Supporting Information

The Pharmacokinetics of Chelerythrine Solution and Chelerythrine Liposomes after Oral Administration to Rats
Weifeng Li, Wei Xing, Xiaofeng Niu, Ping Zhou, Ting Fan

Affiliation
School of Medicine, Xi'an Jiaotong University, Xi'an, PR China

Correspondence
Xiaofeng Niu; Weifeng Li
School of Medicine, Xi'an Jiaotong University
No. 76 Western Yanta Road
Xi'an City, Shaanxi Province 710061
PR China
Phone: +86 29 826 551 39
Fax: +86 29 826 551 38
niuxf@mail.xjtu.edu.cn; liwf@mail.xjtu.edu.cn
Methods

Chromatographic System

A Shimadzu LC-2010A liquid chromatograph equipped with a SPD-10AVP ultraviolet absorption detector, a class-VP work station, and a Diamonsil ODS C$_{18}$ column (5 μm, 150 × 4.6 mm) maintained at 25°C was used for the analysis. The mobile phase consisted of acetonitrile-0.1% (v/v) in aqueous phosphoric acid (H$_3$PO$_4$; Merck pro analysis, 85%) (30:70, v/v PH: 3.8 ± 0.2). The solvent flow rate was 1.0 mL/min, and the detection wavelength was 270 nm. The data were acquired and processed with a Class-VP Ver. 6.0 chromatography data system.

HPLC Determination

Stock solutions

The standard stock solutions were prepared by dissolving 6.0 mg CHE and DHCHE in 10 mL methanol to furnish a nominal concentration of 0.6 mg/mL. The IS stock solution was prepared by dissolving 6.0 mg berberine in 10 mL methanol to furnish a nominal concentration of 0.6 mg/mL. All stock solutions were stored at 4 °C but equilibrated to room temperature before use.

Preparation of calibration plot and quality-control samples
CHE standard solutions (0.02, 0.1, 1.0, 5.0, 10.0, 15.0, 20.0 μg/mL) and DHCH standard solutions (0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 μg/mL) were prepared by spiking 200 μL blank rat plasma with appropriate amounts of the standard stock solution prepared as described above. Quality-control (QC) samples of CHE (0.1, 5.0, and 15.0 μg/mL) and DHCH (0.05, 0.5 and 5.0) were prepared independently in the same manner. A solution containing 10.0 μg/mL of IS was also prepared in methanol.

**Sample preparation**

Plasma sample (100 μL) was mixed with IS (10.0 μg/mL, 100 μL) and methanol (100 μL) on a vortex-mixer for 1 min, then the mixed sample was extracted with 2 mL chloroform by mechanical shaking for 3 min. The extraction was centrifuged at 10'000 g for 10 min, and then the supernatant was transferred to a clean test tube and evaporated to dryness in a water bath at 40°C under a stream of nitrogen. The residue was reconstituted with 100 μL mobile phase. After centrifugation at 4000 g for 10 min, a 40 μL sample of the supernatant was injected into the HPLC system.

**Bioanalytical method validation**

The method validation assays were performed according to the guidelines for chemicals in non-clinical pharmacokinetic studies [17], recommended by Ministry of Health, PR China, which included selectivity, linearity,
limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, stability, and recovery.

To assess the selectivity of the present method, drug-free plasma samples from six different rats of six batches were extracted and analyzed to assess the potential endogenous interferences. Any apparent response at the retention times of CHE, DHCHE, and IS was compared to the response at the LOQ and the response at the working concentrations for CHE, DHCHE, and IS, respectively. This determines whether there are any interfering peaks at the retention times of CHE, DHCHE, and IS.

To evaluate linearity, spiked plasma standard solutions at seven concentrations in the range 0.01–20.0 μg/mL were prepared in triplicate and analyzed separately three times. The LODs and the LOQs of CHE and DHCHE were determined using a signal-to-noise ratio (S/N) of each peak more than 3 and 10, respectively. The calibration curves were constructed using weighted (1/x²) linear least-squares regression analysis of the observed CHE and DHCHE to IS peak-area ratios against concentration. Unknown sample concentrations were calculated from the linear regression equation for the calibration plot of peak area ratio against concentration.

QC samples at three levels of CHE (0.1, 5.0, and 15.0 μg/mL) and DHCHE (0.05, 0.5, and 5.0μg/mL) were assayed to check the accuracy and intra and inter-day precision of the method. For accuracy and
intra-day precision, QC samples (six replicates at each level) were
detected and calculated using a calibration curve prepared on the same
day. For inter-day precision, QC samples were analyzed over four
different days (six replicates at each level per day). Accuracy was
expressed as the percentage of the mean calculated concentrations to the
nominal concentrations, and precision was evaluated as relative standard
deviceation (RSD).

Stability experiments were carried out in plasma tissues under different
conditions. The stability of CHE and DHCHE were evaluated at low (0.1
μg/mL), medium (5.0 μg/mL), and high (15.0 μg/mL) concentrations in
triplicate. All stability results were evaluated by measuring the area
response (CHE/IS or DHCHE/IS) of stability samples against the initial
concentration as determined for freshly prepared samples. The samples
were considered stable when the calculated concentrations in QC samples
were within 85-115% of the nominal concentrations. For the short-term
temperature stability, samples were kept at room temperature (25°C) for 4
h before sample preparation. The freeze-thaw stability of CHE and
DHCHE were determined over three freeze-thaw cycles. In each cycle,
the frozen samples were thawed at room temperature for 1 h and refrozen
for 24 h. The long-term stability was evaluated after keeping the samples
frozen at -20°C for 30 d. The processed samples were stored in the
refrigerator at 4°C. Duplicates at each level QC samples were analyzed
over 2 d.

The recovery of the liquid-liquid extraction was evaluated at the three QC levels of CHE and DHCHE for 10.0 μg/mL IS. It was determined by comparing the mean peak areas (n=6 at each concentration) obtained from plasma samples spiked before extraction with those from plasma samples spiked after extraction.

Results

Selectivity of the proposed method was assessed by comparing chromatograms of blank plasma with CHE and IS-spiked plasma at the LOQ levels. No interference peak (S/N≥3) in blank plasma at the or near the retention time of CHE, DHCHE, and IS was observed just as shown in Fig. 4; it indicated that the extraction procedure was capable of obtaining highly purified samples which in turn ensured a high selectivity of the HPLC method.

The LOD and LOQ of CHE or DHCHE in plasma by HPLC were both found to be 1.0 ng/mL and 4.0 ng/mL, respectively. Concentrations below the LOQ were detected with unacceptable accuracy and precision. The calibration curve was assessed based on a plot of the ratio of peak areas for CHE/IS and DHCHE/IS. A representative regression equation for the calibration plot of CHE was Y=0.0753X+0.0621 over the range 0.02-20
μg/ml, with a correlation coefficient of 0.998. For DHCHE, the
calibration plot was $Y=0.0682X+0.0425$ over the range 0.01-10ug/mL,
and the correlation coefficient was 0.994

The intra and inter-day precision were assessed by analyzing quality
control samples at three QC concentrations in six duplicates. Tables 1
and 2 show the intra and inter-day precision and accuracy results of CHE
and DHCHE, respectively. The accuracy of CHE was in range
94.0-99.7% (intra-day) and 92.0-100.9% (inter-day) with the precision
(RSD) of 1.11-8.51% (intra-day) and 0.93-9.78% (inter-day) in plasma,
while the accuracy of DHCHE was in range 94.0-97.8% (intra-day) and
96.0-102.2% (inter-day) with the precision (RSD) of 1.08-4.26%
(intra-day) and 1.70-8.33% (inter-day). The values obtained were lower
than the limits required for biological sample analysis, which
demonstrates that both precision and accuracy meet the requirements of
the current Chinese Pharmacopoeia (2010) [1].

The stability tests of CHE and DHCHE were made to cover the real
conditions that samples may experience. The results are summarized in
Table 3. The short-term temperature stability results showed that the
corresponding accuracies of CHE and DHCHE were 96.0%, 98.6%,
96.2% and 93.0%, 97.5%, 99.0% for samples spiked with 0.1 μg/mL, 5.0
μg/mL, and 15.0 μg/mL, respectively, which demonstrated that CHE in
plasma was stable in room temperature (25°C) for at least 4 h. After three
freeze-thaw cycles, the corresponding accuracies of CHE were 93.0%, 97.9%, and 99.3%, respectively, while the corresponding accuracies of DHCHE were 93.0%, 98.2%, and 97.6% at the corresponding concentrations, which indicated that CHE and DHCHE were stable in rat plasma during three freeze-thaw cycles. After storage at -20 °C for 30 days the corresponding accuracies of CHE were 91.0%, 99.6%, and 100.9%, and the DHCHE were 88.0%, 95.9%, and 98.4%, respectively. These results indicated that CHE and DHCHE in plasma were stable at least for 2 weeks when stored at -20°C.

The recoveries of the proposed method were evaluated, and the results showed that extraction recoveries of CHE and DHCHE from rat plasma were 83.3±2.8%, 88.7±3.3%, 89.7±1.6% and 86.3±3.1%, 89.4±1.9%, 91.8±1.4% for concentrations of 0.1 μg/mL, 5.0 μg/mL, and 15.0 μg/mL, respectively. Mean recovery of CHE and DHCHE were 87.2% and 89.2%. Recovery of IS was 85.4±4.8% at the concentration (10.0 μg/mL) used in the method.