Research Article

An UPLC-MS/MS Method for Simultaneous Quantification of the Components of Shenyanyihao Oral Solution in Rat Plasma

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1. Introduction

Shenyanyihao oral solution, a famous traditional Chinese preparation, was created by experts in traditional Chinese medicine from the school of Wumen based on the pathological characteristics of chronic nephritis, and it is now the internal preparation of the Traditional Chinese Medicine Hospital of Suzhou province, China. It has been used clinically for nearly two decades and is demonstrated to be efficient in improving the clinical symptoms and reducing proteinuria in patients with chronic nephritis [1]. Shenyanyihao oral solution mainly consists of 30 g of *Campanumoea pilosula* Franch, 10 g of *Atractylodes lancea* (Thunb.) DC, 10 g of *Atractylodes macrocephala* Koidz, 30 g of *Herba Hedyotidis Diffusa*, 30 g of *Pyrrosia lingua* (Thunb.) Farwell, 15 g of *Solanum septemlobum* Bunge, 30 g of *Semen Coix Campanumoea pilosula* Franchi*acryma-jabi* L. var. *frumentaceae* Makino, 20 g of *Scutellaria baicalensis* Georgi, 30 g of *Plantago depressa* Willd., 20 g of *Leonurus japonicus* Houtt., 15 g of polyporus, 10 g of *Angelica sinensis* (Oliv.) Diels, 30 g of *Salvia miltiorrhiza* Bunge, 10 g of *Ligusticum chuanxiong* Hort, and 15 g of *Wolfiporia cocos* [2]. The pharmacological activities of *Rhizoma Atractylodis*, *Rhizoma Atractylodis Macrocephalae*, and *Poria cocos*, including strengthening the spleen and expelling dampness, are attributed to their effective constituents [3–5]. *Rhizoma Atractylodis*, a traditional Chinese medicine, has antibacterial, antiviral, anti-inflammatory, and anticancer activities, and it has been widely used for treating fever, cold, phlegm, and edema in China. *Rhizoma Atractylodis Macrocephalae*, the dried root of a *Compositae* plant, has been widely used for its digestive, diuretic, and antihidrotic activities. Previous research demonstrated that *Rhizoma Atractylodis Macrocephalae* and its compounds could also exert immunoregulation,
anti-inflammation, and antidiabetic activities in experimental models [6]. Furthermore, *Hedyotis diffusa*, *Pyrrosia lingua*, and Uncooked Kernels were reported to have the effect of clearing away heat and toxic materials and promoting diuresis [7, 8]. *Hedyotis diffusa*, a traditional Chinese medicine belongs to the Rubiaceae family, has been widely used for the treatment of various inflammation-related diseases, including appendicitis, arthritis, rheumatism, and urethral infection in China. In addition, the activities of Leonurus, *Radix Salviae miltiorrhizae*, and Angelica primarily focus on improvement of nourishing blood, promoting blood circulation, and resolving dampness [9–20]. A previous study has shown that the combined application of Angelica and *Radix Salviae miltiorrhizae* could exert the function of activating blood and promoting the production of new blood [12].

Shenyanyihao oral solution is an effective prescription in the treatment of chronic nephritis in China. However, the mechanism and pharmacokinetics of the oral solution in chronic nephritis remain obscure.

In the present study, we first analyzed the material basis of Shenyanyihao and the main components were selected for pharmacokinetic research. Then, a sensitive, efficient, and precise UPLC-MS/MS method was developed to simultaneously determine the analytes and ISs in plasma samples of Shenyanyihao oral solution, including stachydrine, Danshensu, chlorogenic acid, protocatechuic acid, plantamajoside, aesculetin, isoquercitrin, ferulic acid, baicalin, and baicalein were purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). Carbamazepine (IS+, purity > 98%) and acetaminophen (IS-, purity > 98%) were obtained from the National Institute for Food and Drug Control (Beijing, China). Acetonitrile, methanol, and formic acid (UPLC grade) were from Merck Company (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA).

2. Experimental

2.1. Chemicals and Reagents. Standards including stachydrine, Danshensu, chlorogenic acid, protocatechuic acid, plantamajoside, aesculetin, isoquercitrin, ferulic acid, baicalin, and baicalein were purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). Carbamazepine (IS+, purity > 98%) and acetaminophen (IS-, purity > 98%) were obtained from the National Institute for Food and Drug Control (Beijing, China). Acetonitrile, methanol, and formic acid (UPLC grade) were from Merck Company (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA).

2.2. Animals. Male Sprague-Dawley rats (180–220 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and housed in an environmentally controlled room with a natural light-dark cycle for 7 days before the experiment was carried out. The male Sprague-Dawley rats were randomly given a dose of 10 g/kg/day of Shenyanyihao oral solution by oral administration for pharmacokinetic experiments [13]. All animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of the Second Military Medical University.

2.3. Preparation of Calibration Standards and QC Samples. Stock solutions of the compounds and the internal standards (ISs) were weighed accurately and dissolved in methanol at

Figure 1: Representative total ion chromatogram of Shenyanyihao oral solution in ESI positive and negative modes.
The working standard solutions were obtained by mixing the stock solutions of the compounds and then diluting them with acetonitrile to a series of appropriate concentrations. The ISs were mixed and prepared by diluting the stock solutions with acetonitrile to concentrations of 10 ng·ml⁻¹ as work solutions. All solutions were stored at 4°C during analysis. Calibration standards were prepared by spiking the working solution into blank rat plasma at different concentrations. Quality control (QC) samples for validation were prepared similar to the calibration standard samples to obtain three different concentrations.

### 2.4. Sample Preparation

The protein precipitation with methanol was applied for sample preparation. 50 μl of plasma spiked with 50 μl of mixed IS solution were thoroughly mixed with 100 μl methanol by vortexing for 30 s.

| Identification | RT (min) | Formula | Herbs | Identification | RT (min) | Formula | Herbs |
|----------------|----------|---------|-------|----------------|----------|---------|-------|
| Arginine       | 0.61     | C₆H₁₄N₄O₂ | Chinese angelica | Isoquercitrin  | 11.94    | C₂₁H₂₆O₁₁ | Hedyotis diffusa |
| Stachydrine    | 0.65     | C₆H₁₃NO₂ | Herba leonuri | Dan phenolic acid B | 11.99 | C₂₆H₃₂O₁₆ | The root of red-rooted salvia |
| Rhamnose       | 0.65     | C₆H₁₄O₆  | Hedyotis diffusa | Rosemary acid | 12.35   | C₁₈H₁₄O₈ | The root of red-rooted salvia |
| Glutamate      | 0.68     | C₆H₁₄NO₂ | Chinese angelica | Baicalin | 13.22 | C₂₂H₁₈O₁₁ | Scutellaria baicalensis |
| Aspartic acid  | 0.69     | C₆H₁₄NO₂ | Hedyotis diffusa | High front glycosides | 14.68 | C₂₂H₂₂O₁₁ | Plantain herb |
| Palmitic acid  | 0.79     | C₁₆H₃₂O₂ | *Poria cocos* | Digitalis glycosides | 14.76 | C₃₁H₄₀O₁₅ | Plantain herb |
| Uridine        | 1.06     | C₆H₁₂N₄O₆ | Agaric polyergus | Red sandalwood of trifoliate bean | 15.00 | C₂₅H₂₅O₁₀ | *Pyrosia lingua* |
| Adenosine      | 1.13     | C₆H₁₄N₄O₄ | Agaric polyergus | Rhubarb phenol | 15.13 | C₁₈H₁₀O₄ | Agaric polyergus |
| Leucine        | 1.18     | C₆H₁₄NO₂ | Chinese angelica | Han baiacin | 15.18 | C₂₆H₂₉O₁₁ | Scutellaria baicalensis |
| Guanosine      | 1.26     | C₁₀H₇N₅O₅ | Herba leonuri | Wood butterfly | 16.28 | C₁₆H₁₄O₅ | Scutellaria baicalensis |
| Phenylalanine  | 2.22     | C₁₆H₃₂O₂ | Herba leonuri | Celery | 18.24 | C₁₈H₁₆O₅ | Plantain herb |
| Hydroxymethylfurural | 2.41 | CₐH₂₉O₅ | *Codonopsis pilosula* | Hispidulin | 18.57 | C₁₈H₁₂O₆ | Plantain herb |
| Syringic acid  | 2.96     | C₆H₁₄O₅  | Herba leonuri | Salvia diol | 21.49 | C₁₈H₁₆O₅ | The root of red-rooted salvia |
| Danshensu      | 2.99     | C₆H₁₂O₅  | The root of red-rooted salvia | Emodin methyl ether | 23.81 | C₁₈H₁₂O₅ | Agaric polyergus |
| Leonurine      | 3.18     | C₁₄H₂₂NO₄ | Herba leonuri | 2-Hydroxyflavones | 23.88 | C₁₈H₁₀O₅ | Agaric polyergus |
| Hydroxybenzaldehyde | 3.22 | C₆H₁₄O₅  | Agaric polyergus | Daidzein | 24.17 | C₁₈H₁₀O₄ | Agaric polyergus |
| Vanillic acid  | 3.22     | C₆H₁₂O₄  | Chinese angelica | Methyl rosemary | 24.90 | C₁₈H₁₈O₈ | The root of red-rooted salvia |
| Caffeic acid   | 4.02     | C₆H₁₂O₄  | The root of red-rooted salvia | Emodin methyl ether | 24.92 | C₁₈H₁₂O₅ | Agaric polyergus |
| Tryptophan     | 4.11     | C₁₄H₂₂N₂O₂ | Chineseangelica | Hydroxytanshinone | 25.53 | C₁₈H₁₈O₄ | The root of red-rooted salvia |
| Protocatechuic acid | 4.54 | C₇H₆O₃  | The root of red-rooted salvia | Stearic acid | 26.88 | C₁₈H₃₆O₂ | Plantain herb |
| Chlorogenic acid | 5.43  | C₁₆H₁₄O₉ | *Pyrosia lingua* | Implicit tanshinone | 26.91 | C₁₈H₂₉O₃ | The root of red-rooted salvia |
| Aesculetin     | 6.52     | C₈H₁₄O₄  | Chinese angelica | Dihydrotanshinone | 27.09 | C₁₈H₁₄O₃ | The root of red-rooted salvia |
| Rutin          | 9.39     | C₂₇H₃₅O₁₆ | *Pyrosia lingua* | Salvia miltiorrhiza new quinone | 27.26 | C₁₈H₁₆O₃ | The root of red-rooted salvia |
| Baicalein      | 10.10    | C₁₅H₁₀O₆ | *Hedyotis diffusa* | Methyl salvianate | 27.37 | C₁₈H₁₈O₅ | The root of red-rooted salvia |
| Ferulic acid   | 10.32    | C₁₀H₁₄O₄ | Chinese angelica | Tanshinone | 27.64 | C₁₈H₁₂O₃ | The root of red-rooted salvia |
| Scutellar      | 10.67    | C₂₁H₁₀O₁₂ | *Scutellaria baicalensis* | Isocryptotanshinone | 27.65 | C₁₈H₂₀O₃ | The root of red-rooted salvia |
| Purple oxalic acid B | 11.35 | C₂₇H₂₂O₁₂ | The root of red-rooted salvia | Phthalic anhydride | 27.87 | C₁₈H₃₄O₄ | Chinese angelica |
2.5. Equipment and LC-MS/MS Conditions. The analytes in plasma were measured by a simple and sensitive UPLC-MS/MS method. Chromatographic analysis was performed on an Agilent 1290 Infinity UPLC system consisting of a binary pump, a surveyor autosampling system, and a thermostatted column compartment. An Agilent Poroshell 120 EC-C18 column (3.0 mm × 100 mm, 2.7 μm) was used for chromatographic separation. The mobile phases of A (acetonitrile) and B (0.1% formic acid aqueous solution) were eluted at a flow rate of 0.4 ml/min with the following gradient conditions: 0-5 min, 50% A; 5-6 min, 50%-90% A; and 6-10 min, 90% A. The column temperature was maintained at 25°C, the autosampler was conditioned at 4°C, and the injection volume was 5 μl. The analysis time was 10 min per sample. An Agilent 6470 tandem mass spectrometer (Agilent Technologies, USA) equipped with an Agilent Jet Stream Technology (AJS) electrospray source interface (ESI) was used for MS detection. The mass spectrometric detection was optimized simultaneously in the positive and negative ion modes by multiple reaction monitoring (MRM). The main MS parameters of the ionized chamber are as follows: capillary voltage at 4000 V (positive)/3500 V (negative), gas temperature at 350°C, drying gas flow at 11 l/min, nebulizer pressure at 40 psi, sheath gas temperature at 400°C, and sheath gas flow at 11 l/min. Data acquisition and analysis were performed using the Agilent MassHunter WorkStation version B.07.00.

2.6. Method Validation. The analytical method was validated for specificity, linearity, matrix effects, extraction recovery, precision, accuracy, and stability. The specificity of the method was tested by analyzing blank plasma, blank samples spiked with the analytes, and actual samples after oral administration. Plasma samples from rats after oral administration were analyzed for endogenous interference.

Blank plasma was added with the compound stock solutions to prepare a series of calibration standard samples. Calibration curves were generated with peak area ratios of the analytes to IS vs. concentration using 1/x weighting. The lower limits of quantitation (LLOQ) were defined as the lowest plasma concentration in the calibration curve.

Replicates of QC samples at three levels were prepared for intraday assay accuracy and precision. The same procedure was performed for 3 consecutive days to determine interday precision and accuracy. The precision was described as relative standard deviation (RSD), and the accuracy was exhibited as relative error (RE).

The matrix effect was determined by comparing the responses of the postextracted standard QC samples with the response of analytes from neat standard samples at three different QC concentrations. The extraction recovery was evaluated by comparing the peak areas of analytes in the extracted plasma samples with those in nonprocessed samples at three different QC concentrations.

The stability was evaluated to cover the anticipated conditions that the samples might be exposed to during storage and handling using QC samples in different conditions. Three levels of QC samples were prepared for analysis under different storage conditions, including short-term stability at room temperature for 3 h, postpreparative stability at the autosampler for 24 h, three freeze-thaw cycles at -80°C, and long-term stability at -80°C for 30 days. Both precision (RSD) and accuracy (RE) should be below 15%.

2.7. Pharmacokinetic Study Protocol. The rats were administered orally with Shenyanyihao oral solution. Blood samples from the ophthalmic venous plexus taken at 0.08, 0.17, 0.25, 0.33, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 h after oral administration and at 0 h (predose) were collected (0.2 ml) into heparinized centrifuge tubes. The blood samples were immediately centrifuged at 4000 rpm at 4°C for 5 min, and the supernatant plasma was transferred into another tube and stored at -80°C until treatment.

2.8. Data Analysis. Pharmacokinetic parameters were analyzed by a noncompartmental method using the WinNonlin7.0 pharmacokinetic program (Pharsight Corp., USA). The maximum concentration (C_{max}) and time to reach the maximum concentration (T_{max}) were measured from values...
Figure 2: Continued.
obtained from the concentration-time curve. All results were expressed as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Method Development and Material Basis of Shenyanyihao Oral Solution.

Pure acetonitrile was utilized as the precipitant for biosamples. A combination of acetonitrile and methanol mixture was used to evaluate the recoveries and matrix effects. The high extraction efficiency was produced by methanol. Different mobile phases were evaluated to improve LC separation and enhance mass sensitivity of analytes. Modifiers such as formic acid and ammonium acetate were added with different concentrations. The signal intensity of the components was acquired using acetonitrile and formic acid aqueous solution. The components were well isolated under the optimized gradient conditions.

To optimize MS parameters, pure compounds in methanol were individually injected into a MS instrument using MS/MS with MRM mode. The [M + H]^+ and [M – H]^− were used as the predominant ions for analytes in the Q1 spectrum for the different ionization modes in each component. MS/MS working parameters such as precursor-to-product ion pair and collision energy were optimized to obtain the highest intensity of deprotonated molecules of the analytes and ISs. There was no endogenous interference in the actual samples under the optimum conditions. Finally, the analytes were successfully separated and their sensitivity was sufficiently enhanced in this study.

We conducted a material basis analysis of Shenyanyihao. First, we searched the literature related to the chemical composition of relevant medicinal materials and consulted the information of relevant chemical substances based on the literature reports. Then, we established a database of known chemical compositions through the formula-database-generator software (Agilent Technologies, USA). Next, we searched the total ion current mass spectrum of the medicinal materials in the database, and recorded the retention

![Figure 2: Representative chromatograms of analytes: (a) typical chromatograms of the blank plasma; (b) chromatograms of LLOQ; (c) chromatograms of plasma samples with different components after oral administration. Correspondence between compounds and characters in the graph are as follows: (1) stachydrine, (2) Danshensu, (3) chlorogenic acid, (4) protocatechuic acid, (5) plantamajoside, (6) aesculetin, (7) isoquercitrin, (8) ferulic acid, (9) baicalin, (10) baicalein, (IS +) carbamazepine, and (IS −) acetaminophen.](image-url)
Table 3: Calibration curves and LLOQ of the components in plasma.

| Compounds  | Calibration curve          | Linear range (ng/ml) | Correlation coefficient (r) | LLOQ (ng/ml) |
|------------|----------------------------|---------------------|-----------------------------|--------------|
| Stachydrine | $y = 0.0028x + 0.0007$     | 5-2500              | 0.999                       | 5.00         |
| Danshensu  | $y = 0.0013x - 0.0001$     | 1-500               | 0.999                       | 1.00         |
| Chlorogenic acid | $y = 0.0100x - 0.0007$ | 0.1-50              | 0.999                       | 0.10         |
| Protocatechuic acid | $y = 0.0056x + 0.0006$ | 0.1-50              | 0.999                       | 0.10         |
| Plantamajoside | $y = 0.0200x - 0.0001$  | 0.05-25             | 0.999                       | 0.05         |
| Aesculetin  | $y = 0.0269x + 0.0023$     | 0.1-50              | 0.999                       | 0.10         |
| Isoquercitrin | $y = 0.0131x - 0.0005$ | 0.1-50              | 0.999                       | 0.10         |
| Ferulic acid | $y = 0.0028x - 0.0056$   | 1-500               | 0.999                       | 1.00         |
| Baicalin    | $y = 0.0116x - 0.0056$     | 5-2500              | 0.999                       | 5.00         |
| Baicalein   | $y = 0.00097x - 0.0003$    | 1-500               | 0.999                       | 1.00         |

Table 4: Precision and accuracy of the components in rat plasma sample.

| Compounds   | Concentration (ng/ml) | Intraday $(n=5)$ | Interday $(n=15)$ |
|-------------|-----------------------|-----------------|-------------------|
|             | Precision (RSD%) | Accuracy (RE%) | Precision (RSD%) | Accuracy (RE%) |
| Stachydrine | 10.00 | 4.17 | -6.93 | 3.51 | -5.79 |
|             | 250.00 | 6.16 | 2.35 | 5.18 | 0.19 |
|             | 2000.00 | 1.92 | 2.69 | 2.36 | 3.49 |
|             | 2.00 | 8.51 | -1.41 | 7.74 | 2.93 |
| Danshensu  | 50.00 | 4.37 | 10.51 | 10.31 | -0.86 |
|             | 400.00 | 0.25 | 8.54 | 3.60 | 3.97 |
|             | 0.20 | 5.42 | -7.99 | 5.25 | -3.25 |
| Chlorogenic acid | 5.00 | 3.22 | 9.48 | 1.84 | 10.40 |
|             | 40.00 | 0.29 | 9.97 | 1.56 | 11.60 |
|             | 0.20 | 5.49 | 2.87 | 4.43 | 4.77 |
| Protocatechuic acid | 5.00 | 0.58 | 5.73 | 2.60 | 7.88 |
|             | 40.00 | 1.93 | 10.72 | 1.75 | 9.02 |
|             | 0.10 | 3.91 | 6.67 | 4.78 | 4.19 |
| Plantamajoside | 2.50 | 0.48 | 3.24 | 6.59 | -4.58 |
|             | 20.00 | 1.99 | 6.38 | 4.65 | 0.70 |
|             | 0.20 | 1.84 | 8.45 | 5.95 | 1.74 |
| Aesculetin  | 5.00 | 1.04 | 10.08 | 2.09 | 8.26 |
|             | 40.00 | 0.84 | 6.87 | 2.91 | 8.75 |
|             | 0.20 | 2.61 | 4.58 | 3.53 | 4.19 |
| Isoquercitrin | 5.00 | 1.01 | 5.03 | 0.98 | 5.00 |
|             | 40.00 | 0.76 | 10.59 | 1.31 | 9.82 |
|             | 2.00 | 7.84 | 5.39 | 6.91 | 3.39 |
| Ferulic acid | 50.00 | 1.23 | 7.27 | 1.20 | 6.40 |
|             | 400.00 | 0.42 | 5.69 | 1.48 | 4.06 |
|             | 10.00 | 4.77 | -3.66 | 4.74 | -4.40 |
| Baicalin    | 250.00 | 4.32 | -9.60 | 4.50 | -8.16 |
|             | 2000.00 | 3.08 | -1.17 | 4.21 | -5.10 |
|             | 2.00 | 3.87 | -5.68 | 5.62 | -5.18 |
| Baicalein   | 50.00 | 1.67 | -8.81 | 4.15 | -6.67 |
|             | 400.00 | 4.07 | -4.66 | 4.54 | -4.53 |
time, charge mass ratio, and adducted ion of the retrieved chemical components as shown in Figure 1.

We identified compounds from the original data according to the charge mass ratio and confirmed the mass accuracy of these compounds. Elemental composition analysis was conducted using the isotope peak ratio. After database search and verification, the properties of compounds have been initially identified, and the results are shown in Table 1. At last, we selected 10 compounds from them for pharmacokinetic analysis. Structures and characteristic ion peaks of these 10 compounds were shown in Figure S1 and Table 2.

### 3.2. Method Validation

#### 3.2.1. Specificity

Typical chromatograms of the blank plasma (Figure 2(a)), LLOQ (Figure 2(b)), and plasma samples with different components after oral administration (Figure 2(c)) are shown in Figure 2. The results revealed no significant interference peak around the retention time of the analytes and ISs, which accomplished the guideline of bioanalytical method validation.

#### 3.2.2. Calibration Curve and LLOQ

A linear regression analysis using 1/x weighting was used to evaluate the linearity of the calibration curve \( y = bx + a \). The results demonstrated that the calibration curve of the different components in plasma showed good linearity in the matrix over the concentration ranges (Table 3). The recovery rate, accuracy, and precision in LLOQ are measured as shown in Tables 4 and 5.

#### 3.2.3. Precision and Accuracy

The results for the precision and accuracy are shown in Table 4. The intraday and

| Compounds     | Concentration (ng/ml) | Extraction recovery (%) | RSD% | Matrix effect (%) | RSD% |
|---------------|-----------------------|-------------------------|------|------------------|------|
| Stachydrine   | 10.00                 | 87.10 ± 1.17            | 1.34 | 103.94 ± 4.22    | 4.06 |
|               | 250.00                | 93.12 ± 1.14            | 1.22 | 103.81 ± 6.39    | 6.15 |
|               | 2000.00               | 85.02 ± 2.22            | 2.61 | 88.58 ± 1.70     | 1.92 |
|               | 2.00                  | 84.24 ± 0.68            | 0.81 | 85.03 ± 7.26     | 8.54 |
| Danshensu     | 50.00                 | 89.22 ± 4.94            | 5.54 | 88.22 ± 3.85     | 4.37 |
|               | 400.00                | 85.51 ± 1.04            | 1.22 | 85.98 ± 0.22     | 0.25 |
|               | 0.20                  | 88.03 ± 3.66            | 4.16 | 91.42 ± 8.00     | 8.75 |
| Chlorogenic acid | 5.00               | 89.21 ± 3.08            | 3.45 | 90.33 ± 2.94     | 3.26 |
|               | 40.00                 | 87.72 ± 0.45            | 0.52 | 92.34 ± 0.27     | 0.29 |
|               | 0.20                  | 86.57 ± 0.81            | 2.09 | 85.90 ± 3.10     | 3.61 |
| Protocatechuic acid | 5.00           | 88.33 ± 2.51            | 2.84 | 88.74 ± 0.50     | 0.57 |
|               | 40.00                 | 85.28 ± 0.28            | 0.32 | 88.10 ± 1.69     | 1.92 |
|               | 0.10                  | 87.25 ± 0.88            | 1.00 | 85.52 ± 6.29     | 7.36 |
| Plantamajoside | 2.50                 | 83.12 ± 2.47            | 2.98 | 89.68 ± 0.44     | 0.49 |
|               | 20.00                 | 82.34 ± 1.51            | 1.83 | 89.84 ± 1.80     | 2.00 |
|               | 0.20                  | 83.80 ± 1.60            | 1.91 | 98.30 ± 1.30     | 1.32 |
| Aesculetin     | 5.00                  | 87.13 ± 1.93            | 2.22 | 91.67 ± 0.94     | 1.02 |
|               | 40.00                 | 84.64 ± 1.36            | 1.61 | 95.37 ± 0.80     | 0.84 |
|               | 0.20                  | 87.04 ± 4.16            | 4.78 | 87.79 ± 2.81     | 3.20 |
| Isoquercitrin  | 5.00                  | 89.18 ± 2.83            | 3.18 | 93.97 ± 0.95     | 1.01 |
|               | 40.00                 | 86.62 ± 0.62            | 0.71 | 92.37 ± 0.70     | 0.76 |
|               | 2.00                  | 95.70 ± 5.63            | 5.88 | 94.87 ± 3.82     | 4.02 |
| Ferulic acid   | 50.00                 | 93.47 ± 3.56            | 3.81 | 99.51 ± 1.18     | 1.19 |
|               | 400.00                | 92.69 ± 0.66            | 0.71 | 98.60 ± 0.41     | 0.42 |
|               | 10.00                 | 95.80 ± 3.07            | 3.20 | 103.45 ± 5.20    | 5.02 |
| Baicalin       | 250.00                | 94.92 ± 3.65            | 3.84 | 105.52 ± 4.57    | 4.33 |
|               | 2000.00               | 98.81 ± 2.93            | 2.96 | 108.22 ± 3.33    | 3.08 |
|               | 2.00                  | 82.55 ± 1.71            | 2.07 | 87.40 ± 4.05     | 4.63 |
| Baicalein      | 50.00                 | 84.04 ± 3.97            | 4.72 | 90.89 ± 1.53     | 1.68 |
|               | 400.00                | 87.01 ± 4.56            | 5.24 | 84.47 ± 3.44     | 4.08 |
interday accuracy were within -9.60% to 11.60%, while the intraday and interday precision were less than 10.31%. The results showed that the precision and accuracy were within the acceptable range of analysis.

3.2.4. Extraction Recovery and Matrix Effect. Extraction recovery for all the components and ISs were beyond 82.34% with no significant differences among the three concentrations. In addition, the matrix effect of the analytes ranged from 84.47% to 108.22, which suggested that the method was reliable and no matrix effect occurred (Table 5).

3.2.5. Stability. The stability was determined under different conditions. The results showed that all the components were stable in the plasma of rats at room temperature for 3 h, at 4°C in the autosampler for 24 h, after three freeze-thaw cycles, and at -80°C in a long-term freezer for 30 days (Table 6). In addition, the results showed no significant degradation of analytes under these conditions.

3.3. Pharmacokinetic Study. The validated method for the quantitation of the different components was employed to evaluate the pharmacokinetic behaviours in rat plasma after oral administration of Shenyanyihao oral solution. The major pharmacokinetic parameters were evaluated using noncompartmental calculations performed with the WinNonlin7.0 pharmacokinetic program. The plasma concentration-time profiles of all analytes are presented in Figure 3. Among them, the peak concentration of stachydrine in Figure 3(a) and baicalin in Figure 3(i) exceeded the upper limit of 2500 ng/ml of the standard curve. So, we measured the point beyond the standard curve by matrix dilution and examined the dilution effect. Dilution integrity was assessed by diluting the samples of high concentration (10 and 50 times concentration of ULOQ) to the quantitative range by blank

| Compounds         | Concentration (ng/ml) | Room temperature (3 h, 25°C) | Autosampler (24h, 4°C) | Three freeze/thaw cycles | Long term (30 day, −80°C) |
|-------------------|-----------------------|-------------------------------|------------------------|--------------------------|---------------------------|
|                   |                       | Precision (RSD%) | Accuracy (RE%) | Precision (RSD%) | Accuracy (RE%) | Precision (RSD%) | Accuracy (RE%) | Precision (RSD%) | Accuracy (RE%) |
| Stachydrine       | 10.00                 | 4.62              | -9.23           | 2.66               | -9.94          | 4.44             | -7.37          | 4.69             | -5.94          |
|                   | 250.00                | 0.81              | 0.09            | 4.06               | -6.0           | 2.59             | 6.17           | 3.49             | 2.03           |
|                   | 2000.00               | 4.49              | 3.14            | 3.82               | 3.07           | 3.55             | 5.27           | 3.37             | 4.64           |
|                   | 2.00                  | 5.29              | 7.09            | 5.29               | 7.09           | 6.92             | -2.70          | 8.08             | 6.79           |
| Danshensu         | 50.00                 | 10.89             | -10.20          | 10.89              | -10.20         | 1.58             | -12.52         | 5.55             | -4.84          |
|                   | 400.00                | 2.78              | 4.73            | 2.78               | 4.73           | 0.82             | 0.91           | 1.22             | 2.60           |
|                   | 0.20                  | 6.13              | -3.40           | 1.98               | -1.57          | 5.29             | -3.89          | 4.57             | -0.48          |
| Chlorogenic acid  | 5.00                  | 0.45              | 11.70           | 0.80               | 13.89          | 4.07             | 9.84           | 1.25             | 10.93          |
|                   | 40.00                 | 0.66              | 11.51           | 0.65               | 14.17          | 1.20             | 13.15          | 0.53             | 12.59          |
|                   | 0.20                  | 3.69              | 7.78            | 1.00               | 12.84          | 1.60             | 1.80           | 3.87             | 5.18           |
| Protocatechuic acid| 5.00                  | 2.55              | 10.39           | 1.99               | 5.52           | 1.03             | 8.49           | 3.58             | 8.11           |
|                   | 40.00                 | 0.95              | 10.48           | 1.97               | 8.98           | 2.29             | 8.55           | 0.50             | 7.28           |
|                   | 0.10                  | 2.00              | 9.23            | 5.21               | 7.94           | 2.91             | 6.68           | 7.05             | 4.14           |
| Plantamajoside    | 2.50                  | 1.28              | -90.31          | 5.08               | -91.35         | 2.59             | -91.11         | 3.79             | -90.84         |
|                   | 20.00                 | 1.36              | 5.03            | 1.94               | -0.13%         | 2.87             | -5.26          | 1.20             | -0.43          |
|                   | 0.20                  | 5.16              | -1.16           | 1.51               | 4.88           | 3.02             | 2.69           | 4.20             | -0.62          |
| Aesculetin        | 5.00                  | 2.41              | 7.25            | 0.89               | 8.31           | 1.95             | 9.94           | 1.79             | 6.76           |
|                   | 40.00                 | 2.07              | 11.25           | 4.89               | 9.87           | 1.90             | 7.39           | 3.61             | 7.41           |
|                   | 0.20                  | 2.65              | 3.57            | 2.55               | 4.81           | 2.56             | 8.27           | 1.88             | 0.43           |
| Isoquercitin      | 5.00                  | 0.66              | 5.18            | 0.48               | 5.40           | 1.58             | 4.20           | 0.51             | 5.56           |
|                   | 40.00                 | 0.54              | 11.02           | 2.16               | 11.09          | 2.43             | 10.30          | 0.63             | 8.27           |
|                   | 2.00                  | 3.09              | 5.81%           | 11.12              | 6.79           | 7.50             | 4.93           | 4.72             | 7.22           |
| Ferulic acid      | 50.00                 | 2.37              | 4.96            | 0.67               | 4.55           | 1.47             | 6.41           | 0.99             | 5.32           |
|                   | 400.00                | 0.38              | 3.57            | 2.20               | 4.12           | 1.39             | 4.29           | 0.06             | 2.22           |
|                   | 10.00                 | 1.38              | -6.39           | 4.18               | -5.56          | 5.63             | -0.46          | 4.01             | -6.58          |
| Baicalin          | 250.00                | 0.37              | -10.18          | 1.38               | -11.90         | 2.72             | -0.02          | 4.76             | -5.18          |
|                   | 2000.00               | 7.12              | -3.62           | 4.10               | -6.54          | 5.25             | -7.42          | 3.31             | -5.38          |
|                   | 2.00                  | 6.37              | -3.82           | 2.29               | -7.65          | 1.69             | -3.44          | 2.30             | -10.32         |
| Baicalein         | 50.00                 | 2.29              | -9.87           | 2.99               | -8.08          | 3.72             | -1.39          | 4.60             | -5.23          |
|                   | 400.00                | 5.15              | 0.51            | 4.11               | -5.84          | 3.38             | -0.69          | 3.42             | -8.34          |

Table 6: Stability of the components in rat plasma sample.
biological matrix. Five replicates were analyzed for each dilution level. The results of dilution integrity experiments (10x and 50x) suggest that the accuracy was within ±15%, while the precision was under 10%, which conformed to the requirements of the methodology. The major pharmacokinetic parameters are shown in Table 7.

Various drugs from Shenyanyihao oral solution are used together to make up for the treatment of the diseases,
Table 7: The main pharmacokinetic parameters of the components after oral administration of Shenyanyihao oral solution in rats (n = 6, mean ± SD).

| Parameters     | Stachydrine | Danshensu | Chlorogenic acid | Protocatechu acid | Plantamajoside | Aesculetin | Isoquercitrin | Ferulic acid | Baicalin | Baicalein |
|----------------|-------------|------------|------------------|-------------------|----------------|------------|--------------|--------------|----------|-----------|
| $t_{1/2}$ (h)  | 9.09 ± 0.51 | 3.91 ± 2.34| 16.72 ± 3.32     | 11.35 ± 2.23      | 12.33 ± 3.92   | 14.43 ± 4.17| 26.39 ± 3.89 | 11.84 ± 4.98 | 5.01 ± 0.52 | 11.72 ± 4.78 |
| $T_{\text{max}}$ (h) | 3.20 ± 1.10 | 0.47 ± 0.08 | 1.30 ± 0.57      | 0.50 ± 0.00       | 1.40 ± 0.22    | 1.00 ± 0.00 | 0.50 ± 0.00  | 0.17 ± 0.00  | 2.80 ± 1.10 | 3.60 ± 0.89 |
| $C_{\text{max}}$ (ng/ml) | 2673.0 ± 163.0 | 181.79 ± 59.58 | 3.33 ± 0.52     | 26.19 ± 11.64    | 1.31 ± 0.23    | 1.65 ± 0.15 | 1.65 ± 0.23  | 70.24 ± 19.09 | 2983.4 ± 1344.3 | 14.25 ± 2.59 |
| AUC₀→ₜ (ng/ml∗h) | 39256.8 ± 4031.7 | 556.04 ± 165.66 | 17.05 ± 2.86   | 41.96 ± 11.42    | 3.99 ± 0.44    | 8.23 ± 1.70 | 4.75 ± 0.68  | 76.39 ± 21.43 | 26525.1 ± 15523.7 | 106.77 ± 46.19 |
| AUC₀→∞ (ng/ml∗h) | 40160.0 ± 4088.2 | 569.72 ± 165.31 | 21.95 ± 3.34   | 45.80 ± 12.60    | 5.01 ± 0.71    | 11.64 ± 2.41| 8.98 ± 1.33  | 95.70 ± 25.36 | 26566.8 ± 15520.4 | 128.95 ± 53.59 |
| AUMC₀→∞ (ng/ml∗h²) | 515393.7 ± 55002.7 | 2049.56 ± 1061.35 | 338.84 ± 99.46 | 307.02 ± 152.54 | 66.31 ± 28.63 | 223.19 ± 97.81 | 304.35 ± 69.29 | 1254.35 ± 628.41 | 229094.2 ± 146621.6 | 2190.95 ± 1741.10 |
| MRT₀→∞ (h)     | 12.82 ± 0.25 | 3.56 ± 1.71  | 15.42 ± 3.31     | 6.60 ± 2.26       | 12.76 ± 4.47   | 18.80 ± 5.01 | 33.67 ± 3.63 | 12.50 ± 4.16  | 8.71 ± 2.00 | 15.16 ± 5.91 |
| $V_{d}$ (l/kg)  | 0.76 ± 0.11  | 13.67 ± 8.51 | 1001.03 ± 202.53 | 24.09 ± 4.86      | 79.97 ± 18.00  | 7.31 ± 2.02 | 127.91 ± 13.82 | 18.01 ± 5.2   | 12.89 ± 8.01 | 131.54 ± 23.79 |
| CL (l/kg/h)     | 0.06 ± 0.01  | 2.47 ± 0.86  | 41.83 ± 6.79     | 1.52 ± 0.47       | 4.89 ± 0.77    | 0.36 ± 0.07 | 3.40 ± 0.50  | 1.13 ± 0.39   | 1.71 ± 0.90 | 8.72 ± 3.03 |
including detoxification, dampness, and promotion of blood circulation, which can eliminate the side effects of stubborn dampness heat [14]. Our previous results have confirmed that Shenyanyihao oral solution has a favorable effect on improving the clinical symptoms of the patients with chronic nephritis, which reveals the function of reducing urinary protein, increasing serum albumin, and regulating blood lipids [13]. In addition, we also found that Shenyanyihao oral solution can improve the proteinuria of rats with adriamycin nephropathy and upregulate the expression of nephrin protein in renal tissue. However, the pharmacokinetic study of the components from Shenyanyihao oral solution remains unclear, which sets obstacles on undoing the detailed mechanisms in the treatment of chronic nephritis.

In the present study, the pharmacokinetic parameters of the components in plasma revealed some differences compared with previous researches [16–23]. Danshensu, protocatechuic acid, isoquercitrin, and ferulic acid were quickly absorbed, and their peak concentrations occurred at 0.47 h, 0.50 h, 0.50 h, and 0.17 h, respectively, while stachydrine, baicalin, and baicalein were absorbed much slower than other components for the average $t_{\text{max}}$ values which were 3.20 h, 2.80 h, and 3.60 h, respectively. Furthermore, the average $t_{1/2}$ of Danshensu was 3.91 h in rats, which predicted the most rapid distribution and elimination among the components of Shenyanyihao oral solution. However, the average $t_{1/2}$ result of Danshensu was not in accordance with the data from previous reports [17, 24]. The $C_{\text{max}}$ of stachydrine and baicalin were 2673.0 ng/ml and 2983.4 ng/ml, respectively, and these results were higher than other components of Shenyanyihao oral solution, which denoted higher plasma concentrations of stachydrine and baicalin in rats. Moreover, the AUC0–1 and AUC0–∞ values of Danshensu were 556.04 ng/ml*h and 569.72 ng/ml*h, and these results were much lower than the data in previous studies [25, 26]. In addition, the $V_{\text{d}}$ values of chlorogenic acid and stachydrine were 1001.03 l/kg and 0.76 l/kg, which exhibited the highest and lowest tissue uptake among the components of Shenyanyihao oral solution after oral administration in rats. The results demonstrated that the different partial pharmacokinetic properties of the components might be related to the metabolic enzyme and interaction system in Shenyanyihao oral solution in rats [27, 28]. The analytical methods and pharmacokinetic parameters could be useful for a deep understanding of the detailed mechanisms of the Shenyanyihao oral solution in the treatment of the chronic nephritis in clinics.

4. Conclusion

In conclusion, the present study firstly explored a sensitive, efficient, and precise UPLC-MS/MS method that was developed to simultaneously determine the components and ISs of the Shenyanyihao oral solution in plasma samples. The pharmacokinetic study of the analytes in rat plasma was successfully used by the method after oral administration. The results of the pharmacokinetic parameters were evaluated and analysed to serve as a potential application of the Shenyanyihao oral solution in clinics.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Chunbo Jiang and Guoqiang Liang contributed equally to this work.

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Supplementary Materials

Fig. S1. Structures and characteristic ion peaks of the 10 selected compounds (Supplementary Materials)

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