Relative Determination of Dehydroevodiamine in Rat Plasma by LC–MS and Study on its Pharmacokinetics

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Received 19 April 2011; revised 20 September 2011

The lack of purified standards is a bottleneck on assaying herbs in vitro and in vivo. This present work proposed a strategy of relative quantification that used a herb extract as a relative standard. A rapid and selective liquid chromatography tandem mass spectrometry method was similarly developed and validated for the relative determination of dehydroevodiamine in rat plasma according to the absolute quantification. Protein precipitation was used for the pretreatment of plasma samples. Chromatographic separation was achieved on a Diamonsil C18 column with an isocratic mobile phase of a 70:30 (v/v) acetonitrile–0.3% formic acid mixture at a flow rate of 0.45 mL/min. The assay was validated in the range 100.0–50,000.0 ng/mL (r² = 0.9804), the lowest level of this range being the lower limit of quantification based on 50 μL of plasma. The precision and accuracy were within recommended limits of nominal values. The method was applied to evaluate the comparative pharmacokinetics of dehydroevodiamine in rats following oral administration of Evodia rutaecarpa and Rhizoma coptidis–Evodia rutaecarpa couple. This approach was found to be capable of providing complete pharmacokinetic parameters as well as the typical pharmacokinetic assay calibrated by authentic standards, except for the absolute plasma concentrations.

The pharmacokinetic research of TCM has been seriously blocked by the lack of purified standards. It is impracticable to separate the constituents one by one to obtain standard substances for assays. The present paper demonstrates a relative reference approach in which a herb extract, instead of a purified standard, acted as the reference substance. On this basis, a rapid liquid chromatography–tandem mass spectrometry (LC–MS–MS) method for the determination of dehydroevodiamine in rat plasma was developed and applied to its pharmacokinetic after oral administration of Evodia rutaecarpa and R–E couple powders to rats independent of authentic standards.

**Experimental**

**Materials**

Methanol, formic acid and acetonitrile were of chromatographic grade from the Yuwang Chemical Factory (Shandong, China). Deionized water was purified by use of an Alpha-Q water-purification system (Millipore, Bedford, MA) for the preparation of samples and buffer solution. All other reagents were of analytical grade. Rhizoma coptidis and Evodia rutaecarpa were purchased from the Sifang Pharmacy (Shenyang, China).

**Instrumentation and analytical conditions**

Qualitative analysis was operated on a Thermo-Electron LCQ linear ion-trap mass spectrometer (Thermo-Electron, San Jose, CA) fitted with an electrospray ionization source over the mass range from m/z 50 to 2,000 in the positive ionization mode. Xcalibur 1.2 data analysis system was used. The spray voltage was set to 4.2 kV. The capillary voltage was fixed at 15 V. The heated capillary temperature was fixed at 210°C. Nitrogen used as the sheath and the auxiliary gas was set to 70 and 20 arbitrary units, respectively. The isolation width for MSn was 1.0 Da. The HPLC system consisted of an Agilent 1100 series equipped with an Agilent 1100 series photodiode-array detector (PDA) and autosampler Data Analysis (Agilent, Palo Alto, CA). Chromatographic separation was carried out on a Diamonsil C18 (150 × 4.6 mm i.d., 5 μm, Dikma) with an EasyGuard C18 Security Guard column (8 × 4.0 mm i.d., Dikma). The mobile phases consisted of 5 mM ammonium formate (0.025% formic acid) (A) and acetonitrile (B) using a gradient elution of 25% B at 0 min, 30% B at 5 min, 40% B at 15 min and 55% B at 20 min (v/v), at a flow rate of 0.5 mL/min. Quantitative analysis was performed on a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer (San Jose, CA) equipped with an electrospray ionization (ESI) source in the positive ionization mode. The MS operating conditions were optimized as follows: spray voltage, 4,200 V; heated capillary...
Method linearity was evaluated by analyzing calibration standards in duplicate at each concentration level over three consecutive days. The accuracy and precision were assessed by analyzing QC samples in six replicates at three concentration levels on three validation days. The extraction recovery was evaluated at three concentration levels by comparing the analyte/IS peak area ratios obtained from six plasma samples with the analytes spiked before and after extraction. Matrix effect was evaluated by comparing the analyte/IS peak area ratios obtained from six plasma samples with the analytes spiked after extraction to those for the neat standard solutions at the same concentrations. The stability of dehydroevodiamine in rat plasma at low and high concentration levels was evaluated under a variety of storage and process conditions.

**Pharmacokinetic application**

Five male Sprague-Dawley rats (250 ± 20 g) were fasted for 12 h before the experiment. The rats were split into two groups to complete the crossover design for pharmacokinetic experiment with a washout period of seven days. The powder of *Evodia rutaecarpa* and R-E couple was suspended in 0.1% carboxymethyl cellulose sodium (CMC-Na) aqueous solution and was administered to the rats (0.18g *Evodia rutaecarpa* powder/kg body weight) by oral gavage. Blood samples (150 µL) were obtained from the oculi chorioideae vein before dosing and subsequently at 10, 20, 45, 90, 150, 210, 300, 420, 480, 720 and 1,440 min following administration, transferred to a heparinized eppendorf tube and centrifuged at 12 krpm for 5 min. The supernatants were frozen at −20°C until analysis. Pharmacokinetic parameters were calculated using Drug and Statistics 2.0 (DAS 2.0) (Mathematical Pharmacology Professional Committee of China, Shanghai, China).

**Results and Discussion**

**Quality analysis**

LC–MS was used for the identification of dehydroevodiamine. Through a full-scan ESI mass spectrum in the positive ion mode, dehydroevodiamine was found in the *Evodia rutaecarpa* extract, plasma samples after administration of *Evodia rutaecarpa* and plasma samples after administration of R-E couple. The ion at *m/z* 302 corresponds to dehydroevodiamine at 8.49 min. The MS data indicated the loss of a methyl radical (*m/z* 287) in MS² and then the loss of 14Da (*m/z* 273) in MS³, which were identical to the MS² spectra in literature (13). The chemical structures of dehydroevodiamine and IS are shown in Figure 1. The typical

**Figure 1.** Chemical structures of dehydroevodiamine (A); metronidazole (IS) (B).
full-scan ESI mass spectra of analytes and IS were described in Figure 2.

Quantity analysis

Method validation

Different herb compositions were extracted according to the procedures described previously. Figure 3 demonstrates that the components in *Rhizoma coptidis* did not affect the response of dehydroevodiamine. The typical chromatograms of a blank, a spiked plasma sample with standard extract at the LLOQ and IS at 200 ng/mL (B); a plasma sample 3 h after oral administration of *R–E* couple (C). For all samples, no interference was found at the retention times of the analyte or the IS.

Typical equation of the calibration curve using weighted (1/\(x^2\)) least squares linear regression was \(Y = -6.496 \times 10^{-3} + 8.283 \times 10^{-3}X\), \(r^2 = 0.9804\); over the range 100.0 ~ 50,000.0 ng/mL. The calibration curves showed excellent linearity in rat plasma. The precision and accuracy data corresponding to lower limit of quantification (LLOQ) for dehydroevodiamine were shown in Table I. The values of accuracy and precision were within recommended limits. The extraction recoveries determined for dehydroevodiamine were shown in Table I.

![Figure 2. Product ion mass spectra of [M]^+ ions of dehydroevodiamine (A); metronidazole (IS) (B).](image)

![Figure 3. Representative SRM chromatograms for dehydroevodiamine (100.0 ng/mL) in extract solution of *Rhizoma coptidis* (A); *Evodia rutaecarpa* (B); *R–E* couple (C).](image)

![Figure 4. Representative SRM chromatograms for analytes in a blank plasma sample (A); a blank plasma spiked with standard extract at the LLOQ and IS at 200 ng/mL (B); a plasma sample 3 h after administration of *R–E* couple (C).](image)

![Figure 5. Mean plasma concentration–time curves of dehydroevodiamine after oral administration of *Evodia rutaecarpa* and *R–E* couple powders.](image)

<table>
<thead>
<tr>
<th>Added (ng/mL)</th>
<th>Found (ng/mL)</th>
<th>RSD%</th>
<th>Relative error</th>
<th>Recovery %</th>
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<tbody>
<tr>
<td>100 (LLOQ)</td>
<td>82.46</td>
<td>17.9</td>
<td>-17.5</td>
<td>92.3</td>
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<tr>
<td>200</td>
<td>180.81</td>
<td>14.2</td>
<td>-9.6</td>
<td>91.7</td>
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<tr>
<td>2,000</td>
<td>1,826.54</td>
<td>8.1</td>
<td>-8.7</td>
<td>82.6</td>
</tr>
<tr>
<td>40,000</td>
<td>34,920.42</td>
<td>8.8</td>
<td>-12.7</td>
<td>5.8</td>
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</table>
in Table I with acceptable variance. The matrix effect at the concentration of 2,000 ngH/mL for dehydroevodiamine and 200 ng/mL for IS in six different sources of rat plasma were 113.4 ± 5.9%. The ionization suppression/enhancement was negligible.

The stability study showed that dehydroevodiamine was stable in plasma at room temperature (25°C) for 4 h (relative error; RE < 10.7%), after three freeze–thaw cycles (RE < 8.1%) and after the reconstitution at 25°C for 24 h (RE < 0.5%).

Results of pharmacokinetic study

The method described previously was applied to a pharmacokinetic study in which plasma concentrations of dehydroevodiamine were determined for 24 h after oral administration of Evodia rutaecarpa and R–E couple (0.18g Evodia rutaecarpa powder/kg body weight). The mean plasma concentration–time profiles (n = 5) are shown in Figure 5.

Pharmacokinetic parameters are listed in Table II. According to definitions, the pharmacokinetic parameters except AUC and C_{max} are independent of absolute plasma concentrations and therefore independent of standards. AUC and C_{max} are not comparable between different analytes but comparable between the same analyte in different samples.

The plasma concentration of dehydroevodiamine after oral administration of R–E couple was much higher than that after oral administration of Evodia rutaecarpa alone. Apparently, Rhizoma copitidis can enhance the absorption and AUC of Evodia rutaecarpa.

The appearance of multiple blood concentration peaks in Figure 5 is likely because the absorption rate of alkaloids was limited by their dissolution rate from herb powders. In addition, distribution re-absorption and enterohepatic circulation might contribute to multiple blood concentration peaks of dehydroevodiamine after oral administration of R–E couple.

Conclusions

In this study, dehydroevodiamine was tentatively identified by multi-stage tandem mass spectrometry. An LC–MS-MS assay was developed for the determination of dehydroevodiamine in rat plasma using the herb extract as the reference substance. The LC–MS-MS assay was applied to the comparative pharmacokinetic studies of dehydroevodiamine in plasma. This approach is well suited for the determination of the compounds without standards.

### References


### Table II

Mean Pharmacokinetic Parameters of Dehydroevodiamine in Rat Plasma (n = 5)

<table>
<thead>
<tr>
<th>Formula</th>
<th>( AUC_{0-\infty} ) (µgH/L h)</th>
<th>( AUC_{0-\infty} ) (µgH/L h)</th>
<th>MRT(0-\infty) (h)</th>
<th>( MRT_{0-\infty} ) (h)</th>
<th>( t_{1/2} ) (h)</th>
<th>( t_{max} ) (h)</th>
<th>C_{max} (µgH/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evodia rutaecarpa</td>
<td>68.130 ± 17.451</td>
<td>68.134 ± 19.162</td>
<td>4.6 ± 0.7</td>
<td>4.6 ± 0.8</td>
<td>1.6 ± 0.4</td>
<td>3.5 ± 3.0</td>
<td>15,383 ± 7,166</td>
</tr>
<tr>
<td>Zuojinwan</td>
<td>186.698 ± 46.442</td>
<td>186.715 ± 3.9211</td>
<td>4.9 ± 0.7</td>
<td>4.9 ± 3.6</td>
<td>1.8 ± 5.4</td>
<td>1.5 ± 1.1</td>
<td>40,992 ± 21,052</td>
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</table>

### Relative Determination of Dehydroevodiamine in Rat Plasma by LC–MS and Study on its Pharmacokinetics

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