Pharmacokinetics and bioavailability of curdione in mice by UPLC-MS/MS

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Received: January 19, 2022 • Accepted: April 22, 2022

ABSTRACT
A UPLC-MS/MS method was developed to determine curdione in the mouse blood, and the pharmacokinetics of curdione in mice after intravenous (5 mg kg$^{-1}$) and oral (20 mg kg$^{-1}$) administration were studied. The HSS T3 column was used for separation, and column temperature was set at 40 °C. Multiple reaction monitoring (MRM) mode were used for determination of curdione. Blood samples were taken from the caudal vein of Institute of Cancer Research (ICR) mice after administration of curdione. It showed a good linear relationship in the range of 1–500 ng mL$^{-1}$ ($r$ > 0.998); the intra-day precision was <13%, the inter-day precision was <15%, and the accuracy was 90%–105%, the recovery was >77%, and the matrix effect was 97%–107%. The half-life was relatively short, and the bioavailability was 6.5%. The developed method was suitable for the pharmacokinetics of curdione in mice.

KEYWORDS curdione, UPLC-MS/MS, pharmacokinetics, bioavailability, mouse

INTRODUCTION
Zedoary Curcuma is commonly called Wenshu and pengzedoary Curcuma [1, 2]. It is the dry rhizome of Curcuma Zedoariae, turmeric or Guangxi Curcuma [3–5]. Curdione, is a sesquiterpene compound with antitumor activity isolated from Zedoary turmeric extract and Zedoary turmeric oil [6–9]. Curdione is one of the main active components of Curcuma oil and a commonly used Chinese medicine quality control index. It has a good clinical effect on cervical cancer; it is also effective for vulvar cancer, skin cancer and lip cancer [10–15]. Recently, it has been reported that curdione has anti-inflammatory and hepatoprotective effects [16, 17], it has significant anticoagulant and antithrombotic effects [18]. It also found that curdione can inhibit thrombin-induced platelet activation and aggregation [9].

There were several LC-MS or LC-MS/MS methods developed for determination of curdione in rat or rabbit plasma [19–24], however, no UPLC-MS/MS method developed for determination of curdione in mice and its pharmacokinetics. Chromatographic detection methods have long analysis time, poor selectivity for complex matrices, low sensitivity, and cannot achieve effective detection of the drugs; UPLC-MS/MS due to its fast analysis speed, it has the advantages of high sensitivity and strong specificity [25–30]. In this study, the protein precipitation method was used to extract curdione in blood samples, and an UPLC-MS/MS method was established for the determination of curdione in mouse blood with high sensitivity and high specificity, and this method was successfully applied to the in vivo pharmacokinetics of curdione after gavage and intravenous injection in mice.
**EXPERIMENTAL**

**Chemicals and reagents**

Curdione (purity >98%, batch number must-20051212, Fig. 1) was purchased from Chengdu Manster Pharmaceutical Co., Ltd. (Chengdu, China), midazolam (internal standard, purity ≥98%, batch number FE04082004) was purchased from Merck Co., Ltd (Darmstadt, Germany). Acetonitrile (batch No. 1714630343) and methanol (batch No. 1716507345) were purchased from Merck Co., Ltd. (Darmstadt, Germany).

**UPLC-MS/MS method**

Xevo TQ-S micro triple quadrupole mass spectrometer and Acquity I-class ultra-high performance liquid chromatography (waters Corp, Milford, Ma, USA) was for sample analysis.

UPLC HSS T3 (2.1 × 100 mm, 1.7 μm) column was used for separation. The column temperature was set at 40°C. The mobile phase was composed of acetonitrile and 0.1% formic acid. The flow rate was set at 0.4 mL min⁻¹ and the elution time was 4 min. The gradient elution procedure: 0–0.2 min, acetonitrile 10%; 0.2–1.2 min, acetonitrile 10%–84%; 1.2–2.0 min, acetonitrile 84%; 2.0–2.5 min, acetonitrile 84%–10%; 2.5–4.0 min, acetonitrile 10%.

The capillary voltage was set at 2.0 kV, the temperature of ion source was 150°C and the temperature of desolvation was 400°C. Quantitative analysis was carried out by MRM in electron spray ionization (ESI) positive mode, curdione m/z 237.40 → 134.99 (cone voltage 36V, collision voltage 14V, Fig. 2) and internal standard m/z 326.20 → 291.4 (cone voltage 30V, collision voltage 25V).

**Preparation of reference solution**

The stock solution of curdione (1.0 mg mL⁻¹) and midazolam (0.1 mg mL⁻¹) was prepared with methanol-water (50:50, v/v). Curdione stock solution was diluted with methanol to prepare a working solution of 50 ng mL⁻¹ of internal standard midazolam.

**Standard curve and quality control samples**

The curdione blood standard curve was prepared by adding curdione working solution into blank mouse blood. The curdione concentrations in mouse blood were 1, 5, 20, 50, 200 and 500 ng mL⁻¹, respectively. The quality control (QC) samples were prepared in the same manner as the blood samples in the standard curve. The three blood concentrations were 4, 40 and 400 ng mL⁻¹.

**Sample handling**

Blood sample (20 μL) was thawed in 1.5 mL tube, then 100 μL acetonitrile (including internal standard 50 ng mL⁻¹) added, and vortex mixing for 1.0 min, and high-speed centrifugation at 13,000 r/min for 10 min. The supernatant 80 μL was taken in the injection bottle, and 2 μL was used for UPLC-MS/MS analysis.

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Fig. 1. Chemical structure of curdione

Fig. 2. Mass spectrum of curdione
Method validation. The selectivity was evaluated by analyzing the blank mouse blood spiked with curdione and internal standard, blank mouse blood, and a mouse sample after oral administration.

A series of standard solutions with different concentrations was prepared (1 ng mL\(^{-1}\)–500 ng mL\(^{-1}\)). Peak area ratios of curdione to internal standard were plotted against analyte concentrations, with a weighting factor of the reciprocal of the concentration (1/x). The lower Limit of quantitation (LLOQ) was defined as the lowest concentration on the calibration curves.

The precision and accuracy were evaluated blood samples at three QC sample concentration levels (4, 40 and 400 ng mL\(^{-1}\)). The precision was determined by measuring QC samples for three consecutive days (n = 6). The accuracy was determined by measuring between the average and real values of QC samples for three consecutive days (n = 6).

The recovery was determined by comparing the measured peak area of QC samples with that of the corresponding standard. The matrix effect was determined by comparing the peak area of the standard solution in treatment blank mouse blood with the that of the corresponding standard solution.

The stability of curdione in mouse blood was studied by analyzing QC samples placed under three storage conditions. Including long-term stability (−20 °C, 30 days), short-term stability (2 h at room temperature), freeze-thaw stability (−20 °C to room temperature) [31].

Pharmacokinetics

ICR mice (male, weight 20–22 g) were obtained from the animal experiment center of Wenzhou Medical University. The rearing conditions of mice were controlled at a temperature of 23°C–25°C, a relative humidity of 50%–60%, and the animal room was kept in the dark for 12 h every day. Before the experiment, the mice were adaptively reared for 7 days, with free access to food and water. Six mice were given intravenously (5 mg kg\(^{-1}\)) and another six mice were given orally (20 mg kg\(^{-1}\)). 20 μL blood samples were taken from the caudal vein of mice into 1.5 mL tube pretreated with heparin at 0.0833, 0.5, 1, 2, 3, 4, 6, 8 and 12 h after administration of curdione, frozen at −20 °C. The blood drug concentration data were analysis by DAS 2.0 software (China Pharmaceutical University) [32].

RESULTS AND DISCUSSION

Method development

The choice of positive and negative mode for ESI was often applied in methodological research. Curdione was more suitable for ESI positive electrode detection with higher sensitivity. The optimization of liquid chromatography conditions, in which the relative chromatographic behavior

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Fig. 3. Ultra-high performance liquid chromatography-mass spectrometry of curdione and internal standard (IS) in mouse blood. (a) a blank mouse blood, (b) a blank mouse blood with curdione and IS, (c) a mouse sample
of chromatographic column and mobile phase plays a decisive role [33–35]. In this experiment, different chromatographic columns, such as BEH C18 and HSS T3, with different mobile phase composition, were tried. The results showed that HSS T3 (2.1 mm × 100 mm, 1.8 μm) under the mobile phase of acetonitrile-0.1% formic acid, the peak time was appropriate, and the peak effect was the best. UPLC-MS/MS was applied to the quantitative detection of curdione in blood, compared with traditional HPLC or GC analysis, it only needed only 4 min to complete the analysis of one blood sample. The sensitivity was high (the LLOQ was 1.0 ng mL⁻¹), and only a small volume of samples was required.

**Method validation.** Figure 3 showed that the retention time of curdione and internal standard were 2.74 and 1.97 min, respectively. No obvious endogenous substances interfere with the detection.

The standard curve equation of curdione in mouse blood was $y = 0.0185x + 0.0135$, $r = 0.9995$. Where $y$ represents the peak area ratio of curdione to internal standard, and $x$ represents the concentration of curdione in blood. The LLOQ of curdione in mouse blood was 1.0 ng mL⁻¹.

The intra-day precision of curdione in the blood of mice was less than 13%, the intra-day precision was less than 15%, the accuracy was 90%–105%, the recovery was >77%, and the matrix effect was 97%–107%, which meets the pharmacokinetic research requirements of curdione (Table 1).

The variation of curdione in the blood of stable was within ±13% and RSD was within 14% at room temperature for 2h, −20 °C for 30 days and in the freeze-thaw stability test, indicating that curdione was stable.

### Pharmacokinetics

The blood concentration time curve of curdione in mice after administration was shown in Fig. 4. The non-compartmental model was used to fit the pharmacokinetic parameters, Table 2. Curdione has a short half-life in mice, indicating that its metabolism was fast. Bioavailability is closely related to drug efficacy, especially for drugs with narrow therapeutic index, small dose, low solubility and emergency use, the change in bioavailability has a particularly serious impact on clinical efficacy [36–38]. When the bioavailability changes from low to high, could lead to poisoning and even life-threatening. On the contrary, it will not achieve the desired curative effect and delay the treatment. The influence of bioavailability should be considered when analyzing the ineffectiveness, inefficiency or poisoning of drug therapy in clinical analysis. At present, there is no literature report on the bioavailability of curdione by UPLC-MS/MS. Considering the cost and operability of human pharmacokinetic experiments, mice were selected as the experimental animal model in this work to preliminarily investigate the bioavailability characteristics of curdione, to provide a reference for the design of clinical experiments. The bioavailability of curdione in mice (20 mg kg⁻¹) was 6.5%, indicating that its oral bioavailability was relatively low.

<table>
<thead>
<tr>
<th>Group</th>
<th>$\text{AUC}_{(0-t)}$ (ng mL⁻¹ h)</th>
<th>$\text{AUC}_{(0-\infty)}$ (ng mL⁻¹ h)</th>
<th>$t_{1/2}$ (h)</th>
<th>$\text{CL}_{z/F}$ (L h⁻¹ kg⁻¹)</th>
<th>$V_{z/F}$ (L kg⁻¹)</th>
<th>$C_{\text{max}}$ (ng mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iv (5 mg kg⁻¹)</td>
<td>91.2±13.7</td>
<td>95.3±15.9</td>
<td>3.5±2.1</td>
<td>53.8±9.5</td>
<td>259.8±124.7</td>
<td>136.1±41.1</td>
</tr>
<tr>
<td>po (20 mg kg⁻¹)</td>
<td>23.8±6.8</td>
<td>25.4±6.8</td>
<td>3.1±1.6</td>
<td>841.9±266.9</td>
<td>3864.8±2515.7</td>
<td>6.4±1.7</td>
</tr>
</tbody>
</table>

Fig. 4. Blood concentration-time curve of curdione after administered intravenously (iv, 5 mg kg⁻¹) and orally (po, 20 mg kg⁻¹) in mice.
CONCLUSION

A UPLC-MS/MS method was established, with MRM in ESI positive ion mode were used to determine the blood curdione concentration of mice after intravenous and oral administration. The results showed that curdione metabolized rapidly in mice and had low oral bioavailability of 6.5%.

ACKNOWLEDGEMENTS

This work was supported by the Young Talent Project of Wenzhou Medical University.

REFERENCE