

# Determination and Pharmacokinetic Comparisons of Atractylodin after Oral Administration of Crude and Processed *Atractylodis rhizoma*

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## ABSTRACT

**Background:** In traditional Chinese medicine, *Atractylodis rhizoma* is the dried rhizome of *Atractylodes lancea* (Thunb.) DC. or *Atractylodes chinensis* (DC.) Koidz. After being processed, the dryness of *A. rhizoma* decreased, and the function of tonifying spleen increased. Therefore, the processed *A. rhizoma* is the best choice of clinical application. As the main active components, polyethylene alkynes exhibits various desirable pharmacological effects including anti-inflammatory, anti-bacterial and anti-arrhythmia activity. However, there is no report on the pharmacokinetic comparisons of atractylodin, one of polyethylene alkynes, in bio-samples after oral administration of crude and processed *A. rhizoma* until now. The *in vivo* study of active components of *A. rhizoma* would be necessary and helpful for clinical application and clarification of processing principle.

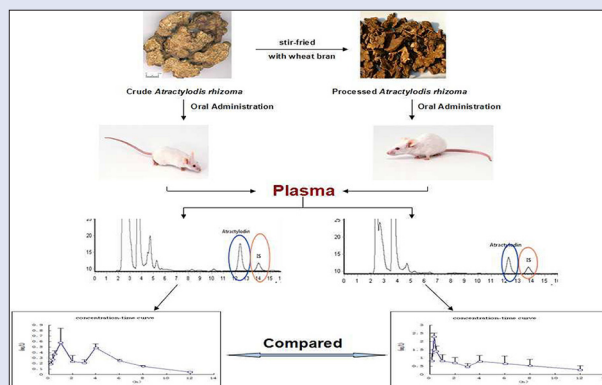
**Objective:** To compare the pharmacokinetic parameters of atractylodin after oral administration of crude and processed *A. rhizoma*, and clarify the processing principle of *A. rhizoma*. **Materials and Methods:** Plasma concentrations of atractylodin in rats were determined by reversed-phase high-performance liquid chromatogram and the main pharmacokinetic parameters were estimated with Drug and Statistics 2.0 Software Package (Chinese Pharmacological Society, Shanghai, China). **Results:** The  $AUC_{0-12}$ ,  $AUC_{0-\infty}$ ,  $T_{max}$  and  $C_{max}$  of processed *A. rhizoma* were increased significantly ( $P < 0.05$ ) compared with that in crude *A. rhizoma* after using Student's *t*-test. **Conclusions:** Processing *A. rhizoma* with wheat bran by stir-frying can promote and accelerate the absorption of atractylodin.

**Key words:** Atractylodin, *Atractylodis rhizoma*, crude and processed, pharmacokinetics, polyethylene alkynes

## SUMMARY

- In this paper, a RP-HPLC method with UV detection for quantification of atractylodin (a main active component in *Atractylodis Rhizoma*) in rat plasma has been developed and applied to a preliminary pharmacokinetic study of atrac-

tylodin after oral administration of crude and processed *Atractylodis Rhizoma* respectively. The result indicates that processing *Atractylodis Rhizoma* with wheat-bran can promote and accelerate the absorption of atractylodin.



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## INTRODUCTION

In China, *Atractylodis rhizoma*, the dried rhizome of *Atractylodes lancea* (Thunb.) DC. or *Atractylodes chinensis* (DC.) Koidz., is widely used for the treatment of rheumatic diseases, digestive disorders, mild diarrhea, and influenza.<sup>[1]</sup> In clinic, *A. rhizoma* is often processed by stir-frying with wheat bran with the aim of reducing its dryness and increasing the function of tonifying spleen.<sup>[2,3]</sup> In order to clarify the influence of processing on pharmacological properties of *A. rhizoma*, an investigation was carried out to compare the pharmacokinetics of typical constituent after oral administration of crude *A. rhizoma* and processed ones. A simple, rapid and sensitive high-performance liquid chromatogram (HPLC) with ultraviolet (UV) detection was developed and validated for the determination of atractylodin in rat plasma. Atractylodin is one of polyethylene alkyne components.<sup>[4,5]</sup> Some literature reported HPLC method for determination its content in *A. rhizoma*.<sup>[6-8]</sup> There is no report on a pharmacokinetic study of atractylodin after oral administration of crude and processed *A. rhizoma* in animals or in human until now. The *in vivo* study of atractylodin, an active components of *A. rhizoma*, would be necessary

and helpful for further clinical application and explanation the processing mechanism.

## MATERIALS AND METHODS

### Materials and reagents

*A. rhizoma* was identified by Professor LiFeng (Liaoning University of TCM) according to the standards of Chinese Pharmacopoeia 2010. The processed *A. rhizoma* comes from the same batch *A. rhizoma* and were

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stir-fried with wheat bran. The herb was stored in a cool and dry place. Atractylodin (purity, 98%) was supplied by National Institute for Food and Drug Control (Beijing, China). The IS called physcion (purity, 98%) was supplied by the national institute for food and drug control (Beijing, China). The chemical structures of atractylodin and IS are shown in Figure 1. HPLC grade acetonitrile was purchased from Fisher Scientific Company (New Jersey, USA), pure water was supplied by Wahaha Company (Hangzhou, China). Analytical grade ethanol and chloroform were from Baierdi Company (Beijing, China).

### Liquid chromatographic condition

The liquid chromatographic (LC) system consisted of an LC-10AD pump (Shimadzu, Kyoto, Japan) with a 20  $\mu$ L loop (Cotata, CA, USA) and an SPD-10 A ultraviolet-visible detector (Shimadzu, Kyoto, Japan). A LC-10AD workstation was used for data acquisition. A Diamonsil C<sub>18</sub> analytical column (250 mm  $\times$  4.6 mm; 5  $\mu$ m) from Dikma Technologies (Dalian, China) was used. The mobile phase consisted of acetonitrile and water (76:24, v/v), and it was delivered with the flow rate of 1 mL/min. The detection wavelength was set at 340 nm. All the measurements were performed at 25°C, and the sample injection volume was 20  $\mu$ L.

### Preparation of *Atractylodes rhizoma* ethanol extract

*A. rhizoma* (50 g) was crushed into powder and soaked into 600 mL of 95% ethanol for 24 h and then percolated at 2 mL/min. Ethanol was evaporated to near dryness under reduced pressure to get the residue. Distilled water was added to the residue and then vortexed. The final concentration of *A. rhizoma* solution was 2 g/mL.<sup>[9]</sup> The sample was stored in dry and dark place before use.

### Animals

All the studies on animals were in accordance with the Guidelines for the Care and Use of Laboratory Animals. Healthy Sprague-Dawley rats (250  $\pm$  20 g) were purchased from The Medical University of Benxi (Benxi, China) and acclimated in the laboratory for 1-week before to the experiments. The rats were maintained in an air-conditioned animal quarter at a temperature of 22°C  $\pm$  2°C and a relative humidity of 50%  $\pm$  10%. Rats for oral ingestion were fasted for 12 h with free access to water. Rats were oral administration crude and processed *A. rhizoma* at a single dose of 40 g/kg (The concentration of the extracted sample solution was 2 g/mL), respectively.

### In vivo pharmacokinetic study of atractylodin

#### Drug administration and blood sampling

For plasma samples, 18 rats were randomly assigned to three groups for pharmacokinetic investigation ( $n = 6$ /group). The blood sample (0.3 mL) was collected at 0, 0.17, 0.33, 0.5, 1, 2, 3, 4, 6, 8, and 12 h. All samples were immediately transferred into heparinized tubes and centrifuged for 5 min at 10,000 rpm. The supernatant was stored at -20°C and dark place until analysis.

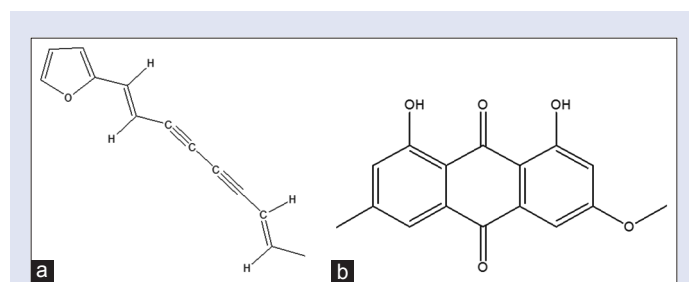


Figure 1: Chemical structures of (a) atractylodin and (b) physcion

### Preparation of plasma sample

For plasma samples, the 200  $\mu$ L of rat plasma was mixed with 20  $\mu$ L IS. After the protein was precipitated with 1000  $\mu$ L of acetonitrile in 1.5 mL polypropylene tube by vortexing for 2 min, the sample was centrifuged at 10,000 rpm for 5 min. The supernatant was transferred into a 5.0 mL tube and was added with 1000  $\mu$ L of chloroform, extract and the under organic phase was transferred to another tube and evaporated to dryness at 40°C with nitrogen. The resulting extract was dissolved in 50  $\mu$ L of methanol, and vortex mixed for 2 min. After centrifugation at 10,000 rpm for 5 min, 20  $\mu$ L supernatant was injected for analysis.<sup>[10]</sup> For each time, 20  $\mu$ L was injected into HPLC system. Then, the concentration of atractylodin was determined.

### Method validation

#### Specificity

The selectivity of the method was demonstrated by comparing chromatograms of blank plasma samples (without IS) obtained from rats, plasma samples spiked with the analytes and IS, and plasma samples after an oral dose. All blank plasma samples were prepared and analyzed to ensure the absence of interfering peaks.

#### Calibration curve

The standard curve in the plasma of atractylodin was linear in the range from 0.029 to 5.800  $\mu$ g/mL.

#### Recovery and stability

The extraction recoveries of atractylodin were determined at the low, medium, and high level of quality control (QC) samples. Recoveries were calculated by comparing the observed peak area ratios in bio-samples to those nonprocessed standard solutions at the same.

The stability of atractylodin in plasma was determined under different storage or handling conditions. Short-term stability was assessed by analyzing QC samples kept at room temperature for 8 h. Freeze-thaw stability was evaluated at three consecutive freeze-thaw cycles. Long-term stability was studied by assaying samples following a period of 10-day of storage at -20°C.

#### Precision

The precision of the method were assessed by determination of quality control samples ( $n = 5$ ) on three different validation days. To determine intra-day precision, the assays were carried out on standard solutions of the analyte at different times during the same day. Inter-day precision was determined by assaying the standard solutions of the analyte over three consecutive days.

### Data analyses

Data were processed by noncompartmental method using Drug and Statistics 2.0 Software Package (Chinese Pharmacological Society, Shanghai, China).

## RESULTS

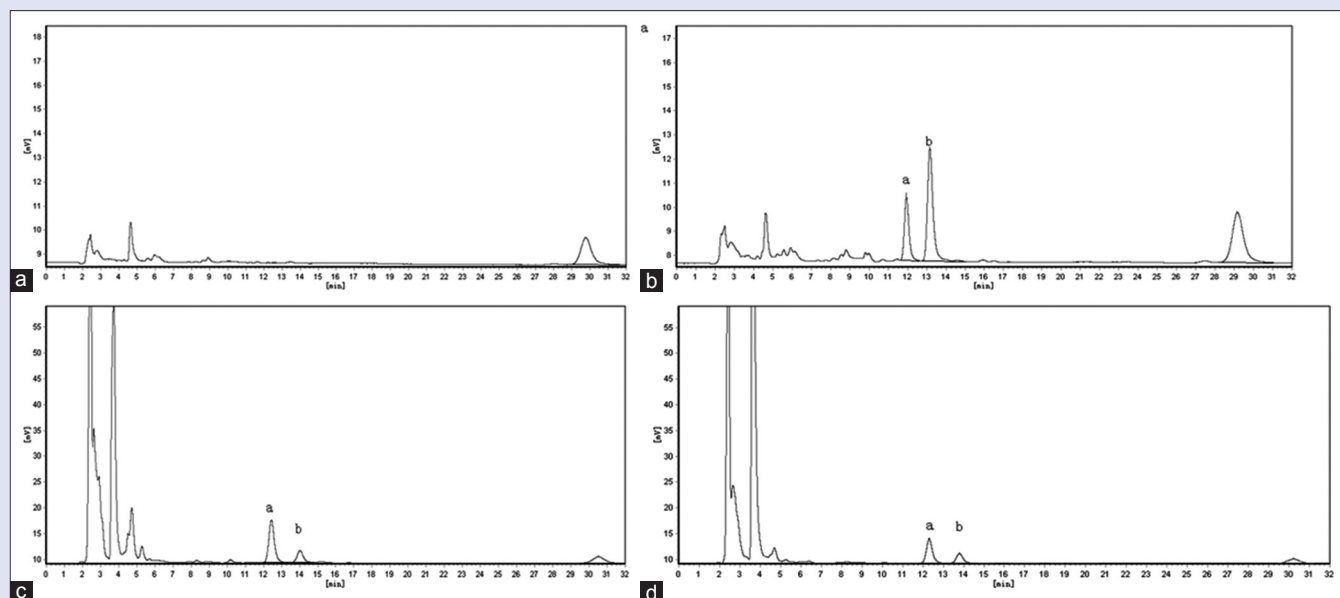
### Method validation

#### Specificity

Figure 2 shows that no interference peaks from endogenous constituents were detected.

#### Linearity of calibration curve

The calibration curves were linear in the determined concentration ranges. And the detection limited for analyses in the range 0.029–5.800  $\mu$ g/ml for atractylodin with a correlation coefficient at 0.9932. The mean standard curves were typically described by the equations:  $Y = 1.292X + 0.033$  for atractylodin.



**Figure 2:** Chromatograms of blank plasma (a); blank plasma spiked with atractylodin 20  $\mu$ L and IS 20  $\mu$ L (b); rat plasma sample (1-h) after oral administration of crude *A. rhizoma* 40 g/kg (c); rat plasma sample (1-h) after oral administration of processed *A. rhizoma* 40 g/kg (d)

### Recovery and stability

The recoveries of atractylodin at each quality control level 2.900, 0.580, 0.029  $\mu$ g/mL were  $83.39\% \pm 6.97\%$ ,  $80.92\% \pm 7.17\%$ , and  $81.71\% \pm 4.82\%$ , respectively. The extraction recoveries determined for atractylodin were consistent and reproducible. Stability of analysis showed no significant sample loss over 12 h at room temperature, three freeze-thaw cycles, and 10 days storage condition. The RE of three conditions was within  $\pm 15\%$ .

### Precision and accuracy

The precision data for atractylodin was given in Table 1.

### Pharmacokinetics study

Plasma concentrations of atractylodin were determined after oral administration of crude and processed *A. rhizoma*. The pharmacokinetic parameters were given in Table 2. As shown in Figures 3 and 4, Table 2, there were significant differences of  $C_{max}$ ,  $T_{max}$ ,  $AUC_{0 \rightarrow t}$ ,  $AUC_{0 \rightarrow \infty}$  and CL.

## DISCUSSION

The assay was applied to a preliminary pharmacokinetic experiment in the rat after oral administration of 40 g/kg crude and processed *A. rhizoma* to rats, respectively. Mean concentration-time curves were shown in Figures 3 and 4. The pharmacokinetic parameters were shown in Table 2.

A significant result of this study was found that atractylodin showed double peaks after oral administration, which demonstrated that a hepatoenteral circulation may exist. For after oral crude *A. rhizoma* the absorption peaks in rat plasma at 1.0 h and 4.0 h, respectively. The  $C_{max}$  was  $0.625 \pm 0.234$  mg/L. And for after oral processed *A. rhizoma* the absorption peaks at 0.34 h and 4.0 h, respectively. The  $C_{max}$  is  $2.299 \pm 0.225$  mg/L. So, the time of absorption peak was 0.67 h in advanced and the concentration of rat plasma was increased after processing. The value of  $T_{max}$  and  $T_{1/2}$  indicated that the atractylodin was rapidly distributed but slowly eliminated. The reason for this result also requires further study.

**Table 1:** The precision data of atractylodin

	Intra-day			Inter-day		
	Measured concentration (mg/L)	Accuracy (%)	Precision RSD (%)	Measured concentration (mg/L)	Accuracy (%)	Precision RSD (%)
2.900	2.826 $\pm$ 0.0517	97.45	1.83	92.724 $\pm$ 0.068	92.72	2.53
0.580	0.550 $\pm$ 0.0154	94.83	2.71	0.557 $\pm$ 0.013	95.95	2.26
0.029	0.027 $\pm$ 0.0007	93.10	2.62	0.026 $\pm$ 0.001	91.26	3.83

RSD: Relative standard deviation

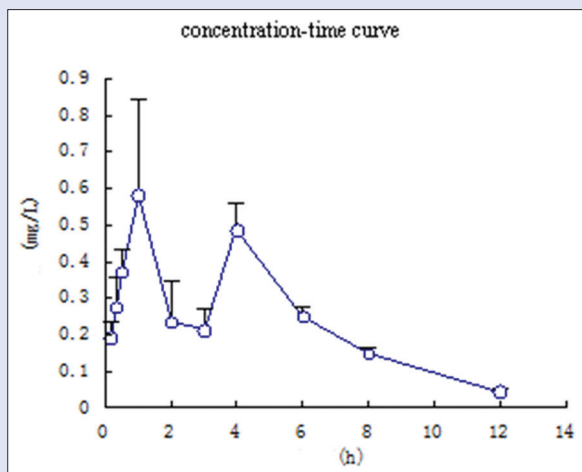
**Table 2:** The pharmacokinetic parameters of atractylodin of crude and processed *Atractylodis rhizoma* at a dose of 40 g/kg to rats, respectively

Parameters	Crude	Processed
$AUC_{(0-t)}$ (mg/L $\times$ h)	2.856 $\pm$ 0.212	7.497 $\pm$ 3.431
$AUC_{(0-\infty)}$ (mg/L $\times$ h)	3.013 $\pm$ 0.250	11.504 $\pm$ 7.254
MRT $_{(0-t)}$ (h)	4.278 $\pm$ 0.220	4.517 $\pm$ 1.056
MRT $_{(0-\infty)}$ (h)	4.836 $\pm$ 0.386	7.0672 $\pm$ 3.238
CLz/F	6.676 $\pm$ 0.569	2.568 $\pm$ 1.727
$t_{1/2z}$ (h)	2.387 $\pm$ 0.361	5.123 $\pm$ 1.875
$T_{max}$ (h)	2.200 $\pm$ 1.643	0.334 $\pm$ 0.000
$C_{max}$ (mg/L)	0.625 $\pm$ 0.234	2.299 $\pm$ 0.225

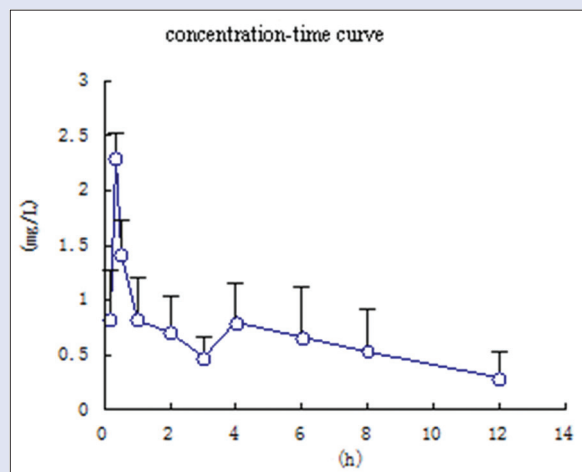
AUC: Area under concentration-time curve; MRT: Mean resident times;  $t_{1/2}$ : Half-life;  $T_{max}$ : Time for maximum of drug concentration;  $C_{max}$ : Maximum of drug concentration

## CONCLUSION

A simple, specific and rapid reversed phase-HPLC method with UV detection for quantification of atractylodin in rat plasma has been developed for the first time. It has been successfully applied to a preliminary pharmacokinetic study of atractylodin after oral administration of 40 g/kg crude and processed *A. rhizoma* respectively. The data have a significant difference ( $P < 0.05$ ). The result indicates that processing *A. rhizoma* with wheat bran by stir-frying can promote and accelerate the absorption of atractylodin.



**Figure 3:** Mean plasma concentration-time curve of atractylodin after oral administration of crude *Atractylodis rhizoma* (40 g/kg) to rats. (Mean  $\pm$  standard deviation,  $n = 6$ )



**Figure 4:** Mean plasma concentration-time curve of atractylodin after oral administration of processed *Atractylodis rhizoma* (40 g/kg) to rats. (Mean  $\pm$  standard deviation,  $n = 6$ )

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## Conflicts of interest

There are no conflicts of interest.

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