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# Metabolic profiles and pharmacokinetics of picroside I in rats by liquid chromatography combined with electrospray ionization tandem mass spectrometry

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ABSTRACT

Picroside I is an iridoid glycoside derived from *Picrorhiza kurroa* Royle ex Benth and *Picrorhiza scrophulariiflora* Pennell and characterized by many biological activities. In this study, a fast, selective, and sensitive UHPLC-MS/MS method was developed and validated to determine picroside I in rat plasma. Analytes were separated by using an ACQUITY UPLC® BEH C18 (2.1 × 50 mm, 1.7 μm) column at a running time of 2 min. Selected reaction monitoring (SRM) transitions were *m/z* 491.1 → 147.1 for picroside I and *m/z* 511.1 → 235.1 for the internal standard in a negative ion mode. The established UHPLC-MS/MS method achieved good linearity for picroside I within the range of 0.1–500 ng/mL. The validated method was successfully applied for the pharmacokinetic analysis of picroside I in rats after oral administration. Fifteen metabolites of picroside I were tentatively identified through ultra-high-performance chromatography/tandem quadrupole time-of-flight mass spectrometry, and four metabolites were identified by comparing with the standards. Besides, nine of these metabolites were discovered for the first time. The proposed metabolic pathways of picroside I *in vivo* can be divided into four parts, namely, phase I reaction of picroside I, including hydroxylation and deoxygenation; phase II reaction of picroside I, including glucuronidation, sulfation, and methylation; phase I biotransformations of metabolites, such as reduction and hydroxylation; and phase II biotransformations of metabolites, such as glucuronidation and sulfation. These results could offer insights into the effectiveness and toxicity of picroside I.

## 1. Introduction

Picroside I is an iridoid glycoside that can be isolated from *Picrorhiza kurroa* Royle ex Benth and *Picrorhiza scrophulariiflora* Pennell [1, 2]. Picroliv, which is a hepatic protectant found in India, contains approximately 60% picroside I and picroside II at a 1:1.5 ratio [3, 4]. Picroliv can protect the liver against galactosamine-, carbon tetrachloride-, thioacetamide-, and aflatoxin $B_1$-induced damage [5–8]. The antioxidant and stabilizing actions on the cell membranes of hepatocytes are main reason for the hepatoprotective activity of picroliv [9, 10]. As the main constituent of picroliv, picroside I exhibits many biological activities, including anti-hepatotoxic [11], anti-oxidant [3], anti-inflammatory [12], anti-tumor [13], and P-glycoprotein stimulation [14]. Moreover, picroside I may enhance the neurite outgrowth stimulated by basic fibroblast growth factor, staurosporine, or dibutyryl cyclic adenosine monophosphate in PC12D cells through a mitogen-activated protein kinase signaling pathway [15]. Picroside I can be well docked into the activity site of CYP3A4, which is an enzyme that metabolizes phase I drugs [16].

The main aspects of *in vivo* drug action are pharmacokinetics and drug metabolism, which play important roles in drug discovery and development [17]. Compared with pharmacodynamic investigations, limited efforts have been devoted to studying the pharmacokinetics and metabolism of picroside I. A few studies have applied liquid chromatography tandem-mass spectrometry (LC-MS/MS) method to determine picroside I in plasma; however, these studies have been hampered by either a complex sample pretreatment method or a long running time [18, 19]. Therefore, a fast and reliable method must be developed for pharmacokinetic studies on picroside I.

Upadhyay et al. [20] found eight picroside I metabolites, including picroside I-glucuronide, -sulfate, -hydroxylated products, in rat liver microsomes and hepatocyte cultures. However, they utilized low-resolution mass spectrometry and thus failed to provide information on mass fragmentation because of technical limitations. Recent

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investigations on drug metabolism have widely involved an ultra-high-performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry (UHPLC-Q/TOF-MS/MS) system, which can obtain the accurate tandem mass spectra of drug metabolites and is a useful tool for structural confirmation [21]. The high-resolution mass spectrometry component of this system allows a reliable identification of metabolites *in vivo*.

In this study, a simple, fast, selective, and sensitive LC-MS/MS method was developed and validated to detect picroside I in rat plasma. The established method was successfully applied to pharmacokinetically analyze picroside I orally administered to rats. Picroside I was also subjected to metabolic studies *in vivo* through high-resolution mass spectrometry for the first time. Fifteen metabolites of picroside I were tentatively identified in rat bile, urine, serum, and feces through UHPLC-Q/TOF-MS/MS. The structures of these metabolites were confirmed by the obtained tandem mass spectra. The proposed metabolic pathways of picroside I *in vivo* contained phases I and II reaction metabolites of picroside I and product-related metabolites.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Picroside I and picroside II (internal standard [IS], structure shown in Fig. 1) with 98% purity were purchased from Nanjing GOREN Bio-Technology Co., Ltd. (Nanjing, China). Catalpol (purity > 96%), cinnamic acid (purity > 99%), 3-phenylpropionic acid (purity > 99%) and *p*-coumaric acid (purity > 98%) were obtained from Sigma-Aldrich Co., LLC (St. Louis, MO, USA). LC-MS-grade acetonitrile, methanol, and formic acid were procured from Sigma-Aldrich Co., LLC. Water was acquired from a Milli-Q Advantage A10 purification system (Millipore Corporation, Billerica, MA, USA). All other chemicals were of analytical grade.

### 2.2. Animals and sampling

Male Wistar rats (7–8 weeks old, 240 ± 10 g) were obtained from the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine (Shanghai, China). The rats were kept under controlled conditions (room temperature, 22 ± 2 °C; relative humidity, 60 ± 5%) at a 12 h/12 h day/night cycle for 7 days before the experiments. All of the animal experiments were approved by the Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine. The rats were subjected to fasting overnight before the experiments and given free access to water.

Six rats were used for pharmacokinetic analysis. After picroside I (dissolved in normal saline) was orally administered at a dose of 100 mg/kg, blood samples (0.2 mL) were collected in heparin tubes at 0, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, and 12 h. The blood samples were then centrifuged at 5000 ×*g* for 10 min to obtain the plasma, and the plasma samples were stored at −80 °C until analysis.

The serum, bile, urine, and fecal samples of six rats were collected for metabolic analysis. The rats were orally administrated with picroside I at a dose of 100 mg/kg. Blood samples (200 μL) were collected through coagulation-promoting tubes at 0, 0.167, 0.5, 1, 2, 4, 8, and 12 h post-dosing. The blood samples were then centrifuged at 5000 ×*g* for 10 min to obtain the serum. The urine and fecal samples were collected at 12 h pre-dosing and at 0–24 h post-dosing in metabolic cages. After the rats were anesthetized by 25% urethane (intraperitoneal injection, 4 mL/kg), the bile samples were collected at 6 h pre-dosing and at 0–12 h post-dosing. These samples were stored at −80 °C until analysis.

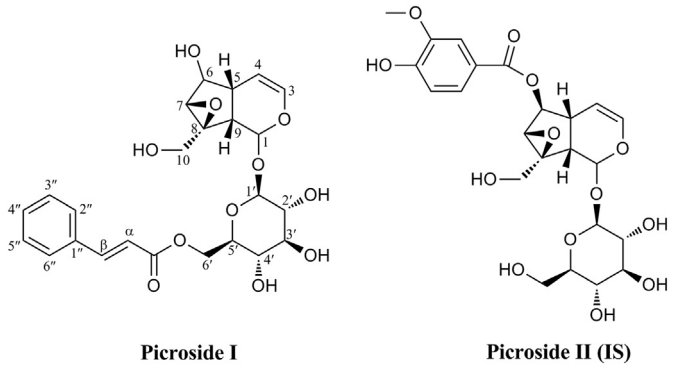


**Fig. 1.** Chemical structures of picroside I and picroside II (IS).

### 2.3. Pharmacokinetic analysis

#### 2.3.1. LC-MS/MS conditions

This study used an LC-MS/MS system, namely, a Nexera X2 UHPLC system (Shimadzu Co., Kyoto, Japan) coupled with an AB SCIEX QTRAP® 6500 mass spectrometer (Redwood City, CA, USA). The analytes were separated by using an ACQUITY UPLC® BEH C18 (2.1 × 50 mm, 1.7 μm) column (Waters, Dublin, Ireland) at 40 °C. The guard column (ACQUITY UPLC® BEH C18) was placed before the inlet of the analytical column. The mobile phases consisted of (A) water and (B) acetonitrile. The gradient elution was as follows: 18% → 28% B at 0–0.8 min, 28% → 60% B at 0.8–1.2 min, 60% → 90% B at 1.2–1.6 min, 90% → 2% B at 1.6–2 min. The flow rate was set at 0.4 mL/min. The auto-sampler tray was maintained at 10 °C, and the sample injection volume was 5 μL.

The mass spectrometer was operated under negative electrospray ionization (ESI) mode. The MS conditions were set as follows: gas (nitrogen) temperature, 550 °C; curtain gas, 30 psi; ion source voltage, −4500 V; collisionally activated dissociation, medium level; and ion source gas 1 and 2, 55 psi. The SRM transitions (Table S1) were *m/z* 491.1 → 147.1 for picroside I and *m/z* 511.1 → 235.1 for the IS. Data were acquired and analyzed using Analyst 1.6.3 (AB SCIEX LLC.).

#### 2.3.2. Sample pretreatment

In this procedure, 50 μL of IS solution (100 ng/mL) was added to 50 μL of each plasma sample and mixed for 20 s. Afterward, 200 μL of acetonitrile was added to the mixture and vortexed for 1 min to precipitate the protein. The mixture was centrifuged at 15000 ×*g* for 10 min, and the clear supernatant was dried under $N_2$ gas. The residue was re-dissolved in 50 μL of 20% acetonitrile and centrifuged at 15000 ×*g* for 10 min. Finally, 5 μL of the solution was injected into the LC-MS/MS system.

#### 2.3.3. Method validation

The established method was validated for selectivity, linearity, accuracy, precision, extraction recovery, matrix effects, and stability in accordance with the USFDA guidelines [22].

The selectivity of this method was assessed by comparing the chromatograms of blank plasma samples from six rats, blank plasma samples from rats spiked with picroside I and the IS, and plasma samples from rats orally administered with 100 mg/kg picroside I.

Calibration curves were prepared by spiking the blank plasma samples with a standard solution to obtain the concentration curves equal to 0.1, 1, 5, 25, 50, 100, 200, and 500 ng/mL of picroside I and 100 ng/mL of the IS. The calibration curves were calculated by plotting the peak area ratio of the analyte to the IS (*y*) *versus* the nominal analyte concentrations (*x*) and evaluated by a weighted ($1/x^2$) least square regression. The curves should have a correlation coefficient (*r*) of at least > 0.99. The LLOQ was defined as the lowest concentration of the analyte whose signal-to-noise (S/N) ratio was > 10:1, which should be determined at an accuracy of ± 20% and a precision of < 15%. Carry-over was assessed by injecting blank samples after highest calibration standard in six replicates.