Research Article

Determination of (4E,6E,12E)-Tetradecatriene-8,10-diyne-1,3-diyl Diacetate in Rat Plasma and Tissues by HPLC-UV Method and Their Application to a Pharmacokinetic and Tissue Distribution Study

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In China Atractylodis Rhizoma is widely used for the treatment of rheumatic diseases and digestive disorders. Stir-frying with wheat bran is the most common processing method. In order to clarify the influence of processing on pharmacological properties of Atractylodis Rhizoma, an investigation was carried out to compare the pharmacokinetics and tissue distribution of typical constituent after oral administration of raw Atractylodis Rhizoma and processed ones. A simple, rapid, and sensitive high performance liquid chromatography with UV detection was developed and validated for the determination of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate in rat plasma. A chromatography was carried out on Diamonsil C\textsubscript{18} (250 × 4.6 mm; 5 µm) analytical column, using a mobile phase which consisted of acetonitrile and 0.1% phosphoric acid water (60:40, v/v) at a flow rate of 1.0 mL.min\textsuperscript{-1}. The wavelength was set at 336 nm. The LLOQ of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate was 0.00143 µg·mL\textsuperscript{-1}. Both accuracy and precision were satisfactory. The pharmacokinetic results showed that the $T_{\text{max}}$ was 1 hour in advance and the $C_{\text{max}}$ was increased after processing. Tissue distribution showed that the highest level was in spleen. And the concentrations in the spleen were increased after stir-frying with bran.

1. Introduction

In traditional Chinese medicine, Atractylodis Rhizoma is the dried root and stems from Atractylodes lancea (Thunb.) DC. or Atractylodes chinensis (DC.) Koidz. The medicinal herb is widely known as Cangzhu in China. And it is widely used for the treatment of rheumatic diseases, digestive disorders, mild diarrhea, and influenza [1]. In clinic, Atractylodis Rhizoma is often processed by stir-frying with wheat bran with the aim of reducing its dryness and increasing the function of tonifying spleen [2, 3].

Atractylodis Rhizoma is rich of essential oil including sesquiterpenes and polyethylene alkynes, which are the main active components in this medicine [4]. Recent researches have shown that polyethylene alkynes exhibit various desirable pharmacological effects including anti-inflammatory, antibacterial, and antiarrhythmic activity [5]. (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate is one of polyethylene alkyne components. Some literature reported HPLC method for determination its content in Atractylodis Rhiomzoma [6–8].

However, there have been few methods available for its quantification in biosamples and few reports on its pharmacokinetic study and tissues distribution until now. The in vivo study of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate, an active component of Atractylodis Rhiroma, would be necessary and helpful for further clinical application and explanation of the processing mechanism. The present paper developed a new and simple RP-HP LC method for quantification of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate in rat plasma and tissues after oral administration of raw and processed Atractylodis Rhiroma, respectively. This fully validated method was successfully applied to a pharmacokinetic and tissue distribution study of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate in rats for the first time.
2. Experimental

2.1. Chemicals and Reagents. The (4E,6E,12E)-tetracatriene-8,10-diyne-1,3-diyldiacetate (purity, 98%) was supplied by Traditional Chinese Medicine Standardization Research Center (Shanghai, China). The IS called Emodin (purity, 98%) was supplied by the National Institute for Food and Drug Control (Beijing, China). The chemical structures of (4E,6E,12E)-tetracatriene-8,10-diyne-1,3-diyldiacetate and IS are shown in Figure 1.

HPLC grade acetonitrile was purchased from Fisher Scientific Company (New Jersey, USA) and Pure water was supplied by Wahaha Company (Hangzhou, China). Analytical grade ethanol and chloroform were from Baieri Company (Beijing, China). Atractylodis Rhizoma was identified by Professor Li Feng (Liaoning University of TCM) according to the standards of Chinese Pharmacopoeia 2010. The processed Atractylodis Rhizoma comes from the same batch Atractylodis Rhizoma. The herb was stored in a cool and dry place.

2.2. Preparation of Atractylodis Rhizoma Solution. Atractylodis Rhizoma (50 g) was crushed into powder and soaked into 600 mL of 80% ethanol for 24 h and then percolated at 2 mL min⁻¹, and Ethanol was evaporated to near dryness under reduced pressure to get the residue. Distilled water was added into the residue and then vortexed for 10 min. The final concentration of Atractylodis Rhizoma solution was 2 g mL⁻¹ [9]. The sample was stored in dry and dark place before use.

2.3. Apparatus and HPLC Conditions. The liquid chromatographic system consisted of an LC-10 AD pump (Shimadzu, Kyoto, Japan) with a 20 µL loop (Cotata, CA, USA) and an SPD-10A ultraviolet-visable detector (Shimadzu, Kyoto, Japan). A LC-10 AD workstation was used for data acquisition. A Diamonsil C₁₈ analytical column (250 x 4.6 mm; 5 µm) from Dikma Technologies (Dalian, China) was used. The mobile phase consisted of acetonitrile and 0.1% phosphoric acid water (60:40, v/v) at a flow rate of 1 mL min⁻¹. The detection wavelength was set at 336 nm. All the measurements were performed at room temperature, and the injection volume was 20 µL.

2.4. Preparation of Standard Solution and Quality Control Samples. Stock solutions of (4E,6E,12E)-tetracatriene-8,10-diyne-1,3-diyldiacetate and IS with concentrations of 0.143 mg mL⁻¹ and 0.0504 mg mL⁻¹, respectively, were prepared in methanol and stored at −20°C and dark place until use. When we used the standard solution, the working concentration of (4E,6E,12E)-tetracatriene-8,10-diyne-1,3-diyldiacetate and IS were 286 µg L⁻¹ and 5.04 µg L⁻¹, respectively. Calibration standards of (4E,6E,12E)-tetracatriene-8,10-diyne-1,3-diyldiacetate were prepared by spiking the appropriate amount of the working solutions into 200 µL blank rat plasma or tissue homogenates. To plasma samples, the final concentrations of calibration standard samples were 0.003575, 0.00715, 0.0143, 0.03575, 0.0715, 0.143, and 0.2145 µg mL⁻¹. Quality control (QC) samples were prepared at low, medium, and high concentrations of 0.00357, 0.03575, and 0.0715 µg mL⁻¹. To tissue homogenates, the final concentrations of calibration standard samples were 0.00143, 0.00375, 0.00715, 0.0143, 0.0286, 0.03575, and 0.0715 µg mL⁻¹. Quality control (QC) samples were prepared at low, medium, and high concentrations of 0.00375, 0.03575, and 0.0715 µg mL⁻¹ for tissue homogenates.

2.5. Sample Preparations. For plasma samples, the 200 µL of rat plasma was mixed with 20 µL IS (0.00504 mg mL⁻¹). After protein was precipitated with 1,000 µL of acetonitrile in 1.5 mL polypropylene tube by vortexing for 2 min, the sample was centrifuged at 10,000 rpm min⁻¹ for 5 min. The supernatant was transferred into a 5.0 mL tube and added with 1,000 µL 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 µL of methanol, and vortex mixed for 2 min. After centrifugation at 10,000 rpm min⁻¹ for 5 min, 20 µL supernatant was injected for analysis [10–12]. For tissue homogenate, each weighed tissue sample was thawed and the homogenized in ice-cold physiological saline (2 mL). Then a 200 µL of tissue homogenate was taken and processed further like the plasma samples.

2.6. Method Validation

2.6.1. Specificity. The selectivity of the method was demonstrated by comparing chromatograms of blank plasma samples and tissue homogenate (without IS) obtained from rats and plasma samples and tissue homogenate spiked with the analytes and IS and plasma samples and tissue homogenate.
Figure 2: Chromatograms of blank plasma (a); blank plasma spiked with (4E,6E,12E)-tetradecatriene-8,10-diynyl-1,3-diyldiacetate 20 μL (0.143 μg mL⁻¹) and IS 20 μL (0.00504 mg mL⁻¹) (b); rat plasma sample (4 h) after oral administration of raw Atractylodis Rhizoma 40 g kg⁻¹ (c); rat plasma sample (4 h) after oral administration of processed Atractylodis Rhizoma 40 g kg⁻¹ (d); Chromatograms of blank tissue homogenate (e); blank tissue homogenate with (4E,6E,12E)-tetradecatriene-8,10-diynyl-1,3-diyldiacetate 20 μL (0.286 μg mL⁻¹) and IS 20 μL (0.00504 mg mL⁻¹) (f); spleen sample (2 h) after oral administration of raw Atractylodis Rhizoma 40 g kg⁻¹ (g); spleen sample (2 h) after oral administration of processed Atractylodis Rhizoma 40 g kg⁻¹ (h).

after an oral dose. All blank plasma samples and tissue homogenates were prepared and analyzed to ensure the absence of interfering peaks.

2.6.2. Calibration Procedure. The linearity of the method was assessed by plotting calibration curves in plasma at seven concentration levels in triplicate on three consecutive days. The lower limit of quantification (LLOQ) was defined as the lowest concentration of the calibration curve that was measured with accuracy and precision by analyzing samples in six replicates at the concentration of 0.00143 μg mL⁻¹ for (4E,6E,12E)-tetradecatriene-8,10-diynyl-1,3-diyldiacetate.

2.6.3. Accuracy and Precision. Intraday precision and accuracy were evaluated by analysis of the three QC samples with six determinations per concentration at the same day, whilst the interday precision and accuracy were measured over three consecutive days. The precision was defined as the relative standard deviation (RSD%), while accuracy was determined by calculating the percentage deviation observed in the analysis of QC samples and expressed by relative
2.6.4. Extraction Recovery and Stability. The extraction recoveries of (4E,6E,12E)-tetradeacatriene-8,10-diyne-1,3-diyldiacetate were determined at low, medium, and high level of QC samples. Recoveries were calculated by comparing diacetate were determined at low, medium, and high level recoveries of (4E,6E,12E)-tetradeacatriene-8,10-diyne-1,3-diyldiacetate in plasma and tissues are shown in Figure 2. The retention time of Emodin (IS) was about 16.30 min and (4E,6E,12E)-tetradeacatriene-8,10-diyne-1,3-diyldiacetate was about 30.45 min. It was indicated that analytes and IS were well separated and no interferences were detected from endogenous substances or metabolites.

2.7. Applications in Pharmacokinetic Studies. All the studies on animals were in accordance with the Guidelines for the Care and Use of Laboratory Animals. Healthy Sprague-Dawley rats (250 ± 20 g) were purchased from The Medical University of Dalian (Dalian, China) and acclimated in the laboratory for one week before to the experiments. Rats for oral ingestion were fasted for 12 h with free access to water. Rats were oral administration raw and processed Atractylodis Rhizoma at a single dose of 40 g·kg⁻¹, respectively.

For plasma samples, twelve rats were randomly assigned to two groups for pharmacokinetic investigation (n = 6 per group). The blood sample (0.5 mL) was collected at 0, 0.17, 0.33, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h. All samples were immediately transferred into heparinized tubes and centrifuged for 5 min at 10,000 rpm·min⁻¹. The supernatant was stored at −20°C and dark place until use.

3. Results and Discussion

3.1. Method Validation

3.1.1. Specificity. The representative chromatograms for determination of (4E,6E,12E)-tetradeacatriene-8,10-diyne-1,3-diyldiacetate in plasma and tissues are shown in Figure 2. The retention time of Emodin (IS) was about 16.30 min and (4E,6E,12E)-tetradeacatriene-8,10-diyne-1,3-diyldiacetate was about 30.45 min. It was indicated that analytes and IS were well separated and no interferences were detected from endogenous substances or metabolites.

3.1.2. Linearity of Calibration Curve and Lower Limit of Quantification. The calibration curves were linear over the concentration range of 0.003575–0.2145 μg·mL⁻¹ in rat plasma and 0.00143–0.0715 μg·mL⁻¹ in tissue homogenates by weighted (1/x²) linear least-squares regression method. The correlation coefficient values of the calibration curves were over 0.9900. The RE of the back-calculated values of the standards from their nominal values were constantly within 15% for all values, including the LLOQ. The LLOQ measurement showed the respective averages 0.003575 μg·mL⁻¹ with RSD 7.68% for rat plasma and 0.00143 μg·mL⁻¹ with RSD 8.68% for tissue homogenates. Typical linear regression equations, correlation coefficients in plasma and each tissue were listed in Table 1.

3.1.3. Precision and Accuracy. The intraday and interday precision and accuracy were assessed by analyzing six aliquots of

| Sample         | Calibration curves | r      | Linear range            |
|----------------|--------------------|--------|-------------------------|
| Plasma         | Y = 23.628X + 0.044 | 0.9950 | 0.00361–0.18814         |
| Heart          | Y = 13.259X + 0.031 | 0.9958 | 0.00149–0.07322         |
| Liver          | Y = 14.351X + 0.064 | 0.9951 | 0.00144–0.06925         |
| Spleen         | Y = 12.936X + 0.028 | 0.9951 | 0.00146–0.07431         |
| Lungs          | Y = 14.704X + 0.059 | 0.9956 | 0.00143–0.07872         |
| Kidney         | Y = 12.936X + 0.028 | 0.9951 | 0.00143–0.06716         |
| Stomach        | Y = 15.161X + 0.012 | 0.9964 | 0.00143–0.06786         |
| Large intestine| Y = 11.271X + 0.019 | 0.9952 | 0.00146–0.08179         |
| Small intestine| Y = 12.956X + 0.037 | 0.9985 | 0.00144–0.07339         |
low, medium, and high concentration samples. The intraday precision of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate in rat plasma and tissues ranged between 1.83 and 6.71% with RE of $-14.47\sim-7.81\%$ and the interday precision ranged between 1.57 and 8.68% with RE of $-14.38\sim-5.13\%$.

3.1.4. Recovery and Stability. The extraction recoveries of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate ranged between 1.57 and 8.68% with RE of $-14.47\sim-7.81\%$ and the interday precision ranged between 1.57 and 8.68% with RE of $-14.38\sim-5.13\%$.

The extraction recoveries of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate ranged from 86.6% to 91.6% in plasma and tissue samples and the method recovery ranged from 85.6% to 94.9%, while the recovery of IS was above 80%. These data indicated that the biosample preparation procedure was satisfied and can achieve the acceptable extraction recovery. Stability of analytes 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 were within ±15%.

3.2. Pharmacokinetics of (4E,6E,12E)-Tetradecatriene-8,10-diyne-1,3-diyl Diacetate in Rats. The assay was applied to a preliminary pharmacokinetic experiment after oral administration of 40 g kg$^{-1}$ raw and processed Atractylodis 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 is finding that (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate showed double peaks after oral administration, which demonstrated that a hepatocentral circulation may exist. For raw Atractylodis Rhizoma the absorption peaks in rat plasma was at 2 h and 4 h, respectively and the $C_{\text{max}}$ was 38 ± 24 μg L$^{-1}$. And for processed Atractylodis Rhizoma the absorption peaks was at 1 h and 3 h, respectively and the $C_{\text{max}}$ is 42 ± 17 μg L$^{-1}$. So the time of absorption peak was 1 hour in advance and the concentration of rat plasma was increased after processing. The value of $T_{\text{max}}$ and $T_{1/2}$ indicated that the (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate was rapidly distributed but slowly eliminated. The reason for this result also requires further study.

3.3. Tissue Distribution Study. The tissue concentrations of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate determined at 0.5, 2, 4, and 8 h after oral administration raw and processed Atractylodis Rhizoma at a dose of 40 g kg$^{-1}$ are shown in Table 3 and Figures 5 and 6, which indicated that (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate could be distributed to all collected tissues, for raw Atractylodis Rhizoma, the concentrations of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate in tissue were distributed, followed by spleen, liver, small intestine, heart, stomach, large intestine, kidney, and lungs; for processed Atractylodis Rhizoma, the concentrations of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate in tissue were distributed, followed by spleen, heart, kidney, liver, lungs, stomach, small intestine, large intestine. Relatively, the concentrations of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate of raw and processed Atractylodis Rhizoma was higher in the spleen. And the concentrations in the spleen were increased after stir-frying with bran. The results showed that processing can promote the rate of absorption of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate.

4. Conclusion

A simple, specific, and rapid RP-HPLC method with UV detection for quantification of (4E,6E,12E)-tetradecatriene-8,10-diyne-1,3-diyl diacetate in rat plasma has been developed for the first time. It has been successfully applied
Table 2: The pharmacokinetic parameters of \((4E,6E,12E)\)-tetradecatriene-8,10-diyn-1,3-diyl diacetate of raw and processed \(Atractylodis Rhizoma\) at a dose of 40 g kg\(^{-1}\) to rats, respectively \((n = 6)\).

| Parameters          | Raw            | Processed       |
|---------------------|----------------|-----------------|
| AUC\(0-t\) (mg h L\(^{-1}\)) | 0.260 ± 0.137  | 0.265 ± 0.100   |
| AUC\(0-\infty\) (mg h L\(^{-1}\)) | 0.317 ± 0.157  | 0.303 ± 0.111   |
| MRT\(0-t\) (h)      | 7.706 ± 0.888  | 7.384 ± 0.86    |
| MRT\(0-\infty\) (h) | 14.86 ± 6.813  | 12.22 ± 3.364   |
| \(t_{1/2z}\) (h)    | 9.905 ± 7.382  | 8.047 ± 3.347   |
| \(T_{max}\) (h)     | 2.333 ± 0.816  | 1.000 ± 0.817   |
| \(C_{max}\) (mg L\(^{-1}\)) | 0.038 ± 0.024  | 0.042 ± 0.017   |

Table 3: The tissue concentrations of \((4E,6E,12E)\)-tetradecatriene-8,10-diyn-1,3-diyl diacetate after oral administration raw and processed \(Atractylodis Rhizoma\) at a dose of 40 g kg\(^{-1}\) to rats, respectively \((\mu g/g)\).

| Tissues                  | 0.5 h Raw | 0.5 h Processed | 2 h Raw  | 2 h Processed | 4 h Raw  | 4 h Processed | 8 h Raw  | 8 h Processed |
|--------------------------|-----------|-----------------|----------|---------------|----------|---------------|----------|---------------|
| Heart                    | 125.31    | 137.68          | 128.97   | 244.30        | 77.35    | 61.62         | 35.94    | 30.56         |
| Liver                    | 80.37     | 158.37          | 155.10   | 208.56        | 158.07   | 173.38        | 115.10   | 128.98        |
| Spleen                   | 170.71    | 119.41          | 253.18   | 292.69        | 186.45   | 204.92        | 17.34    | 30.66         |
| Lung                     | 69.35     | 70.01           | 46.54    | 174.26        | 84.79    | 100.26        | 40.20    | 60.61         |
| Kidney                   | 108.76    | 105.54          | 76.15    | 242.21        | 81.83    | 84.78         | 24.51    | 43.17         |
| Stomach                  | 112.31    | 107.95          | 119.77   | 122.96        | 65.22    | 78.53         | 43.17    | 59.93         |
| Large Intestine          | 20.33     | 30.68           | 79.02    | 54.78         | 112.25   | 143.34        | 166.77   | 176.64        |
| Small Intestine          | 91.25     | 106.29          | 139.04   | 115.53        | 191.22   | 159.02        | 44.91    | 54.78         |

Figure 5: The concentration-time profile of \((4E,6E,12E)\)-tetradecatriene-8,10-diyn-1,3-diyl diacetate in tissues after oral administration of raw \(Atractylodis Rhizoma\) at a dose of 40 g kg\(^{-1}\) to rats \((n = 6)\).

Figure 6: The concentration-time profile of \((4E,6E,12E)\)-tetradecatriene-8,10-diyn-1,3-diyl diacetate in tissues after oral administration of processed \(Atractylodis Rhizoma\) at a dose of 40 g kg\(^{-1}\) to rats \((n = 6)\).

to a preliminary pharmacokinetic and tissue distribution study of \((4E,6E,12E)\)-tetradecatriene-8,10-diyn-1,3-diyl diacetate after oral administration of 40 g kg\(^{-1}\) raw and processed \(Atractylodis Rhizoma\), respectively. We found that the \(T_{max}\) have significant difference \((P < 0.05)\), and other pharmacokinetics have no significant difference after using Student’s t-test. The result indicated that processing can promote and accelerate the absorption and the concentration of \((4E,6E,12E)\)-tetradecatrien-8,10-diyn-1,3-diyl diacetate is the highest in the spleen Which proved that the traditional theory of processing \(Atractylodis Rhizoma\) can increase its function of tonifying the spleen.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

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