ABSTRACT

Background: Atractylodis rhizoma is one of the most often used drugs in traditional Chinese medicine. Stir frying with wheat bran is the most common processing method. To clarify the principle of processing, an experiment was carried out to compare the tissue distribution of typical constituent after oral administration of raw A. rhizoma and processed ones.

Objective: To compare the tissues distribution of atractylodin after oral administration of raw and processed A. rhizoma and clarify the processing principle of A. rhizoma.

Materials and Methods: High-performance liquid chromatogram with ultraviolet detection was developed and validated for the determination of atractylodin in rat tissues.

Results: The atractylodin in raw and processed A. rhizoma was distributed in all tissues involved in this study.

Conclusions: The concentration of atractylodin in it is the highest in the stomach and small intestine.

Key words: Atractylodin, Atractylodis rhizoma, crude and processed, tissue distribution

SUMMARY

In this paper, a simple, specific, and rapid reversed phase-high-performance liquid chromatogram method with ultraviolet detection for quantification of atractylodin in rat tissue has been developed for the first time. The result indicates that the concentration of atractylodin in it is the highest in the stomach and small intestine.

Abbreviations used: IS: Internal standard substance; A. Rhizoma: Atractylodis rhizoma; RSD: Relative standard deviation; HPLC: High performance liquid chromatography.

INTRODUCTION

Atractyloidis rhizoma (Chinese name Cang Zhu) is the dried rhizome of Atractylodes lancea (Thunb.) DC. or Atractylodes chinensis (DC.) Koidz, which was first recorded in the ancient pharmaceutical book “Shen Nong Ben Cao Jing” and has been used since antiquity to treat rheumatic diseases, digestive disorders, mild diarrhea, and influenza. 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. It has been reported that A. rhizoma contains rich of essential oil including sesquiterpenes and polyethylene alkynes which are the...
main active components. They are the main active components. Recent
researches have shown that polyethylene alkyne exhibits various desirable
pharmacological effects including anti-inflammatory, antibacterial, and
antiarrhythmic activity.[6]

Atractylodin is one of the polyethylene alkyne components and main
bioactive components in *A. rhizoma*. In previous researches, our
group has studied the pharmacokinetics of atractylodin after oral
administration of raw and processed *A. rhizoma*. The research in
this paper is the continuation of previous research and to study the
tissue distribution of atractylodin after oral administration of raw and
processed *A. rhizoma*. This paper developed a simple high-performance
liquid chromatogram (HPLC) method to determine the concentration
of atractylodin in rat tissues and to compare the concentration in different
tissues after oral administration of raw and processed *A. rhizoma*, which
clarified preliminary the influence of processing on tissue distribution of
*A. rhizoma*. [9-11]

**MATERIALS AND METHODS**

Materials and reagents

*A. rhizoma* (purchased from Herb Planting Base, Hubei, Luotian,
China) was identified by Professor Li Feng (Liaoning University of
TCM) according to the standards of Chinese Pharmacopoeia 2010.
The processed *A. rhizoma* comes from the same batch *A. rhizoma*.
The herb was stored in a cool and dry place. Atractylodin (purity,
98%) was supplied by the National Institute for Food and Drug
control (Beijing, China). The Internal standard substance (IS) called
physcion (purity, 98%) was supplied by the National Institute for Food
and Drug control (Beijing, China). Pure water was supplied by Wahaha
Company (Hangzhou, China). HPLC grade acetonitrile was purchased
from Fisher Scientific Company (New Jersey, USA). Analytical grade
ethanol and chloroform were from Baierdi Company (Beijing, China).
High-speed homogenizer was purchased from Putian Instrument
Manufacturing Co., Ltd. (Changzhou, China).

High-performance liquid chromatography

condition

The HPLC system consisted of an LC-10AD Pump, SPD-10A UV
spectrophotometric detector (Shimadzu, Kyoto, Japan) with a
20 μL loop (Cotati, CA, USA), and a workstation for data collection.
Separation was performed on a Diamonsil C18 analytical column
(5 μm, 250 mm × 4.6 mm) from Dikma Technologies (Beijing, China).
The mobile phase consisted of acetonitrile and water (75:25, v/v). The
detection wavelength was set at 340 nm and flow rate was 1 mL/min.
All the measurements were performed at 25°C, and the sample injection
volume was 20 μL.

Preparation of Atractylodis rhizoma solution

*A. rhizoma* (50 g) was crushed into powder and soaked into 600 mL
of 95% ethanol for 24h and then percolated at 2 mL/min. After
evacuating the solvent under reduced pressure, the residue was
redissolved in water and then vortexed. The final concentration of
*A. rhizoma* solution was 2 g/mL. The sample was stored in dry
and dark place before use.

Processed *A. rhizoma* (50 g) was prepared with same method.

Animals

Healthy cleaning grade Sprague-Dawley rats (290 g ± 10 g) were
purchased from the Animal Center of Benxi Chang Sheng Biotechnology
Co. Ltd., (Benxi, China) and conventionally raised a week before the
experiment. The rats were maintained in an air-conditioned animal
quarter at a temperature of 22°C ± 2°C, humidity of 50% ±10%, and
12-h light/dark cycle. Rats were deprived of food overnight before the
experiment but were allowed free access to water. All experiments were
conducted in accordance with the Guidelines for the Care and Use of
Laboratory Animals.

In tissue distribution study of atractylodin

Drug administration and tissue sampling

For tissue sample, 45 rats were divided into nine groups (*n* = 5 per group)
randomly. Rats were oral administration raw and processed *A. rhizoma*
at a single dose of 20 g/kg. Heart, liver, spleen, lung, kidney, stomach,
small intestine, and large intestine were collected at 10 min, 1.5, 4, and
8 h. Tissue samples were weighed 0.2 g rapidly, rinsed with physiological
saline to remove the blood or content, blotted on filter paper, and then
stored at −20°C and dark place before analysis.

Preparation of tissue sample

For tissue sample, each weighed tissue sample was thawed and then
homogenized in ice-cold physiological saline (1 mL). Then, a 200 μL
tissue homogenate (homogenate time 10 s/time, gap 30 s, 3–5 times,
ice water bath) was taken and mixed with 20 μL IS. After protein was
precipitated with 1000 μL of acetonitrile in 1.5 mL polypropylene tube
by vortexing for 2 min, the sample was centrifuged at 8910 ×g for 5 min.
The supernatant was transferred into a 5.0 mL tube and added with 1000
μL of chloroform, extract and the under organic phase was transferred
to another tube and evaporated to dryness at 40°C with nitrogen.
The residue was reconstituted in 50 μL methanol, and centrifugation at
8910 ×g for 5 min, 20 μL supernatant was injected into HPLC system
for analysis.

Method validation

Specificity

The selectivity of the method was demonstrated by comparing
chromatograms of tissue homogenate spiked with the analytes and IS,
tissue homogenate after an oral dose. All blank tissue homogenates were
prepared and analyzed to ensure the absence of interfering peaks.

Calibration curve

The linearity of the method was assessed by plotting calibration curves in
tissue homogenate at three concentration levels. The calibration curves
were linear over the concentration range of 0.029–5.80 μg/mL in tissue
homogenates of atractylodin.

Recovery

The recoveries of atractylodin were determined at low, medium, and
high level of sample. The data indicated that the biosample preparation
procedure was satisfied and can achieve the acceptable extraction
recovery.

Stability

The stock solution stabilities for the atractylodin and the IS and short-term
stability were assessed by analyzing samples kept at room temperature
for 1 h and 24 h, respectively. Long-term stability was studied by assaying
samples following a period of 30-day of storage at −20°C.

Precision

Intraday precision was evaluated by analysis of the five samples with three
determinations per concentration at the same day. Interday precision
was determined by assaying the standard solutions of the analysis over 3
consecutive days.
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Data analyses
HPLC analysis procedure was applied to analyze tissue distribution of atractylodin.

RESULTS
Method validation

Specificity
Figure 1 shows that no interference peaks from endogenous constituents were detected.

Linearity of calibration curve
The calibration curves were linear over the concentration range of 0.029–5.80 μg/mL in tissue homogenates of atractylodin. The correlation coefficient values of the calibration curve were over 0.9900. Typical linear regression equations and correlation coefficients in each tissue are listed in Table 1.

Recovery and stability
The extraction recoveries of atractylodin ranged from 82.402% to 89.744% in tissue samples. The data are listed in Table 2. The stock solution stabilities for the analyte and IS did not significant differences. The data are listed in Table 3.

Stability of analysis showed no significant sample loss over 24 h at room temperature, three freeze-thaw cycles, and 30 days storage condition. The RSD of three conditions was within ± 15%. The data are listed in Table 4.

Table 1: The linear regression analysis of atracylodin in rat tissue

| Tissues     | Standard curves | r      | Linear range(μg/mL) |
|-------------|-----------------|--------|---------------------|
| Heart       | Y=2.846X+0.102  | 0.9955 | 0.029–5.80          |
| Liver       | Y=3.732X+0.207  | 0.9908 | 0.029–5.80          |
| Spleen      | Y=3.175X-0.140  | 0.9950 | 0.029–5.80          |
| Lung        | Y=2.965X-0.055  | 0.993  | 0.029–5.80          |
| Kidney      | Y=2.286X+0.055  | 0.9943 | 0.029–5.80          |
| Large intestine | Y=2.135X+0.428 | 0.9902 | 0.029–5.80          |
| Small intestine | Y=3.737X-0.013 | 0.9966 | 0.029–5.80          |
| Stomach     | Y=2.700X+0.048  | 0.9932 | 0.029–5.80          |

Table 2: The recovery of atractylodin in rat tissue (n=3)

| Concentration (μg/mL) | Mean(%) | RSD (%) |
|-----------------------|---------|---------|
| Heart                 |         |         |
| 0.029                 | 85.635  | 6.996   |
| 0.580                 | 82.402  | 3.945   |
| 5.800                 | 89.744  | 2.111   |
| Liver                 |         |         |
| 0.029                 | 83.266  | 3.742   |
| 0.580                 | 85.913  | 2.136   |
| 5.800                 | 85.419  | 5.174   |
| Spleen                |         |         |
| 0.029                 | 85.462  | 3.393   |
| 0.580                 | 85.924  | 2.242   |
| 5.800                 | 88.027  | 3.265   |
| Lung                  |         |         |
| 0.029                 | 87.884  | 1.923   |
| 0.580                 | 85.419  | 4.327   |
| 5.800                 | 85.202  | 3.005   |
| Kidney                |         |         |
| 0.029                 | 85.613  | 3.485   |
| 0.580                 | 85.064  | 2.453   |
| 5.800                 | 84.435  | 1.019   |
| Large intestine       |         |         |
| 0.029                 | 86.054  | 3.459   |
| 0.580                 | 85.949  | 3.944   |
| 5.800                 | 87.814  | 1.832   |
| Small intestine       |         |         |
| 0.029                 | 83.529  | 1.476   |
| 0.580                 | 87.015  | 3.810   |
| 5.800                 | 88.195  | 2.841   |
| Stomach               |         |         |
| 0.029                 | 83.788  | 2.390   |
| 0.580                 | 85.394  | 2.992   |

Figure 1: (a) Chromatograms of blank tissue homogenate. (b) Blank tissue homogenate with atractylodin 20 μL and IS 20 μL. (c) Spleen sample (1.5 h) after oral administration of raw Atractylodis rhizoma 20 g/kg. (d) Spleen sample (1.5 h) after oral administration of processed Atractylodis rhizoma 20 g/kg
The concentration-time profile of atractylodin after oral administration of 20 g/kg raw and processed Atractylodes rhizoma at a dose of 20 g/kg to rats.

### Table 3: The stock solution stabilities for atractylodin and the IS (n=6)

| Time (h)/Peak area (A) | 0 | 4 | 8 | 12 | 24 | 48 | Mean | RSD% |
|------------------------|---|---|---|----|----|----|------|------|
| Atractylodin           | 8803.1 | 8811.5 | 8790.2 | 8781.1 | 8799.4 | 8791.3 | 8796.1 | 0.12 |
| IS                     | 21745 | 21721 | 21733 | 21716 | 21672 | 21659 | 21708 | 0.15 |

### Table 4: The stability of atractylodin in rat tissue (n=5)

| Tissue | RSD (%) |
|--------|---------|
| Heart  | 0.029 μg/mL | 0.580 μg/mL | 5.800 μg/mL |
| Liver  | 7.002 | 1.730 | 2.504 |
| Spleen | 6.170 | 2.007 | 2.357 |
| Lung   | 3.752 | 1.361 | 2.316 |
| Kidney | 3.232 | 1.913 | 1.626 |
| Large intestine | 4.156 | 2.445 | 2.106 |
| Small intestine | 3.980 | 1.386 | 1.307 |
| Stomach | 4.337 | 1.375 | 2.067 |

### Table 5: The tissue concentrations of atractylodin after oral administration raw Atractylodis rhizoma at a dose of 20 g/kg to rats (n=5)

| Tissue (μg/g) | 10 min | 1.5h | 4h | 8h | Mean | RSD % |
|---------------|--------|------|----|----|------|-------|
| Heart         | 283.781 | 253.131 | 202.018 | 183.150 | 183.150 | 183.150 |
| Liver         | 1137.556 | 692.906 | 209.169 | 728.843 | 728.843 | 728.843 |
| Spleen        | 326.318 | 245.568 | 601.706 | 164.200 | 164.200 | 164.200 |
| Lung          | 585.987 | 502.812 | 410.743 | 1428.825 | 1428.825 | 1428.825 |
| Kidney        | 153.743 | 291.956 | 359.706 | 138.693 | 138.693 | 138.693 |
| Large intestine | 232.512 | 749.400 | 290.031 | 560.663 | 560.663 | 560.663 |
| Small intestine | 2249.675 | 527.906 | 462.938 | 318.243 | 318.243 | 318.243 |
| Stomach       | 4717.862 | 4446.906 | 3436.469 | 2516.869 | 2516.869 | 2516.869 |

### Table 6: The tissue concentrations of atractylodin after oral administration processed Atractylodis rhizoma at a dose of 20 g/kg to rats (n=5)

| Tissue (μg/g) | 10 min | 1.5h | 4h | 8h | Mean | RSD % |
|---------------|--------|------|----|----|------|-------|
| Heart         | 216.056 | 112.825 | 102.350 | 180.987 | 180.987 | 180.987 |
| Liver         | 1143.706 | 271.150 | 201.331 | 305.993 | 305.993 | 305.993 |
| Spleen        | 307.350 | 210.393 | 505.693 | 242.443 | 242.443 | 242.443 |
| Lung          | 193.087 | 129.656 | 220.818 | 14.938 | 14.938 | 14.938 |
| Kidney        | 189.712 | 169.575 | 172.568 | 211.493 | 211.493 | 211.493 |
| Large intestine | 59.743 | 287.413 | 227.181 | 556.219 | 556.219 | 556.219 |
| Small intestine | 1285.381 | 406.888 | 557.825 | 482.981 | 482.981 | 482.981 |
| Stomach       | 4666.162 | 2666.687 | 3168.821 | 1530.475 | 1530.475 | 1530.475 |

### Accuracy and precision

Accuracy was assessed by analyzing six aliquots of low, medium, and high concentration samples. Accuracy of atractylodin in tissues ranged from 85.00% to 96.80%. The precision data for atractylodin were not exceed 5%.

### Tissue distribution study

The tissue distribution of atractylodin determined at 10 min, 1.5, 4, and 8 h after oral administration raw and processed A. rhizoma a dose of 20 g/kg is shown in Tables 5 and 6 and Figures 2, 3.

### DISCUSSION

The assay was applied to a tissue distribution experiment in the rat after oral administration of 20 g/kg raw and processed A. rhizoma, respectively. The tissue distribution was shown in Tables 5 and 6. The atractylodin in raw and processed A. rhizoma was distributed in all tissues, such as heart, liver, spleen, lung, kidney, large intestine, small intestine, and stomach. The concentration of atractylodin in raw and processed A. rhizoma is the highest in the stomach and small intestine which proved that the theory of A. rhizoma can strengthen spleen-stomach and improve its function of digestion and elimination. The concentration of atractylodin in different tissues after oral processed A. rhizoma decreased, the reason needs further research. Atractylodin is one of the main components in volatile oils of A. rhizoma. Volatile oils are both “dry” (side effect) components and active components. After being processed, the content of volatile oils was decreased, so the “dry” effects can be weakened while the therapeutic effects can be improved relatively. In this study, the IS of rhubarb, emodin, and physcion was studied. Finally, the moderate retention time and no interference peaks from endogenous constituents are physcion.

### CONCLUSIONS

A simple, specific, and rapid reversed phase-HPLC method with ultraviolet detection for quantification of atractylodin in rat tissue has been developed for the first time. It has been successfully applied to a tissue distribution study of atractylodin after oral administration of 20 g/kg raw and processed A. rhizoma, respectively. The atractylodin in raw and processed A. rhizoma was distributed in all tissues and the concentration of atractylodin is the highest in the stomach and small intestine. The concentration of atractylodin in processed A. rhizoma decreased, but its relative concentration is higher in the stomach and small intestine than other tissue.

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Nil.
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Conflicts of interest

There are no conflicts of interest.

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Figure 3: The concentration-time profile of atractylodin after oral administration processed Atractylodis rhizoma at a dose of 20 g/kg to rats.