopril was stable under conditions of storage and in the course of processing. Table II shows the results of stability. The QC plasma samples were stable for 24 h at room temperature (Table II). In the long-term stability study, the plasma samples spiked with the QC plasma samples also showed no loss of the analyte when they were stored for 30 days at −20°C (Table II). The stock solutions were stable for at least 1 month. The final stability test was demonstrated after 4 freeze-thaw cycles. No significant deterioration of the analyte was observed under any of these conditions (Mean relative errors were 1.8–6.0%, n = 5).

**Bioequivalence study**

The method was successfully applied to the bioequivalence study of 2 lisinopril formulations in 18 healthy Chinese volunteers after an oral dose of 20 mg. Mean plasma concentration-time profiles of lisinopril was presented in Figure 3. The main pharmacokinetic parameters were calculated and showed in Table IV. The bioequivalence of drugs was determined with respect to AUC\(_{0–48}\), AUC\(_{0–\infty}\), \(C_{max}\), \(T_{max}\), \(T_{1/2}\), and \(C_{max}/AUC_{0–48}\) where AUC\(_{0–48}\) is the area under curve at 48 h, AUC\(_{0–\infty}\) is the area under curve at infinite time, \(C_{max}\) is the maximum (measured) drug concentration in the blood plasma, \(T_{max}\) is the time to attaining \(C_{max}\), \(T_{1/2}\) is half-elimination time, and \(C_{max}/AUC_{0–48}\) is relative absorption rate. As can be seen from Table IV, the pharmacokinetic parameters of test drug (lisinopril) were very close to those of reference drug. In this study in 18 healthy Chinese volunteers, a single, 20 mg dose of test drug was found to be bioequivalent to reference drug based on the rate and extent of absorption.

**Conclusion**

The LC–ESI-MS method for the determination of lisinopril in human plasma has been developed. Method validation has been successfully applied to the bioequivalence study of 2 lisinopril formulations in 18 healthy Chinese volunteers after an oral dose of 20 mg. Mean plasma concentration-time profiles of lisinopril was presented in Figure 3. The main pharmacokinetic parameters were calculated and showed in Table IV. The bioequivalence of drugs was determined with respect to AUC\(_{0–48}\), AUC\(_{0–\infty}\), \(C_{max}\), \(T_{max}\), \(T_{1/2}\), and \(C_{max}/AUC_{0–48}\) where AUC\(_{0–48}\) is the area under curve at 48 h, AUC\(_{0–\infty}\) is the area under curve at infinite time, \(C_{max}\) is the maximum (measured) drug concentration in the blood plasma, \(T_{max}\) is the time to attaining \(C_{max}\), \(T_{1/2}\) is half-elimination time, and \(C_{max}/AUC_{0–48}\) is relative absorption rate. As can be seen from Table IV, the pharmacokinetic parameters of test drug (lisinopril) were very close to those of reference drug. In this study in 18 healthy Chinese volunteers, a single, 20 mg dose of test drug was found to be bioequivalent to reference drug based on the rate and extent of absorption.

**Table II. Reproducibility and Accuracy for Lisinopril of Quality Control Sample in Human Plasma (n = 5)**

<table>
<thead>
<tr>
<th>Nominal conc. (ng/mL)</th>
<th>Mean found conc. (ng/mL)</th>
<th>Precision RSD (%)</th>
<th>Mean relative error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.9</td>
<td>8.2</td>
<td>-2.0</td>
</tr>
<tr>
<td>40</td>
<td>39.3</td>
<td>8.8</td>
<td>-1.8</td>
</tr>
<tr>
<td>160</td>
<td>158.9</td>
<td>5.7</td>
<td>-0.7</td>
</tr>
<tr>
<td><strong>Inter-day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.8</td>
<td>10.3</td>
<td>-4.0</td>
</tr>
<tr>
<td>40</td>
<td>40.4</td>
<td>7.8</td>
<td>1.0</td>
</tr>
<tr>
<td>160</td>
<td>158.4</td>
<td>5.5</td>
<td>-1.0</td>
</tr>
<tr>
<td><strong>Short-term stability for 24 h in plasma at room temperature (RT)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.2</td>
<td>7.5</td>
<td>4.0</td>
</tr>
<tr>
<td>40</td>
<td>41.3</td>
<td>7.2</td>
<td>3.3</td>
</tr>
<tr>
<td>160</td>
<td>155.4</td>
<td>6.4</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Storage in plasma at −20°C for 1 month</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.3</td>
<td>9.5</td>
<td>6.0</td>
</tr>
<tr>
<td>40</td>
<td>38.6</td>
<td>5.6</td>
<td>-3.5</td>
</tr>
<tr>
<td>160</td>
<td>165.7</td>
<td>6.3</td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Four freeze/thaw cycles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.1</td>
<td>7.2</td>
<td>2.0</td>
</tr>
<tr>
<td>40</td>
<td>40.8</td>
<td>6.5</td>
<td>2.0</td>
</tr>
<tr>
<td>160</td>
<td>157.2</td>
<td>4.9</td>
<td>-1.8</td>
</tr>
</tbody>
</table>

**Table III. Recovery in Plasma Samples After Administration of Lisinopril and Spiked with Standard (n = 5)**

<table>
<thead>
<tr>
<th>Sample (ng/mL)</th>
<th>Standard added (ng/mL)</th>
<th>Found (ng/mL)</th>
<th>Recovery (%)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.8</td>
<td>5</td>
<td>28.0</td>
<td>96.6</td>
<td>7.5</td>
</tr>
<tr>
<td>23.2</td>
<td>40</td>
<td>61.9</td>
<td>94.4</td>
<td>7.1</td>
</tr>
<tr>
<td>21.8</td>
<td>160</td>
<td>181.4</td>
<td>98.2</td>
<td>4.8</td>
</tr>
</tbody>
</table>
proved by a variety of tests for matrix effects, extraction efficiency, selectivity, linearity, sensitivity, precision, recovery, and stability. The steps of sample pretreatment were very simple. The total chromatographic run time was less than 3.5 min. The extraction efficiencies were about 80%. The method has several advantages as compared to the previously reported LC–MS–MS methods (20–22). It has advantages such as simple sample pretreatment, small volume plasma samples need and exact results.

Methods (20–22). It has advantages such as simple sample pretreatment, small volume plasma samples need and exact results. The method has been successfully used to the pharmacokinetics and bioequivalence studies.

Acknowledgements

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