A Gas Chromatography–Mass Spectrometry Method for the Determination of Pogostone in Canine Plasma and Its Application to a Pharmacokinetic Study

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In this study, a simple and selective gas chromatography–mass spectrometry method was developed and validated for the determination of pogostone in canine plasma. Liquid–liquid extraction was used to separate pogostone from canine plasma, and the mean extraction recovery rates of pogostone and the internal standard (isoalantolactone) were 80.61 and 75.89%, respectively. Plastic centrifuge tubes were inadequate for the plasma sample treatment procedure because of the adsorption effect of pogostone on the inner surface. The chromatographic separation was performed on a capillary column of Agilent HP-5ms, and the spectrometer was operated in an electron-impact ionization with an electron multiplier voltage mode. The standard curve was linear over the concentration range of 1.02–406 ng/mL ($r > 0.99$). The intra- and interday accuracies for pogostone at four concentrations were 97.77–99.92% and 98.51–100.22%, respectively. The relative standard deviations were <15%. The method was successfully applied to a pharmacokinetic study after the oral administration of pogostone to beagle dogs.

**Materials and methods**

**Materials**

Pogostone (99.8%) was kindly supplied by Liang Xiong in our research group, which was extracted from Pogostemonis Herba. Isoalantolactone used as the internal standard (IS; Figure 1B) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Methanol was purchased from TEDIA Company (Fairfield, IA, USA), and ethyl acetate was obtained from ARK Chemical (Chengdu, China). Water was made by distillation in glass and passage through a Milli-Q water purification system (Millipore, Bedford, MA, USA).

**Equipment and GC–MS condition**

GC–MS was performed with an Agilent 7890A GC instrument, an Agilent 5975C mass spectrometer and Agilent ChemStation.
software (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated on a capillary column of Agilent HP-5ms (30 m × 0.25 mm i.d. × 0.25 μm, coated with film 5% phenyl methyl siloxane). The column temperature was at 110°C for injection; the temperature program began at 25°C min⁻¹ to 240°C and held for 1 min, then at 35°C min⁻¹ to 280°C and held for 2 min. The spectrometer was operated in EI ionization, and the inlet ionization source temperature was 230°C. The electron multiplier voltage (EMV) mode with a gain factor of 1.01 was used, and the actual EMV was 1576.47 V. Two product ion mass spectra of the base ion peaks at m/z 168.0 and m/z 190.1 of pogostone, and IS were acquired with selected ion monitoring.

**Preparation of standard and quality control samples**
The standard curves of pogostone in plasma were 1.02, 5.03, 10.2, 50.3, 102, 203 and 406 ng/mL. The concentration of IS in plasma was 0.503 μg/mL. Quality control (QC) samples were
prepared on the day of analysis and in bulk at four concentrations: 1.02 (LLQC), 5.03 (LQC), 50.3 (MQC) and 203 (HQC) ng/mL. The pogostone and IS stock solutions were made in methanol at concentrations of 81.2 and 503.2 µg/mL, stored at 4°C and protected from light for ≤1 month.

**Sample treatment**

Control (blank) plasma was obtained from blank dogs for calibration, and bulk spiked QC samples were removed from the freezer and allowed to thaw to room temperature before extraction. Control plasma with a volume of 270 µL (200 µL of blank plasma spiked with 50 µL of working standard solutions of various concentrations and 20 µL of the IS solution) and sample plasma with a volume of 270 µL (200 µL of sample plasma added to 70 µL of methanol) were prepared for the extraction. Then a sample was extracted using 2 mL of ethyl acetate in a vortex shaker (Vortex Genius 3, IKA-Werke GmbH & Co., Staufen, Germany). The upper organic phase was transferred and

Figure 3. Chromatograms of the base ion peak at m/z 168.0 of Pogostone (A) and the base ion peak at m/z 190.1 of isoalantolactone (B).
evaporated to dryness under a stream of nitrogen at 30°C (N-EVAP 11,155, Organamation Associates, Inc., Berlin, MA, USA). The residue was reconstituted in 100 μL of methanol. The sample was transferred to the glass autosampler vial insert and 1 μL was injected into the chromatographic system. During the whole process of sample treatment, glass centrifuge tubes were used instead of plastic tubes.

**Pharmacokinetic study**

The animal protocol was approved by the Committee of Scientific Research and the Committee of Animal Care of the Chengdu University of Traditional Chinese Medicine (Chengdu, China). Six beagle dogs (three males and three females), weighing 9–11 kg, were purchased from GLP Experiment Center of Sichuan Provincial Hospital (Sichuan, China). All dogs were fasted for 12 h before dosing and for another 4 h after dosing. Water was provided *ad libitum*. Each dog had a lavage of a single dose of pogostone. Blood samples (≈3 mL) were withdrawn from the forelimb veins of each dog before dosing and at 5, 15, 30 and 45 min, and 1, 2, 4, 6, 9, 12 and 16, 24 h after dosing into glass centrifuge tubes (Vacutainer, Zhenjiang Gongdong Medicao Technology Co., Ltd.). The heparinized blood was centrifuged at 4,500 rpm for 10 min and the plasma in the upper layer was transferred and stored at −20°C until analysis. The C<sub>max</sub> and T<sub>max</sub> were observed visually from the plots of pogostone plasma concentration versus time. All data were processed using Phoenix WinNonlin 6.3 (Pharsight Corporation, USA) to construct pharmacokinetic profiles.

**Results**

**Method validation**

The assay method was validated in terms of selectivity, linearity, sensitivity, accuracy, precision, extraction recovery and stability, according to the Food and Drug Administration guidelines (20). The mass chromatograms of GC–MS analysis of pogostone and IS are shown in Figure 2A and B, respectively. The S<sub>r</sub> (%) was calculated as follows:

\[
S_r = \frac{y}{x} \times 100
\]

where \(y\) represents the peak area ratio of pogostone to IS and \(x\) represents the concentration of pogostone in plasma. The calibration curve was fitted by linear least-squares regression analysis with a weighting factor of 1/x^2. The LOQ [signal-to-noise ratio (S/N) ≥ 5] of pogostone was 1.02 ng/mL.

**Precision and accuracy**

The intraday accuracy and precision of the method were evaluated by analyzing five replicates at four different QC levels in 1 day, and the interday accuracy and precision were evaluated by analyzing the QC samples in three different days (Table I). The intra- and interday precision RSDs were within 3.15–5.79 and 2.68–7.13%, respectively. The intra- and interday accuracy ranged from 97.77 to 99.92% and from 98.51 to 100.22%, respectively. The deviations of the mean accuracy were within 15%.

**Extraction recovery**

The extraction recovery for pogostone and IS were evaluated by assaying two groups of samples: (i) neat standard solutions of pogostone and IS in a solution of methanol, and (ii) extracts from plasma spiked with pogostone and IS. Samples of each group were prepared at three QC levels of LQC, MQC and HQC. By comparing the absolute peak areas of the analytes obtained in Groups 1 and 2, the extraction recoveries of the analytes were calculated as extraction recovery (%): RSDs of these values were < 6%. The mean recovery of the IS was 75.89% (n = 15). The extraction recoveries for pogostone and IS shown in Table I. The mean recovery of pogostone was 80.61%, which was treated in glass centrifuge tubes during the entire process (n = 15). The mean recovery of IS was 75.89% (n = 15). The RSDs of these values were < 6%.

**Stability**

The stability of pogostone was assessed, in terms of short term (at room temperature for 14 h), long term (at −20°C for 21 days), three freeze–thaw cycles (from −22°C to 25°C) and autosampler stabilities (at 25°C for 12 days). The samples were processed and analyzed with the freshly prepared samples. All stability experiments were performed at three QC levels of LQC, MQC and HQC in triplicate.

**Table I**

<table>
<thead>
<tr>
<th>Nominal concentration (ng/mL)</th>
<th>Intraday (n = 5)</th>
<th>Interday (n = 3)</th>
<th>Extraction recovery (%)</th>
<th>IS (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSD (%)</td>
<td>Accuracy (%)</td>
<td>RSD (%)</td>
<td>Accuracy (%)</td>
</tr>
<tr>
<td>1.02</td>
<td>5.79</td>
<td>99.92 ± 5.79</td>
<td>7.13</td>
<td>99.45 ± 7.09</td>
</tr>
<tr>
<td>5.10</td>
<td>4.46</td>
<td>99.68 ± 4.45</td>
<td>5.63</td>
<td>100.13 ± 5.64</td>
</tr>
<tr>
<td>51.0</td>
<td>4.18</td>
<td>97.77 ± 4.09</td>
<td>4.70</td>
<td>98.51 ± 4.83</td>
</tr>
<tr>
<td>203</td>
<td>3.15</td>
<td>99.92 ± 3.13</td>
<td>2.68</td>
<td>100.22 ± 2.68</td>
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</tbody>
</table>
Pharmacokinetics

The concentration of pogostone in canine plasma was detectable and quantified up to 16–24 h for all dogs after dosing. Figure 4 depicted the mean plasma pogostone concentration versus time profile in dog plasma. The primary pharmacokinetic parameters are shown in Table III. The results showed that the established method was sensitive enough to detect at least four half-lives of pogostone following its oral administration to beagle dogs at a dose of 1.5 mg/kg.

Discussion

The GC–MS method was successfully used to determine pogostone in canine plasma samples and applied to a pharmacokinetic study. Three organic solvents, diethyl ether, chloroform and ethyl acetate, were evaluated regarding the extraction recovery. Ethyl acetate was found to be more efficient with a recovery rate of ≏80% compared with diethyl ether (50%), and lower toxicity compared with chloroform.

It was worthwhile to note that the extraction recovery rates of pogostone were high in glass centrifuge tubes. Plastic centrifuge tubes, however, were avoided in this study because the adsorption effect of pogostone on the inner surface. The extraction recovery rates using plastic centrifuge tubes (Better Biotechnology Co., Ltd, Jiangsu, China) during the sample treatment were much lower and with big error (Table I). When pogostone was dissolved in organic solvents (hexane and ethyl acetate) and vibrated in a vortex shaker, there was also a loss observed. It has been proposed that adsorption of various drugs to plastic infusion bags acted as a function of time (21, 22). Dahlstrom et al. (23) studied the adsorption of medetomidine to hydrophilic polystyrene plastic. That study found that the electrostatic binding was the most important mechanism for surface interaction. Another study (24) found that the lipophilicity of compounds explained the adsorption of drugs to polystyrene plastic surface. All these results indicated that a loss of pogostone occurred by surface adsorption maybe through lipophilic interactions.

Conclusions

A simple and selective GC–MS method for the quantification of pogostone in dog plasma was developed and validated. The

<table>
<thead>
<tr>
<th>Analyte concentration (ng/mL)</th>
<th>Bias (%)</th>
<th>Analyte concentration (ng/mL)</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short-term stability</td>
<td>Long-term stability</td>
<td>Three freeze–thaw cycle stabilities</td>
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<tr>
<td>5.10</td>
<td>4.69</td>
<td>9.61</td>
<td>8.77</td>
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<tr>
<td>51.0</td>
<td>4.38</td>
<td>6.42</td>
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<tr>
<td>203</td>
<td>4.29</td>
<td>6.84</td>
<td>5.56</td>
</tr>
</tbody>
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