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# Application of a liquid chromatography–tandem mass spectrometry method to the pharmacokinetics, tissue distribution and excretion studies of *Dactylicapnos scandens* in rats

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ABSTRACT

The herbal ingredients of isocorydine and protopine were isolated from *Dactylicapnos scandens*. This study was aimed at developing a liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS/MS) method to quantify isocorydine and protopine in rat plasma and tissues for pharmacokinetic, tissue distribution and excretion studies. Biological samples were processed with ethyl acetate extraction, and corydaline was chosen as the internal standard (IS). The analytes were separated by a $C_{18}$ column and detected with a triple quadrupole mass spectrometer using positive ion ESI in the multiple reaction monitoring (MRM) mode. The MS/MS ion transitions monitored were $m/z$ 342.0 → 278.9 for isocorydine, 354.1 → 188.0 for protopine and 370.0 → 192.0 for IS, respectively. Excellent linearity was observed over the concentration range between 10 and 3000 ng/mL for isocorydine and 10–300 ng/mL for protopine. The lower limit of quantification (LLOQ) was 10 ng/mL for both isocorydine and protopine. This novel method was rapid, accurate, high sensitive and high selective. It was successfully applied to the pharmacokinetic, tissue distribution and excretion studies of *D. scandens*. These preclinical data of *D. scandens* would be useful for the clinical reference.



## 1. Introduction

*Dactylicapnos scandens* (D. Don) Hutch., a perennial herb, is mainly distributed in NW India, Thailand, Yunnan province and Tibet Autonomous Region of China [1]. *D. scandens*, prepared from the dried tubers of *D. scandens* (D. Don) hutch, was a well-known traditional Chinese medicine (TCM) with the Chinese name "Zijinlong", and has been used for the treatments of hypertension, inflammation, blooding and pain for centuries [2]. A variety of alkaloids have been isolated and identified from *D. scandens* so far, and two major constituents were (+) isocorydine and protopine [3–5]. Despite the successful clinical treatments of isocorydine and protopine, their pharmacokinetic behavior, as well as their tissue distribution and excretion, remained unknown.

Several methods have been developed for simultaneous determination of isocorydine and protopine in the root of *D. scandens* using high-performance liquid chromatography (HPLC) method with UV detection [6,7]. However, the sensitivity of both methods was disappointing, as the lower limit of quantification (LLOQ) was between 0.26 and 0.4870 μg for isocorydine and 0.09 and 0.1722 μg for protopine, and neither of methods was established for the purpose of pharmacokinetic study of *D. scandens*. Therefore, establishing a sensitive method to determine isocorydine and protopine in biological samples has become critical for developing clinical treatment with *D. scandens*. Liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) has been commonly used to investigate the pharmacokinetics of TCM due to its high efficiency, sensitivity and selectivity [8–10]. In our study, a LC–ESI-MS/MS method was established for the simultaneous assay of isocorydine and protopine in rat biological samples and was successfully applied to their pharmacokinetic studies. To the best of our knowledge, it is the first time to report the pharmacokinetic, tissue distribution and excretion profiles of major alkaloids *in D. scandens*.

## 2. Materials and methods

### 2.1. Chemicals and reagents

*D. scandens* was purchased from Yunnan (China). Isocorydine hydrochloride (purity > 98%) was purchased from the

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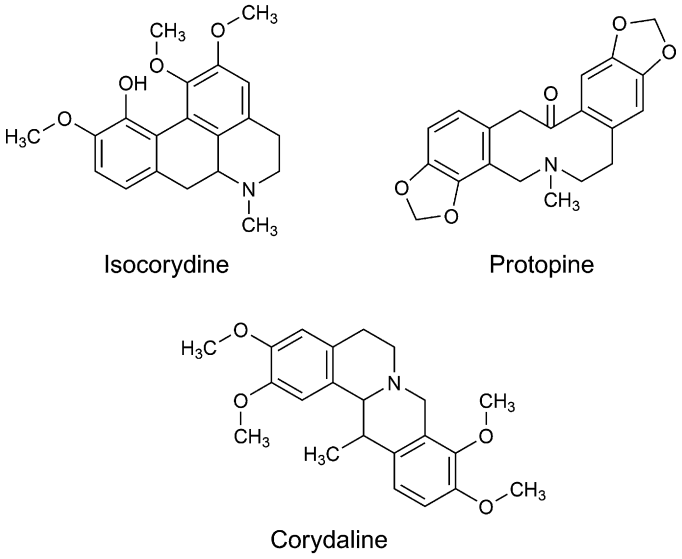


**Fig. 1.** Chemical structures of isocorydine, protopine and IS.

International Laboratory (USA). Protopine (purity > 98%) and corydaline (IS, purity > 98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of isocorydine, protopine and the internal standard (IS) corydaline are shown in Fig. 1. HPLC grade methanol and formic acid were purchased from TEDIA Inc. (USA). Ultra pure ammonium acetate was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Analytical grade ethanol, diethyl ether, dichloromethane and ethyl acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Solutol® HS 15 was purchased from Sigma–Aldrich (USA). Ultrapure water (18.2 MΩ) was obtained from an ELGA–purelab Ultra system (High Wycombe, UK).

### 2.2. Instrumentation

A Waters ACQUITY™ TQD with an ultra performance liquid chromatography (Waters, Milford, MA, USA) was used. Chromatographic separation was achieved on a Zorbax SB–$C_{18}$ (2.1 mm × 100 mm, 3.5 μm; Agilent, USA). Data acquisition and processing were performed using Masslynx 4.1 software and Quanlynx V4.1 (Micromass, Manchester, UK). All centrifugation were performed on an Eppendorf 5415R Refrigerated Microcentrifuge (Eppendorf, Germany).

### 2.3. Animals

Male Sprague–Dawley (SD) rats (200–220 g) were obtained from the Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, China). Animals were bred in a breeding room with temperature at 25 °C, humidity of 50 ± 10%, and a 12 h dark–light cycle. They had free access to water and rodent chow all the time. All the experimental animals were housed under the above conditions for one week for acclimation, and were fasting overnight before experiments. The study was approved by the Animal Ethics Committee of Zhejiang University.

### 2.4. Preparation of D. scandens extract

Decoction pieces of *D. scandens* (66 g) were cold leached for 48 h and ultrasonic extracted at 40 °C for 1 h by 1 L ethanol (95%, v/v). Then the decoction pieces were reflux extracted twice by another 1 L ethanol (95%, v/v) at 85 °C. After filtration, the combined extracting solution was rotary evaporated to dry under reduced pressure to obtain the extract. To calculate the administration dosage, the isocorydine and protopine contents in the extract were determined by HPLC, with the result of 158 and 64.7 mg/g, respectively.

### 2.5. Standard and sample preparation

#### 2.5.1. Preparation of stock and working solutions

The stock solutions of isocorydine (1 mg/mL), protopine (1 mg/mL) and the IS (1 mg/mL) were prepared with methanol, respectively. The stock solution of isocorydine was diluted with water to make a series of working solutions of 0.2, 1, 2, 10, 20, 40 and 60 μg/mL. The stock solution of protopine was diluted with water to make a series of working solutions of 0.2, 0.4, 1, 1.6, 2, 4 and 6 μg/mL. The stock solution of IS was diluted with water to make a 10 μg/mL working solution. Both of the stock and working solutions were kept at 4 °C away from light and brought to room temperature before use.

#### 2.5.2. Preparation of quality control (QC) samples

Series of standard working solutions (10, 50, 100, 500, 1000, 2000 and 3000 ng/mL for isocorydine; 10, 20, 50, 80, 100, 200 and 300 ng/mL for protopine) were prepared by spiking 90 μL blank biological matrix with 5 μL isocorydine and 5 μL protopine working solutions of different concentrations as mentioned above. No IS quality-control (QC) samples at three levels (low: 12 ng/mL for both isocorydine and protopine; medium: 200 ng/mL for isocorydine and 50 ng/mL for protopine; high: 2500 ng/mL for isocorydine and 250 ng/mL for protopine) were also independently prepared in the same way. The calibration working solutions and QC samples were freshly prepared before use.

### 2.6. Sample pretreatment

In the present study, a conventional liquid–liquid extraction (LLE) method was applied to extract isocorydine, protopine and IS from biological samples (plasma, tissue homogenates, urine, feces or bile). Biological samples were taken out from −80 °C storage and thawed at room temperature. Then 10 μL water and 5 μL IS working solution (10 μg/mL) were added to 90 μL biological samples followed by addition of 400 μL ethyl acetate. After vortex for 1 min and centrifugation at 13,000 rpm for 10 min, 300 μL supernatant was collected and evaporated to dryness under vacuum at room temperature. The residue was reconstituted in 100 μL methanol–$H_2O$ (50:50, v/v), followed by vortex for 1 min and centrifuged for 10 min at 13,000 rpm Finally, the supernatant was injected for LC–MS/MS analysis.

### 2.7. Chromatographic conditions

The mobile phase was delivered at a flow rate of 0.3 mL/min using a gradient elution profile consisting of 5 mM ammonium acetate with 0.2% formic acid (A) and methanol with 0.2% formic acid (B). The initial mobile phase composition was A–B (90:10, v/v) with a 1.0 min hold and it was rapidly changed to A–B (10:90, v/v) within 0.2 min, followed by a linear change to A–B (5:95, v/v) over a 1.3 min period, then held for 1.5 min, and finally returned to A–B (90:10, v/v) during 0.5 min and re-equilibrated for 1.5 min

### 2.8. Mass spectrometer conditions

Analytes were detected by MS/MS with an electrospray ionization (ESI)–interface in positive multiple reaction monitoring (MRM) mode. Mass transitions of isocorydine ($m/z$ 342.0 → 278.9), protopine ($m/z$ 354.1 → 188.0) and IS ($m/z$ 370.0 → 192.0) were