Determination and pharmacokinetics of manidipine in human plasma by HPLC/ESIMS

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ABSTRACT: A sensitive HPLC/ESIMS method was established for the determination of manidipine in human plasma and pharmacokinetics study. After basified plasma with ammonia, manidipine and the internal standard (IS) (felodipine) were extracted with n-hexane and separated on a Hypersil ODS2 column with a mobile phase of methanol–5 mM ammonium acetate solution containing 0.1% acetic acid (85:15, v/v). MS determination was performed by electrospray ionization in the selected ion monitoring mode. Manidipine was monitored at m/z 611.4 and IS at m/z 384. The assay had a calibration range from 0.2 to 20 ng/mL and a lower limit of quantification of 0.1 ng/mL. The method has been successfully applied to the pharmacokinetic study in healthy volunteers. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: manidipine; HPLC/ESIMS; pharmacokinetics

INTRODUCTION

Manidipine is a lipophilic, third-generation, highly vaso-selective, dihydropyridine (DHP) calcium channel antagonist having long-lasting activity for the treatment of essential hypertension (Otero and Claros, 2005; Roca-Cusachs and Triposkiadis, 2005). After oral administration of manidipine in human at clinical dose, the plasma concentration of manidipine is very low (Ohkubo et al., 1996). Therefore, development of a sensitive analytical method for manidipine is necessary for pharmacokinetic studies.

In previous studies, HPLC-UV (Miyabayashi et al., 1989) and HPLC-ECD (Eastwood et al., 1990) methods have been reported. However, these methods were not sensitive enough for pharmacokinetic research of manidipine. A more sensitive electrochemical detection method for determination of manidipine in human plasma with a lower LOQ of 0.3 ng/mL has been developed (Ohkubo et al., 1996), but the solid-extraction and column-switching procedures were tedious and costly. In this paper, we report a sensitive and specific HPLC/ESIMS method for the determination of manidipine in human plasma using liquid–liquid extraction.

EXPERIMENTAL

Reagents and materials. Manidipine was kindly donated by Jiangsu Wu Zhong Su Yao Drug Development Co. Ltd. (Nanjing, China) and felodipine was purchased from Jiangsu Lianhuan Pharmaceutical Co. Ltd. (Yangzhou, China) as internal standard (IS). The chemical structures of manidipine and IS are shown in Fig. 1. Methanol was HPLC grade (Tedla Co., Inc., USA). All other reagents and chemicals were of analytical grade.

Apparatus and conditions. Analyses were performed using a Shimadzu HPLC system coupled to a LCMS-2010EV mass spectrometer (Shimadzu, Japan). The analytical column was a Hypersil ODS2 (5 µm, 4.6 × 200 mm i.d., Elite Co., Dalian, China) kept at 28°C in the CTO-20A column oven (Shimadzu, Japan). The mobile phase consisted of methanol–5 mM ammonium acetate solution containing 0.1% acetic acid (85:15, v/v), and the flow-rate was 0.7 mL/min. The detector voltage was set at 1.75 kV. HPLC/ESIMS was performed in positive ion selected ion monitoring (SIM) mode using target ions at m/z 611.4 for minidipine and m/z 384 for the internal standard (IS) (Fig. 2). The mobile phase was degassed using a DGU-20A3 degasser (Shimadzu, Japan) and mixed with a CBM-20A rotary pump (Shimadzu, Japan). Test samples were introduced using a SIL-20AC auto-injector (Shimadzu, Japan) with an effective volume of 10 µL.

Sample preparation. The plasma sample (1 mL) was spiked with 50 µL of felodipine (1 µg/mL, in methanol) as internal standard, basified by addition of 0.1 mL 1% ammonia solution, and vortex-mixed in a glass tube. The mixture was extracted with 4 mL of n-hexane containing 2% isopropyl...
alcohol. Following centrifugation, 3 mL of organic layer was evaporated to dryness in a vacuum pump at 40°C. The residue was dissolved in 100 µL of 90% methanol and loaded into the glass insert of an auto-sampler vial. An aliquot of 10 µL of the sample was injected onto the LC–MS system using an auto-sampler.

**Preparation of calibration samples and quality control samples.** The calibration curve samples were prepared in 1.0 mL of plasma, by adding aliquots (100 µL) of the stock solution of manidipine to drug-free plasma at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 ng/mL. Quality control samples containing three different concentrations (0.5, 2.0 and

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**Figure 1.** Chemical structure of felodipine (IS) and manidipine.

**Figure 2.** MS scan spectra of manidipine (A) and IS (B).
10.0 ng/mL) were prepared in the same way for calibration curve samples. Then the plasma samples were treated according to the sample preparation procedure above.

**Specificity, accuracy, precision and stability.** Six human blank plasma samples were extracted by the sample preparation method described above to assay the specificity. The accuracy of the method was measured by injection of five quality control samples then calculated as a percentage of the observed concentration to the nominal one (% nominal).

The intra-day percentage relative standard deviation (%RSD) was calculated according to the determination of five quality control plasma samples in a day. Otherwise, the inter-day %RSD was calculated according to the determination of the quality control samples daily on five separate days.

Frozen stability was assessed after 0, 3, 5, 7 and 14 days of storage at −20°C; freeze–thaw stability was assessed after 0, 3 and 7 days of storage in a freezer at −20°C and the stability of manidipine in plasma samples was tested after 0, 1 and 2 freeze–thaw cycles (−20°C to ambient temperature). The stability of manidipine in extracts was also examined after 0, 4, 8, 16 and 32 h of storage at ambient temperature.

**Drug administration and sampling.** Two doses of manidipine hydrochloride tablets (10 and 20 mg) were orally administered to eight healthy volunteers. Blood samples (4 mL) were collected by venepuncture at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0 and 12.0 h after administration. Plasma samples were separated by centrifugation at 4000 rpm for 5 min and stored at −40°C until analysis.

**Pharmacokinetic analysis.** The pharmacokinetic analysis of the manidipine was performed using one-compartment methods by 3p87. The area under the plasma concentration vs time curve (AUC) was calculated using the trapezoidal rule.

**RESULTS**

**Chromatography**

The HPLC/MS method described provides good separation of manidipine and felodipine from the other endogenous plasma constituents. There was no interference from IS contributing to the m/z channel of manidipine and vice versa. Figure 3 shows an MS chromatogram of blank human plasma (A); blank human plasma containing manidipine and internal standard (felodipine) (B); and a plasma sample from a volunteer administered a single dose of manidipine with internal standard (C),

![Figure 3. MS chromatogram of manidipine in human plasma; (A) blank human plasma; (B) blank human plasma containing manidipine and internal standard (felodipine); (C) a plasma sample from volunteers administered a single oral dose of manidipine with internal standard. This figure is available in colour online at www.interscience.wiley.com/journal/bmc](image-url)
Table 1. Accuracy and precision for the determination of manidipine in human plasma (n = 5)

<table>
<thead>
<tr>
<th>Nominal concentration (ng/mL)</th>
<th>Intra-day measured concentration (mean ± SD)</th>
<th>Precision, RSD (%)</th>
<th>Inter-day measured concentration (mean ± SD)</th>
<th>Precision, RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.50 ± 0.03</td>
<td>5.39</td>
<td>99.68 ± 5.37</td>
<td>4.88</td>
</tr>
<tr>
<td>2.0</td>
<td>1.97 ± 0.07</td>
<td>3.76</td>
<td>98.65 ± 3.71</td>
<td>7.81</td>
</tr>
<tr>
<td>10.0</td>
<td>9.46 ± 0.46</td>
<td>4.94</td>
<td>94.60 ± 4.67</td>
<td>5.47</td>
</tr>
</tbody>
</table>

Table 2. Stability of manidipine after storage under indicated conditions

<table>
<thead>
<tr>
<th>Nominal concentration (ng/mL)</th>
<th>Ambient (n = 5)</th>
<th>Freezing (n = 5)</th>
<th>Freeze–thaw (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured concentration (mean ± SD)</td>
<td>RSD (%)</td>
<td>Measured concentration (mean ± SD)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.48 ± 0.03</td>
<td>5.33</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td>2.0</td>
<td>1.96 ± 0.04</td>
<td>2.08</td>
<td>1.92 ± 0.07</td>
</tr>
<tr>
<td>10.0</td>
<td>9.25 ± 0.44</td>
<td>4.78</td>
<td>9.55 ± 0.11</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation, average of concentration in 0, 4, 8, 16 and 32 h; ‡ mean ± standard deviation, average of concentration in 0, 3, 5, 7 and 14 days; § mean ± standard deviation, average of concentration in 0, 1 and 2 cycles per week.

the retention times for which were about 5.8 min for manidipine and 5.6 min for IS.

HPLC-MS method validation

Linear least-squares regression analysis of the calibration graph demonstrated linearity in the range 0.2–20 ng/mL. A typical standard curve (r = 0.9996) could be described by the equation As/Ais = 0.2466 C + 0.0105. The limit of detection was 0.1 ng/mL. Both the precision and accuracy of the method were found to be well within the acceptable limits (Table 1). Manidipine was stable in plasma at ambient temperature for up to at least 32 h; it also remained intact at −20°C for up to 2 weeks. With respect to the run-time stability of processed samples, no significant loss of manidipine was observed at ambient temperature, and no degradation was observed after two cycles of freezing and thawing and after two weeks of freezing (Table 2).

Application of the method in pharmacokinetics

The method described above was applied to evaluate the pharmacokinetics of manidipine in human blood plasma, which determined the concentration vs time profile of manidipine in human after a single oral dose (10 and 20 mg) of manidipine (Fig. 4). Further studies are in progress to evaluate the pharmacokinetic parameters of manidipine. In this paper, we studied the clinical pharmacokinetic of manidipine by comparing the pharmacokinetic parameters of two different single doses of manidipine. The main pharmacokinetic parameters AUC0–t, Cmax and T1/2ke are shown in Table 3. The results demonstrated that the present method is suitable and applicable for clinical pharmacokinetic studies of manidipine at clinical doses.

DISCUSSION

In this paper, a highly sensitive and specific HPLC-MS method for the determination of manidipine in human plasma after oral administration in clinical doses has been developed. The liquid–liquid extraction of plasma samples with n-hexane containing isopropyl alcohol is simple. Series measures were taken to improve the...
method: the plasma was basified with 1% ammonia to maintain the stability of manidipine in the extraction procedure, and n-hexane was mixed with isopropyl alcohol to dissolve the emulsification. In conclusion, it was shown that the method could be applied to pharmacokinetic studies for manidipine in clinical investigation.

REFERENCES


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**Table 3. Pharmacokinetic parameters of manidipine in healthy volunteers (n = 8)**

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>$K_e$ (1/h)</th>
<th>$T_{1/2}$ (h)</th>
<th>$T_{max}$ (h)</th>
<th>$C_{max}$ (ng/mL)</th>
<th>$AUC_{0-t}$ (ng/mL h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.44 ± 0.13</td>
<td>1.79 ± 0.50</td>
<td>1.56 ± 0.94</td>
<td>2.64 ± 1.55</td>
<td>7.92 ± 2.89</td>
</tr>
<tr>
<td>20</td>
<td>0.32 ± 0.11</td>
<td>2.32 ± 0.78</td>
<td>2.44 ± 1.18</td>
<td>5.53 ± 2.61</td>
<td>20.36 ± 8.40</td>
</tr>
</tbody>
</table>