

# Plasma Pharmacokinetics and Tissue Distribution of Arctiin and Its Main Metabolite in Rats by HPLC-UV and LC-MS

## Authors

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## Key words

- arctiin
- arctigenin
- HPLC-MS
- pharmacokinetics
- tissue distribution
- *Arctium lappa*
- Compositae

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## Abstract

▼  
 The pharmacokinetic profile of arctiin, the major active lignan in fruits of *Arctium lappa* L., was investigated. Its main metabolite arctigenin was identified by an LC-MS method, and an HPLC-UV technique was developed for the simultaneous quantification of the metabolite and arctiin in plasma and organs. Chromatographic separation was performed on an Agilent™ C<sub>18</sub> HPLC column with acetonitrile and water by linear gradient elution. Arctiin and arctigenin were identified online by LC-MS. The pharmacokinetics and tissue distribution of arctiin and arctigenin were determined for the first time by using a simple, selective, and accurate HPLC method. The  $AUC_{0-t}$  values of arctigenin were larger compared with arctiin after oral administration of arctiin. The concentration of the metabolite was significantly higher than the concentration of arctiin in the stomach

and small intestine in rats after oral administration of arctiin, indicating that the stomach and small intestine were the major organs of arctiin metabolism. These findings could provide support for the clinical studies conducted with *Fructus Arctii*.

## Abbreviations

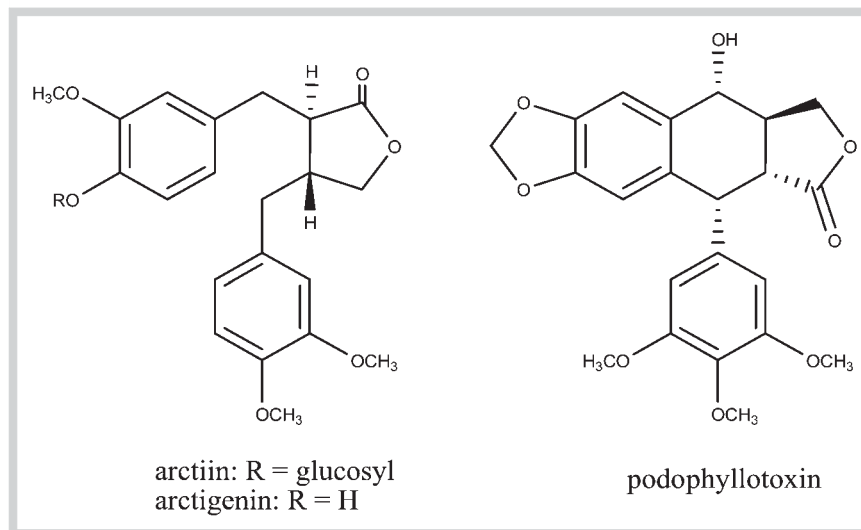
▼  
 TCM: traditional Chinese medicine  
 QC: quality control  
 AUC: area under the plasma level/time curve  
 AIC: Akaike's information criterion

**Supporting information** available online at <http://www.thieme-connect.de/ejournals/toc/plantamedica>

## Introduction

▼  
 Fructus Arctii, a generally-used traditional Chinese medicine prepared from *Arctium lappa* (Compositae), is used for the treatment of colds, throat irritation, mumps, measles, sores, eczema, and cancer. Arctiin, one of the major lignans in Fructus Arctii, has been reported to have enhancing immunological function, and to act as an anti-inflammatory [1], platelet activating factor (PAF) antagonist [2], Ca<sup>2+</sup> antagonist, and antihypertensive [3,4]. In recent studies, it has been shown that arctiin had antitumour activity [5–7]. In addition, it was reported that arctiin could be metabolised into arctigenin by human intestinal bacteria [8], and arctigenin showed, like arctiin, various pharmacological actions in *in vitro* and *in vivo* studies [5–7]. Thus, it is essential to understand the physiological disposition of arctiin.

However, information about arctiin and its metabolite's pharmacokinetic and distribution *in vivo*, which is very important for new drug discovery, has not been dealt with up to now. In order to explore the potential of arctiin as a therapeutic agent, it is necessary to further study the *in vivo* pharmacokinetic and distribution characteristics of arctiin and its metabolite. To our knowledge, there are several methods for determination of arctiin and arctigenin in the plant or extract, such as HPLC with UV [9], FD [10], ESI/MS [11], and MS/MS [12]. Up to now, the pharmacokinetic and tissue distribution of arctiin and arctigenin have not been simultaneously characterised. The purpose of this study was to develop a sensitive, simple, and accurate HPLC method to simultaneously determine the concentrations of arctiin and arctigenin in rat plasma and tissues and to investigate both the



**Fig. 1** Chemical structure of arctiin, arctigenin, and podophyllotoxin.

pharmacokinetic parameters and tissue distribution of arctiin and arctigenin after oral administration of arctiin.

## Material and Methods

### Chemicals and reagents

The fruits of *Arctium lappa* L. were purchased from the Medical Material Co. of the Liaoning province of Shenyang, China in September 2009, and identified by Dr. Wang Bing, Liaoning University of Traditional Chinese Medicine. A voucher specimen (M 518) is deposited at the Herbarium of the Department of Pharmacy, Liaoning University of Traditional Chinese Medicine, China. The purities of arctiin and arctigenin were determined to be up to 98% by HPLC. Podophyllotoxin (>98%), which was used as the internal standard, was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific. The purified water was prepared using a water purification system (Mili-Q Biocel).

### Preparation of arctiin and arctigenin

An aliquot of Fructus Arctii (100 g) was extracted with 80% EtOH (3 × 600 mL, 1 h each). After filtration, the filtrate was evaporated at reduced pressure to afford 12 g of crude residue. Then, the residue was subjected on a column chromatography over silica gel (200–300 mesh, 4 × 30 cm), eluting with CHCl<sub>3</sub> (3 L) and CHCl<sub>3</sub>:MeOH (20:1, 5 L). Fractions with pure arctiin were collected by TLC guidance. After recrystallization, arctiin (4 g) and arctigenin (0.7 g) were yielded, and their structures were further identified by UV, IR, NMR, and HPLC compared with the data in reference [13]. The chemical structures of arctiin and arctigenin are shown in **Fig. 1**.

### Animals

Adult male Sprague-Dawley (SD) rats (purchased from Sippr-BK Experimental Animal Ltd Co., Grade II, Certificate No. 00800161386, n = 36) weighing 200 ± 20 g were used. All of the animals were housed in an air-conditioned room (temperature, 25 °C; relative humidity, 55%) and allowed to freely access food and water. The rats were kept in a breeding room to be acclimated for 4 days before use. At the end of the experiment, pento-

barbital sodium was used for euthanasia of the animals. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). The study protocol was approved by the ethics regulations of the Liaoning University of TCM (131/2010).

### Instrumentation and chromatographic conditions

The metabolites were identified by LC-MS/MS. A Waters Alliance 2695–996 system (Waters Co.) was coupled on-line to a Finnigan TSQ (Thermo Fisher Scientific Inc.) mass spectrometer equipped with an ESI source. The AutoMS operation parameters were as follows: positive ion mode (ESI<sup>+</sup>); nebuliser, 40 psi; auxiliary gas, 20 psi; electrospray voltage, 4.5 kV; capillary temperature, 320 °C; and mass range, 50–1000 m/z.

An Agilent 1100 HPLC system (Agilent) equipped with a quaternary pump, an online degasser, a column-heating assembly, and a UV detector were used for the quantitative determinations. The separation was performed on an Agilent™ C<sub>18</sub> HPLC column (150 mm × 4.6 mm, particle size 5 μm; Agilent Technologies). The mobile phases consisted of acetonitrile (A) and water (B). The applied gradient elution was as follows: solution A–B held at 20:80 (v/v) at first; then was changed linearly to 25:75 (v/v) from 0 min to 12 min, followed by a linear gradient to a solution of 35:65 (v/v) in 14 min. The flow rate was set to 1.0 mL/min throughout the elution; the temperature was set to 30 °C, and the wavelength was set at 280 nm. The injection volume was 20 μL. The chromatographic run time for each analysis was 25.0 min.

### Preparation of stocks, calibration samples, and quality control samples

The stock solutions were prepared by dissolving 5.50 mg of arctiin, 4.14 mg of arctigenin, and 5.36 mg IS in 5 mL methanol, respectively. A series of mixture standard working solutions with concentrations of 0.55, 2.75, 5.50, 11.0, 16.5, 22.0, and 27.5 μg/mL for arctiin and 0.166, 0.828, 1.656, 3.312, 4.968, 6.624, and 8.28 μg/mL for arctigenin were obtained by diluting the mixture of the stock standard solutions with methanol. The IS working solution was prepared by diluting the IS stock solution with methanol. All solutions were stored at 4 °C.

### Sample preparation

Plasma samples (200  $\mu$ L) and tissue homogenates (0.2 g) were spiked with 50  $\mu$ L IS, and the mixtures were extracted with 1000  $\mu$ L acetonitrile by vortex mixing for 3 min. After centrifugation at 4000  $\times$  g for 5 min, the solution was transferred to a polypropylene tube and dried under nitrogen gas at room temperature. The plasma and tissue residue were reconstituted in 50  $\mu$ L and 100  $\mu$ L of methanol, respectively. The injection volume was 20  $\mu$ L for analysis.

### Method validation

The validation has been performed according FDA guidelines.

**Linearity and quantification:** The method was fully validated for its specificity, linearity, lower limits of detection (LLOD), lower limits of quantification (LLOQ), accuracy, and precision. The LLOD was determined during evaluation of the linear range of the calibration curve and is defined as the lowest concentration level resulting in a signal-to-noise ratio of 3 : 1. The LLOQ was determined as the lowest concentration of the analyte in rat plasma and tissue that could be quantified with an inter-assay relative standard deviation (% RSD) lower than 20% and with accuracy rates between 80 and 120%.

**Accuracy and precision:** The precision and accuracy of the method was evaluated by analyzing QC samples with different concentrations. The intraday variability was determined by assaying five replicates on the same day, and the inter-day variability was determined by assaying five replicates on three consecutive days. Precision was defined as the coefficient of variation expressed as percentage. The accuracy of these samples was determined by comparing the calculated concentration obtained from the calibration curve with the known concentration.

**Extraction recovery:** Extraction recoveries from rat plasma and tissue were determined at three concentrations by comparing the peak areas extracted from rat plasma and tissue with those of the same quantities added to methanol.

**Stability:** Stability of arctiin and arctigenin in rat plasma and tissue was assessed with QC samples (n = 3) stored at -20 °C for 30 days. Freeze-thaw stability of arctiin and arctigenin in rat plasma and tissue was investigated with QC samples (n = 3) subjected to three freeze/thaw cycles.

### Pharmacokinetic study and tissue distribution

Three groups of SD rats (n = 6, each group) were assigned to receive an arctiin solution by oral administration at the doses of 25, 50, and 100 mg/kg of arctiin, respectively. Serial blood samples (0.4 mL) were obtained via the rats' orbital vein at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 14, 16, 20, 24, 36, and 48 h after administration and were collected into heparinized centrifuge tubes. The blood samples were immediately centrifuged at 4000  $\times$  g for 10 min at room temperature. The plasma samples were analysed by the previously described methods.

Another three groups of SD rats (n = 6, each group) were executed at 0.5, 1.0, and 3.0 h after oral administration of 100 mg/kg arctiin, respectively. The organ tissues (0.2 g) obtained were raised and further perfused with physiological saline to remove residual blood contaminants and were then homogenised. After centrifugation at 4000  $\times$  g for 10 min, the supernatant was treated using the same HPLC analysis method as the plasma.

### Statistical analysis

The plasma and tissue concentrations of arctiin and arctigenin at different time points were evaluated by means of linear regression analysis. All data were calculated using Microsoft Excel 2003 (Microsoft). The relevant pharmacokinetic parameters were calculated using the computer program DAS 2.0 (Chinese Society of Mathematical Pharmacology, Beijing, China) from the Chinese Pharmaceutical Association.

### Supporting information

Detailed data for method validation are available as Supporting Information.

### Results and Discussion

▼ In our study, the plasma samples were analysed by MS in the ESI positive ion mode by obtaining the full-scan mass spectra after direct injection in the mobile phase solution. The sensitive ions in the full-scan positive ion mass spectra were  $[M + Na]^+$  ( $m/z$  557.1) for arctiin,  $[M + H]^+$  ( $m/z$  372.9) and  $[M - H_2O + H]^+$  ( $m/z$  354.8) for arctigenin, and  $[M - H_2O + H]^+$  ( $m/z$  369.9) for podophyllotoxin (IS) (● Fig. 2); thus, arctigenin and arctiin could be detected.

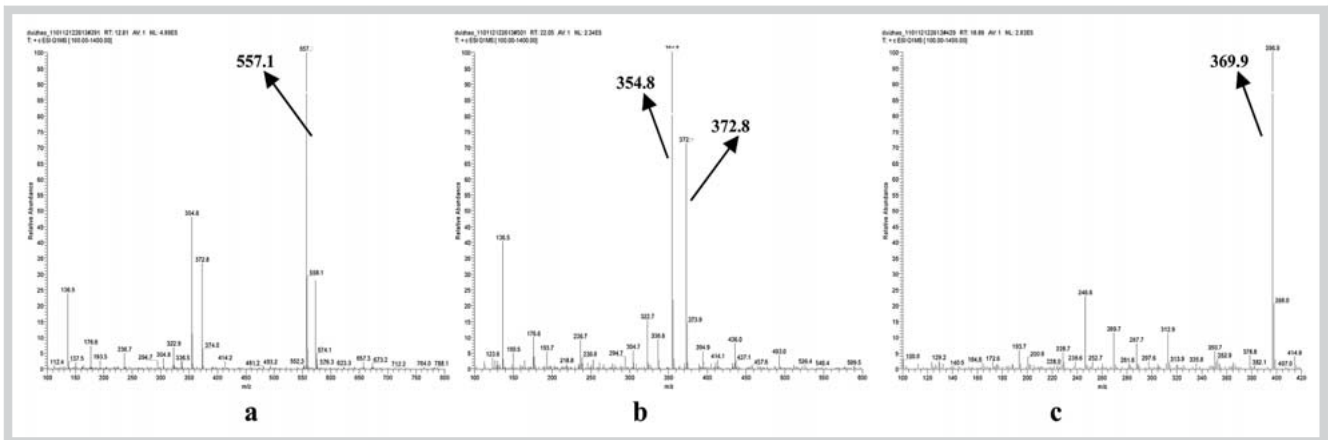
Several organic solvents were tested for precipitating protein and extracting the analytes from rat plasma and organ tissues, including methanol, acetonitrile and methanol-ethyl acetate (1 : 1, v/v). Acetonitrile could well remove proteins and other interfering components in rat plasma and tissue. In addition, 10% salicylsulphonic acid was used to precipitate protein in the experiment. It had a conspicuous effect on precipitating protein; however, it exhibited a bad peak shape. Thus, the plasma and organ samples were prepared by acetonitrile in this study.

The chromatograms of the plasma and organs are shown in ● Fig. 3. Arctiin, podophyllotoxin, and arctigenin were well separated at 12.382, 18.750, and 21.697 min, respectively, with no endogenous interference.

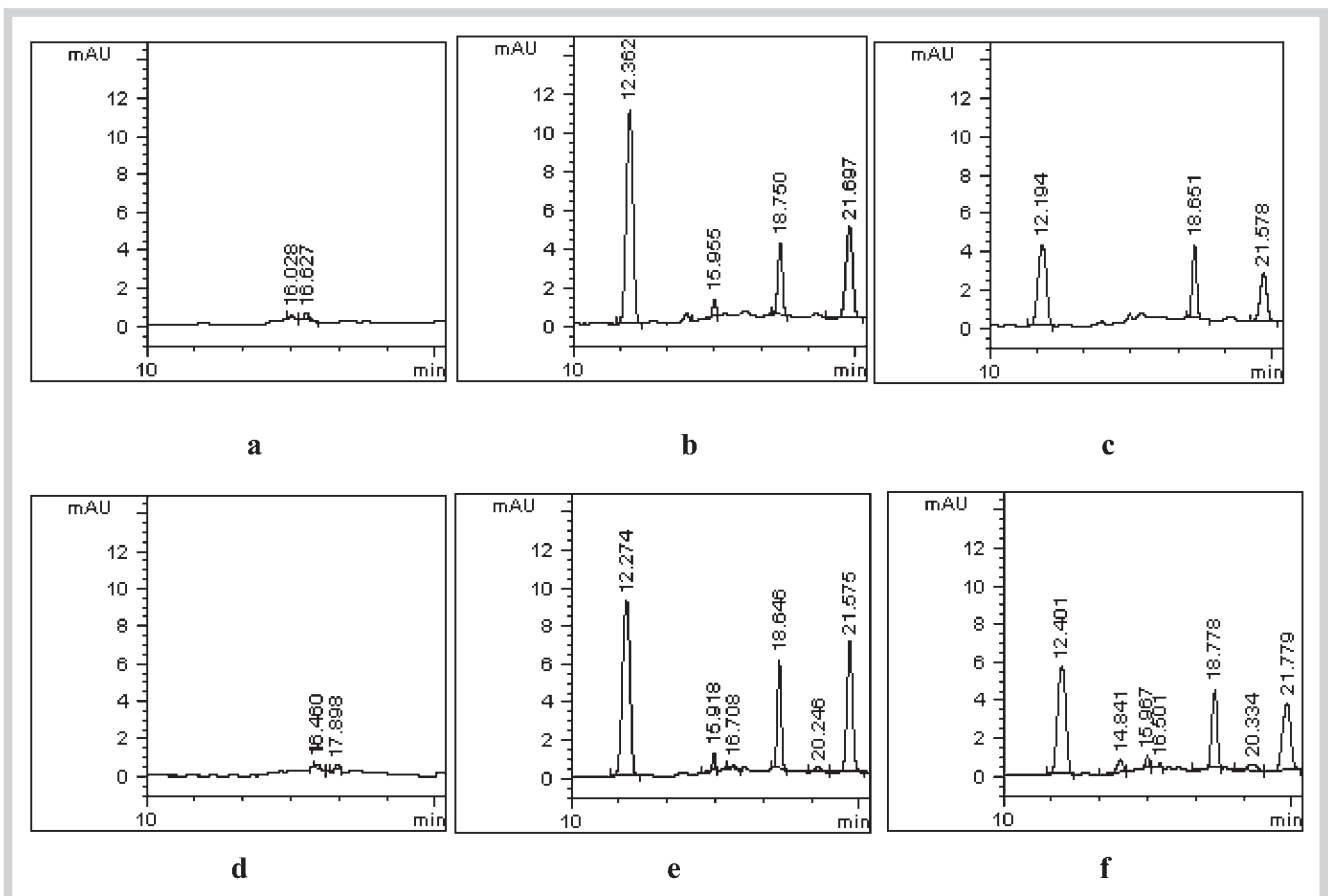
The linear calibration curves were obtained in the given concentration range of arctiin or arctigenin in plasma and tissue samples, respectively. The standard curves were fitted to a first-degree polynomial,  $Y = aX + b$ , where  $Y$  was the peak area of arctiin/IS or arctigenin/IS,  $a$  and  $b$  were constants, and  $X$  was the concentration ( $\mu$ g/mL or  $\mu$ g/g) of arctiin or arctigenin (see Supporting Information). Calibration curves were found to be linear over the calibration range of 0.138–6.88  $\mu$ g/mL for arctiin and 0.041–0.207  $\mu$ g/mL for arctigenin in rat plasma, and 0.128–25.7  $\mu$ g/g for arctiin and 0.166–33.1  $\mu$ g/g for arctigenin in rat tissue. All curves had correlation coefficients of > 0.99. The LLOQ of arctiin and arctigenin were 0.138 and 0.041  $\mu$ g/mL for plasma, and 0.128 and 0.166  $\mu$ g/g for tissue with the RSD < 20%.

The RSD for the intra-day (repeatability) and inter-day precision ranged from 0.530 to 13.1% for the QC standards. The percentage of extraction recoveries of arctiin and arctigenin for the plasma and organ tissue were between 72.4% and 98.9%, respectively (see Supporting Information). These data indicated that the precision of the sample preparation method was satisfactory and resulted in no appreciable matrix effect for arctiin, arctigenin, and IS.

The stability tests were designed by taking into account the anticipated conditions that real samples may experience (see Supporting Information). The RSD of the stability studies were 3.72–13.2%.



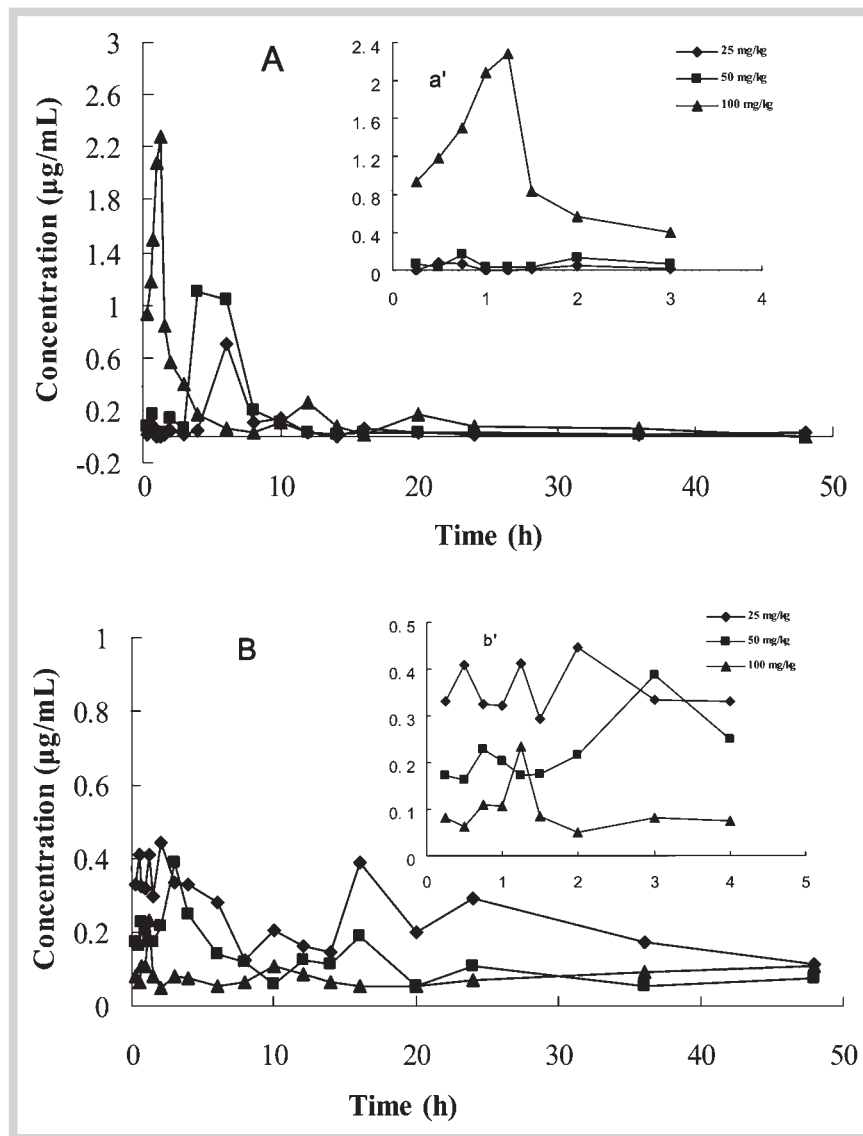
**Fig. 2** Product ion mass spectra of arctiin (a), arctigenin (b), and podophyllotoxin (c).



**Fig. 3** Chromatograms of rat plasma and tissue samples. **a** blank plasma; **b** blank plasma spiked with arctiin, arctigenin, and IS; **c** plasma sample obtained 60 min after oral administration of arctiin at a dose of 100 mg/kg to rat; **d** blank mixed tissue; **e** blank mixed tissue spiked with arctiin, arctigenin, and IS; **f** heart obtained 60 min after oral administration of arctiin at a dose of 100 mg/kg to rat. (Arctiin,  $t_R$  = 12.382 min; IS,  $t_R$  = 18.750 min; arctigenin,  $t_R$  = 21.697 min.)

The method presented here was successfully used to quantify arctiin and arctigenin in rat plasma and organ tissues after oral administration of arctiin. The concentration-time profiles of arctiin and arctigenin are shown in **Fig. 4**. According to the F test and the AIC, a two-compartment pharmacokinetic model fit best to the plasma data of arctiin and arctigenin. The calculated phar-

macokinetic parameters are listed in **Table 1**. After the rats were administered 25, 50, and 100 mg/kg of arctiin, the area of arctiin and arctigenin under the plasma concentration-time curve from 0 h to the time of the last measurable concentration ( $AUC_{0-t}$ ) of the analyte in rats' plasma changed out of proportion to the dosages; the terminal half-life ( $t_{1/2}$ ) did not exhibit a good



**Fig. 4** Mean plasma concentration-time curves of arctiin and arctigenin after oral administration of arctiin to rat at different doses. **A** Arctiin and **B** arctigenin.

**Table 1** Pharmacokinetic parameters for arctiin and arctigenin in SD rats (mean  $\pm$  SD,  $n = 6$ ) after a single oral administration of arctiin.

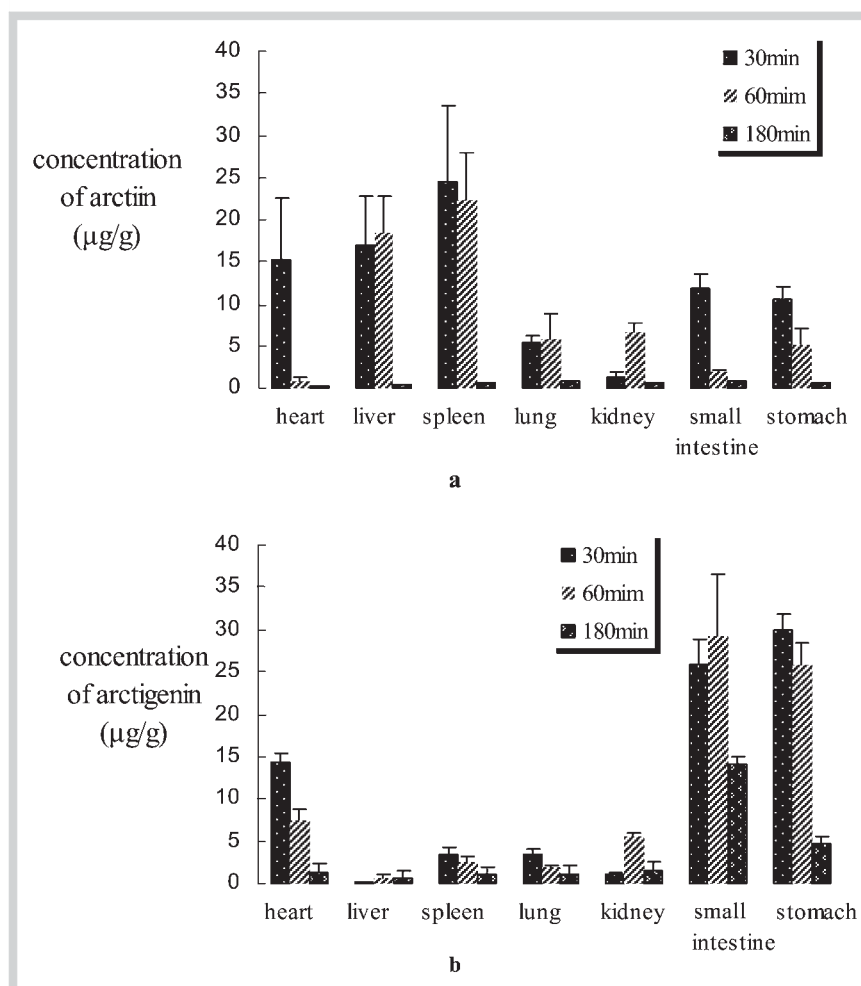
	Dose (mg/kg)	Parameters				
		$T_{max}$ (h)	$C_{max}$ (mg/L)	$T_{1/2}$ (h)	CL (L·kg/h)	$AUC_{0-t}$ (mg·h/L)
Arctiin	25	6.00	0.70 $\pm$ 0.15	2.32 $\pm$ 0.22	16.3 $\pm$ 5.5	3.32 $\pm$ 1.09
	50	4.00	1.59 $\pm$ 0.20	4.70 $\pm$ 1.99	2.70 $\pm$ 0.32	9.34 $\pm$ 1.09
	100	0.83 $\pm$ 0.14	2.50 $\pm$ 1.26	5.18 $\pm$ 1.35	14.6 $\pm$ 3.0	7.07 $\pm$ 1.44
Arctigenin	25	2.67 $\pm$ 1.15	0.64 $\pm$ 0.23	15.9 $\pm$ 1.1	2.12 $\pm$ 0.13	13.6 $\pm$ 5.7
	50	3.50 $\pm$ 0.71	0.65 $\pm$ 0.14	23.5 $\pm$ 1.8	6.50 $\pm$ 0.32	7.70 $\pm$ 0.38
	100	1.00 $\pm$ 0.35	0.23 $\pm$ 0.06	22.3 $\pm$ 1.6	11.8 $\pm$ 0.9	8.46 $\pm$ 0.61

correlation with the administered dose, which suggest that the pharmacokinetics of arctiin and arctigenin in rats obeyed a non-linear processes.

The noncompartmental model was applied to the pharmacokinetic evaluation of arctigenin against the original compound arctiin. Arctiin exhibited a rapid and poor absorption phase followed by a sharp but lasting disappearance. The  $T_{max}$  of three doses for arctigenin were all shorter than for arctiin, suggesting that arctiin may be converted to arctigenin by intestinal bacteria [14] and

arctigenin is absorbed rapidly. The concentration peak values of arctiin were much higher than arctigenin, indicating that arctiin should also be a major compound *in vivo*. The data suggests that arctiin and arctigenin should have pharmacological effects at the same time because there were no significant differences in the  $AUC_{0-t}$  between them.

The tissue concentrations of arctiin and its metabolites determined at 3 time points after oral administration are shown in **Fig. 5**. After the oral administration of arctiin, arctiin and arcti-



**Fig. 5** Contents of arctiin and arctigenin in the organs at different times.

genin were rapidly distributed in all organs in rats. The spleen was the organ where arctiin concentration was the highest after 30 min, followed by the liver, heart, small intestine, stomach, lungs, and kidney. The arctigenin concentration was highest in the stomach and the small intestine, but lowest in the liver, which suggests that arctiin was metabolised in the stomach and the small intestine [15] and not in the liver. Three hours after arctiin administration, the tissue concentrations of the two compounds became markedly reduced in all studied tissues, mainly in the spleen.

Our study is the first evaluation of the plasma pharmacokinetics of arctiin as well as its metabolite arctigenin. Arctiin and arctigenin have been identified in rat plasma by LC-MS and quantified by HPLC-UV. The validated method was simple, fast, reproducible, and suitable for the research of arctiin and arctigenin in rat plasma and organ tissues with podophyllotoxin as the internal standard. The assay utilised an acetonitrile extraction method and a reversed-phase separation with sufficient selectivity and sensitivity. The evaluation of the pharmacokinetics and tissue distribution of arctiin and arctigenin will help further the understanding of their pharmacological activity and clinical use.

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### Conflict of Interest



No conflict of interest exists regarding the submission of this manuscript, and the manuscript was approved by all authors for publication.

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*Please note:* This article was changed according to the following erratum on February 19, 2013: The first names and the last names of all the authors were mistakenly swapped. The authors must read: Fan He, De-Qiang Dou, Yu Sun, Lin Zhu, Hong-Bin Xiao, Ting-Guo Kang