Simultaneous Determination and Pharmacokinetics of Tetrandrine, Fangchinoline, and Cyclanoline in Rat Plasma by Ultra-High Performance Liquid Chromatography-Mass Spectrometry after Oral Administration of Stephaniae Tetrandrae Radix Extract

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Abstract

Objective: The objective of the study was to develop a rapid and sensitive ultra-performance liquid chromatography-tandem mass spectrometric method for the determination of tetrandrine, fangchinoline, and cyclanoline in rat plasma and to investigate their pharmacokinetics after oral administration of Stephaniae Tetrandrae Radix extracts. Methods: Sample pretreatment involved methanol pretreatment and liquid–liquid extraction of ethyl acetate from plasma with methanol. Tramadol was used as the internal standard. The analysis was performed using an high strength silica T3 column (100 mm × 2.1 mm, 1.8 μm) and a gradient elution method consisting of mobile phase solution A (0.1% formic acid in water) and B (acetonitrile) at a flow rate of 0.4 mL/min. The detection was performed using a triple quadrupole tandem mass spectrometer in the multiple reaction monitoring mode and using an electrospray ionization source in the positive ionization mode. Results: High efficiency was achieved with an analysis time of 4 min/sample. The calibration curve linear in the concentration range of 1250 ng/ml (R² ≥ 0.9900) and the lower limit of quantification is 1 ng/ml. The intraday and interday precision (relative standard deviation) values were lower than 9.4. Accuracy (relative error) was within 10.3% at all three quality control levels. Conclusions: This method was successfully applied in pharmacokinetics of tetrandrine, fangchinoline, and cyclanoline in rats after oral administration of Stephaniae Tetrandrae Radix extracts. The maximum plasma concentration (C_max) of tetrandrine, fangchinoline, and cyclanoline was 124.71 ± 16.08, 84.56 ± 3.28, and 57.61 ± 6.26 ng/mL, respectively. The time to reach C_max was 10.39 ± 3.04 for tetrandrine, 10.17 ± 3.04 for fangchinoline, and 6.40 ± 3.16 for cyclanoline. The pharmacokinetic results might help further guide the clinical application of Stephaniae Tetrandrae Radix.

Keywords: Pharmacokinetics, rat plasma, stephaniae tetrandrae radix, ultra-high performance liquid chromatography-mass spectrometry

INTRODUCTION

Stephaniae tetrandrae radix, the dry roots of Stephania tetrandra S. Moore, is a type of traditional Chinese medicine. Its Chinese name is “Fang Ji.” The Chinese Pharmacopoeia stated that it is used to dispel wind, relieve pain, and induce de tumescence. The components responsible for the pharmacological activity of Stephaniae Tetrandrae Radix are tetrandrine, fangchinoline, and cyclanoline. Previous investigations have shown that tetrandrine inhibited the growth of colon cancer cells and induced apoptosis in breast cancer cells. Fangchinoline can promote apoptosis of pancreatic cancer cells, inhibit melanoma cell growth, and inhibit de tumescence. This compound can also inhibit cancer cells.

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the migration of breast cancer cells.\cite{17-12} Cyclanoline is an acetylcholinesterase inhibitor\cite{13} and can inhibit hemolysis of red blood cells and enhance free radical-scavenging activity.\cite{2}

Pharmacokinetics involves the quantitative study of the processes (absorption, distribution, metabolism, and excretion) of a drug undergone in vivo. Liu et al. have studied the pharmacokinetics of tetrandrine in a self nanoemulsifying drug delivery system.\cite{14} Yang et al. have studied the pharmacokinetics of tetrandrine in human blood.\cite{15} Li et al. have studied the development of a quantitative and qualitative method based on ultra high performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC QTOF MS/MS) for evaluating paclitaxel–tetrandrine interaction and its application in a pharmacokinetic study.\cite{16} However, there are no reports on the pharmacokinetics of cyclanoline and the simultaneous determination of the three alkaloids. Therefore, it is of great significance to simultaneously determine these three alkaloids and to compare their pharmacokinetics in rat plasma.

In this study, we developed, for the first time, a UPLC-MS/MS combined with the multiple reaction monitoring (MRM) technique for the simultaneous determination of tetrandrine, fangchinoline, and cyclanoline in rat plasma. The pharmacokinetics of cyclanoline were studied for the first time. Tetrandrine, fangchinoline, and cyclanoline were detected simultaneously for the first time. This method was successfully applied for the pharmacokinetic analysis of the three compounds in rat plasma following the oral administration of Stephaniae Tetrandrae Radix extract. The results of this study will provide useful information for clinical applications and further experimental studies.

**Experimental**

**Materials and reagents**

Herbs were purchased from Shanghai Leiyunshang Pharmaceutical Co., Ltd. (Shanghai, China) and then identified by Professor Zhenyue Wang of Heilongjiang University of Chinese Medicine (Heilongjiang, China) as tetrandrine (110,711), fangchinoline (110,793), and cyclanoline. Stephaniae Tetrandrae Radix extracts were prepared with methanol at concentrations of 49.4, 49.0 and 49.4 μg/mL, respectively, and further diluted with methanol to prepare working solutions of various concentrations. The stock solution was diluting with methanol to prepare the IS working solution (200 ng/mL). The standard solutions for the standard calibration curves were prepared at final concentrations of 1.282, 2.564, 5.128, 51.28, 76.92, 100.4, 150.6, and 200.8 ng/mL for tetrandrine; 1.023, 2.046, 4.092, 40.92, 61.38, 81.62, 122.4, and 163.2 ng/mL for fangchinoline; 1.281, 2.562, 5.124, 51.24, 76.86, 100.3, 150.4, and 200.5 ng/mL for cyclanoline. Quality control (QC) samples were prepared at four concentration levels in blank plasma: high QC (HQC; 160.6, 130.5, and 160.4 ng/mL), medium QC (MQC; 80.32, 65.28, and 80.24 ng/mL), low QC (LQC; 20.08, 16.32, and 20.06 ng/mL), and lower limit of quantification (LLOQ) (1.281, 1.282, and 1.281 ng/mL) for tetrandrine, fangchinoline, and cyclanoline. All stock and working solutions were stored in the freezer at 4°C before analysis.

**Preparation of calibration standards and quality control samples**

Standard mixed stock solutions of tetrandrine, fangchinoline, and cyclanoline were prepared with methanol at concentrations of 49.4, 49.0 and 49.4 μg/mL, respectively, and further diluted with methanol to prepare working solutions of various concentrations. The stock solution was diluting with methanol to prepare the IS working solution (200 ng/mL). The standard solutions for the standard calibration curves were prepared at final concentrations of 1.282, 2.564, 5.128, 51.28, 76.92, 100.4, 150.6, and 200.8 ng/mL for tetrandrine; 1.023, 2.046, 4.092, 40.92, 61.38, 81.62, 122.4, and 163.2 ng/mL for fangchinoline; 1.281, 2.562, 5.124, 51.24, 76.86, 100.3, 150.4, and 200.5 ng/mL for cyclanoline. Quality control (QC) samples were prepared at four concentration levels in blank plasma: high QC (HQC; 160.6, 130.5, and 160.4 ng/mL), medium QC (MQC; 80.32, 65.28, and 80.24 ng/mL), low QC (LQC; 20.08, 16.32, and 20.06 ng/mL), and lower limit of quantification (LLOQ) (1.281, 1.282, and 1.281 ng/mL) for tetrandrine, fangchinoline, and cyclanoline. All stock and working solutions were stored in the freezer at 4°C before analysis.

**Preparation of Stephaniae Tetrandrae radix extracts**

Briefly, 500 g of the dried herb was soaked in 80% (v/v) alcohol for 12 h. After refluxing for 2 h, the herb solution was filtered. This process was repeated three times, and all the filtrates were mixed. Waste slag was concentrated, dried, and then suspended in water to obtain an oral solution. Water was added to prepare 0.3 g/mL crude drug suspension.

**Experimental**

**Materials and reagents**

Herbs were purchased from Shanghai Leiyunshang Pharmaceutical Co., Ltd. (Shanghai, China) and then identified by Professor Zhenyue Wang of Heilongjiang University of Chinese Medicine (Heilongjiang, China) as tetrandrine (110,711), fangchinoline (110,793), and cyclanoline. Stephaniae Tetrandrae Radix extracts were prepared with methanol at concentrations of 49.4, 49.0 and 49.4 μg/mL, respectively, and further diluted with methanol to prepare working solutions of various concentrations. The stock solution was diluting with methanol to prepare the IS working solution (200 ng/mL). The standard solutions for the standard calibration curves were prepared at final concentrations of 1.282, 2.564, 5.128, 51.28, 76.92, 100.4, 150.6, and 200.8 ng/mL for tetrandrine; 1.023, 2.046, 4.092, 40.92, 61.38, 81.62, 122.4, and 163.2 ng/mL for fangchinoline; 1.281, 2.562, 5.124, 51.24, 76.86, 100.3, 150.4, and 200.5 ng/mL for cyclanoline. Quality control (QC) samples were prepared at four concentration levels in blank plasma: high QC (HQC; 160.6, 130.5, and 160.4 ng/mL), medium QC (MQC; 80.32, 65.28, and 80.24 ng/mL), low QC (LQC; 20.08, 16.32, and 20.06 ng/mL), and lower limit of quantification (LLOQ) (1.281, 1.282, and 1.281 ng/mL) for tetrandrine, fangchinoline, and cyclanoline. All stock and working solutions were stored in the freezer at 4°C before analysis.

**Animal experiments**

Healthy male Sprague-Dawley rats were supplied by the ExperimentalAnimal Center of Heilongjiang University of Traditional Chinese Medicine. All animal procedures were carried out in compliance with the guidelines for scientific animal procedures approved by the ethics committee of the Heilongjiang University of Chinese Medicine. Blood samples
were obtained from 12 male rats (weighing approximately 220 ± 20 g) at different time points after oral administration of Stephaniae Tetrandrae Radix extract (0.82 g/kg) through orbital veins. Before administration 12 h, rats fasted but took free water. Blood samples (0.3 mL) of each rat were collected into 1.5 mL heparinized tubes at specific time points (0, 1, 2, 4, 8, 12, 16, 24, 36, 48, and 72 h) after oral administration of the extract. The collected blood samples were centrifuged at 3500 rpm for 10 min and immediately transferred into 2 mL tubes at 4°C and stored at −20°C for analysis.

### Preparation of plasma samples

The plasma samples were thawed at room temperature before we do the further operations. We prepared the samples by mixing 100 μL plasma sample with 10 μL of IS (200 ng/mL) and 200 μL methanol in a 10 mL glass tube. Mixture was subjected to ultrasonication for 3 min and then vortex-mixed for 1 min to fully precipitate the proteins. As a matter of convenience to extract the analyte, 2 mL ethyl acetate was first used for liquid–liquid extraction, then the obtained substance was ultrasonic for 3 min, and finally mixed by eddy current for 3 min. The sample was then centrifuged at 3500 rpm for 5 min to remove particulates. Under 40°C of nitrogen, the upper organic layer was removed and evaporated to dry. Next, methanol (100 μL) was used to dissolve the residue, and the sample was vortex-mixed, the process for a minute. After these samples have been treated, then shift into 1.5 mL Eppendorf tubes, centrifuged at 13,000 rpm (5 min, at 4°C), and the 0.22-μm membrane was used for filtered. Then, a 2-μL aliquot of the sample solution was injected into the UPLC-MS/MS system.

### Method validation

The method was adequately validated according to the Chinese Pharmacopoeia (2020); the validation parameters include specificity and selectivity, linearity, intra- and inter-day precision, accuracy, stability, matrix effects, and recovery. Validation runs were conducted on 3 days in a row. Each validation process included two sets of calibration standards and six replicates of QC samples at three different concentrations.

#### Selectivity

Selectivity is the ability to detect interference from endogenous materials or other components in the plasma sample at the retention time of the three analytes. Blank samples from six rats, spiked blank plasma samples with the three analytes and the IS, and plasma samples collected at 2.0 h in the following oral administration of Stephaniae Tetrandrae Radix extract were used for comparison and analysis. At the retention time of each of the three analytes, no interference peak was detected.

#### Linearity and lower limit of quantification

Seven different concentrations of standard plasma samples were used to establish calibration curves on 3 consecutive days. The linearity of standard plasma sample was determined by plotting the peak area ratio (y) of the analytes to the IS versus the nominal concentration (x) using a linearly weighted (1/
x² least square linear regression method in duplicate. The LLOQ was determined as the lowest concentration point of the calibration curve, and we evaluated the LLOQ precision by measuring the relative standard deviation (RSD). The calibration curves of the three analytes could be quantified with RSD not exceeding 20%.

**Precision and accuracy**

The intra- and inter-day precision and accuracy were validated through six replicates of four concentration levels of QC samples. Among these QC samples (LLOQ, LQC, MQC, and HQC), intraday precision and interday precision were analyzed on the same day and on 3 consecutive days, respectively. RSD was used as an indicator to assess the intra- and inter-day precision, which should not be more than 15%, and the accuracy was estimated by relative error (RE) within a requirement of 85%–115%, except for LLOQ. The accuracy of the LLOQ should be within 80%–120% and <20% of the precision.

**Extraction recovery and matrix effect**

Extraction recoveries were measured through the analysis of the plasma samples at three concentration levels in six replicates, respectively. Extracted samples (A), unextracted samples (B), and postextracted spiked samples (C) were chosen for the evaluation of the recovery and matrix effects in the same assay. The extraction recovery was estimated by comparing the peak areas of the analytes in the QC samples with those in samples spiked after extraction of blank plasma at equal concentrations. The extraction recovery and matrix effect of the IS were similarly evaluated at the same concentration.

**Stability**

QC samples of the three analytes at three QC levels (LQC, MQC, and HQC) were added to drug-free plasma samples. We designed four different assessments of stability: short-term stability (storage for 4 h at room temperature), long-term stability (storage for 2 weeks at −20°C), freeze-thaw stability (three cycles of freezing at −20°C and thawing), and postpreparative stability (storage for 12 h after sample preparation at 4°C). QC samples at a series of concentrations for stability analysis were obtained using a calibration curve established using freshly prepared standard samples.

**Application to pharmacokinetic study**

The DAS 2.1 (Mathematical Pharmacology Professional Committee of China, Shanghai, China) with noncompartmental analysis of plasma concentration versus time data was employed to obtain the pharmacokinetic parameters. By this means, the maximum plasma concentration (Cmax) and the time to reach Cmax (Tmax) were gained intuitively from the measured data. From the linear regression of the terminal points in a semi-log plot of the plasma concentration against time, we got the elimination rate constant (Ke). Subsequently, the formula T1/2 = 0.693/Ke was adopted to obtain the elimination half-life T1/2. The linear trapezoidal rule was used to evaluate the area under the plasma concentration-time curve (AUC0→∞) to the last measurable plasma concentration (C) from 0 h to 24 h. Based on the calculated data above, the area under the plasma concentration-time curve to time infinity (AUC0→∞) was calculated through the formula AUC0→∞ = AUC0→t + C/T1/2 Ke3.

**RESULTS AND DISCUSSION**

Optimization of ultra-high performance liquid chromatography-mass spectrometry analysis

Choosing a suitable ionization mode to obtain precursors and product ions of IS and analytes is the first step in the development of our method. Alkaloids are present in the form of positive ions. Therefore, we used the positive ionization mode to detect the three analytes. Figure 1 shows the ion pairs of the three IS and analytes in the positive ion mode. To obtain the richest relative abundance of precursor and product ions, the parameters of the fragment or and collision energies were optimized. Table 1 lists the MS/MS transitions and energy parameters of all compounds. It is key step for improving the performance of the method to select a proper IS. The IS should exhibit effective separation, stable response, extraction efficiency and benign reproducibility. So tramadol was chosen as the IS.

**Method validation**

**Selectivity**

The retention times of tetrandrine, fangchinoline, cyclanoline, and the IS were 3.37, 3.25, 3.12, and 3.45 min, respectively. Typical chromatograms obtained from blank plasma samples spiked with the analytes and IS as well as plasma samples collected at 2 h after the oral administration of Stephaniae Tetrandrae Radix extract are shown in Figure 2. No endogenous peaks in blank plasma chromatograms. However, the analytes were easily identified in rat plasma. Therefore, the method showed detection selectivity for the three analytes in rat plasma. There were no endogenous components interfering with the analysis in rat plasma under the above conditions of the mobile phase. Under the adjusted liquid phase conditions, our method clearly distinguished the three compounds without interference.

**Linearity and lower limit of quantification**

Table 2 shows the regression equation, correlation coefficients, and linear range of the three analytes. The LLOQ of tetrandrine, fangchinoline, and cyclanoline was 1.282, 1.023, and 1.281 ng/mL, respectively. The standard calibration curves of tetrandrine, fangchinoline, and cyclanoline were linear, as the values of the correlation coefficients exceeded 0.9900 (0.9923 for tetrandrine; 0.9936 for fangchinoline; and 0.9971 for cyclanoline). The range of calibration was suitable for the quantitative detection of the three analytes in rats after the oral administration of Stephaniae Tetrandrae Radix extract.

**Precision and accuracy**

The results of precision and accuracy assessments are listed in
Table 2: The regression equations, linear ranges, and correlation coefficients for the determination of the analytes in rat plasma

| Compounds   | Regression equation | r    | Linear range (ng/mL) | LLOQ (ng/mL) |
|-------------|---------------------|------|----------------------|--------------|
| Tetrandrine | $Y=5.50\times10^{-2}X+0.524$ | 0.9923 | 1.282-200.8          | 1.282        |
| Fangchinoline | $Y=4.91\times10^{-3}X+1.335$ | 0.9936 | 1.023-204.6          | 1.023        |
| Cyclanoline | $Y=8.50\times10^{-3}X+1.746$ | 0.9971 | 1.281-200.5          | 1.281        |

LLOQ: Lower limit of quantification

Table 3: Precision and accuracy of determination of tetrandrine, fangchinoline, and cyclanoline in rat plasma ($n=18$, 6 replicates per day for 3 days)

| Compounds   | Spiked concentration (ng/mL) | Measured (ng/mL) | Intra-day precision RSD (%) | Inter-day precision RSD (%) | Accuracy | RE (%) |
|-------------|------------------------------|------------------|-----------------------------|-----------------------------|----------|--------|
|             |                 |                  |                             |                             |          |        |
| Tetrandrine | 1.282            | 1.032±0.37       | 4.39                        | 9.21                        | 89.73    | −10.27 |
|             | 20.08            | 20.91±0.25       | 9.26                        | 8.34                        | 89.85    | −10.15 |
|             | 80.32            | 81.33±3.62       | 7.81                        | 8.76                        | 90.96    | −9.04  |
|             | 160.6            | 161.8±32.98      | 5.89                        | 7.70                        | 102.54   | 2.54   |
| Fangchinoline | 1.023            | 1.092±0.08       | 8.11                        | 8.94                        | 91.48    | −8.52  |
|             | 16.32            | 16.25±0.06       | 7.88                        | 9.65                        | 89.84    | −10.16 |
|             | 65.28            | 64.29±1.54       | 7.09                        | 7.74                        | 96.28    | −3.72  |
|             | 130.6            | 130.8±15.41      | 4.10                        | 5.65                        | 102.57   | 2.57   |
| Cyclanoline | 1.281            | 1.252±0.37       | 9.19                        | 8.33                        | 92.58    | −7.42  |
|             | 20.06            | 20.51±0.66       | 7.49                        | 6.27                        | 92.68    | −7.32  |
|             | 80.24            | 81.83±7.57       | 2.91                        | 5.87                        | 98.09    | −1.91  |
|             | 160.4            | 161.3±2.98       | 2.83                        | 3.64                        | 101.26   | 1.26   |

RSD: Relative standard deviation, RE: Relative error

Figure 2: Representative multiple reaction monitoring chromatograms of internal standard (1), tetrandrine (2), fangchinoline (3), and cyclanoline (4) in rat plasma. (A) Blank sample (without the three analytes and internal standard). (B) Lower limit of quantification sample (three analytes and internal standard). (C) Quality control sample (three analytes and internal standard). (D) Sample collected from a rat at 2 h after the administration of stephaniae tetrandrae radix extract.
Table 3. The intra- and inter-day precision (RSD %) of the three analytes was in the range of 2.83%–9.56% and 3.64%–11.68%, respectively. The accuracy ranged from −14.27% to 2.54%. Both the RSD and RE were typically within the requirement (<15%) defined by regulatory guidelines for all analytes. The developed method was considered precise and accurate consequently.

Extraction recovery and matrix effects

The extraction recovery and matrix effect data of the three analytes are summarized in Table 4. The extraction recoveries of the three analytes at three QC (LQC, MQC, HQC) levels were in the ranges of 106.3%–113.5%, 102.2%–107.4%, and 100.9%–105.3%, respectively. The RSD and RE were typically within the requirement (<15%) defined by regulatory guidelines for all analytes. The developed method was considered precise and accurate consequently.

Stability

The stability of these three analytes was detected and obtained under four conditions. Table 5 indicates that the three analytes were stable in the spiked plasma under various experimental conditions. All analytes can be stored at −20°C for at least a month stably. The RSD values of all stability studies vary between −9.75% and 11.7%, which met methodological requirements.

Pharmacokinetic study

The pharmacokinetics of the three compounds in 12 rats were analyzed through UPLC-MS/MS after a single oral administration of 0.82 g/kg Stephaniae Tetrandrae Radix extract. The oral dose of rats was obtained by the conversion of human dose in pharmacopoeia. The human dosage of Stephaniae Tetrandrae Radix in the Chinese Pharmacopoeia is 10 g/day; thus, the final oral dosage for rats was calculated as 0.82 g/kg based on the Meeh-Rubn method. Figure 3 shows the average plasma concentration-time curves of the three analytes.

The relevant pharmacokinetic parameters, including the $T_{1/2}$, $C_{\text{max}}$, $T_{\text{max}}$, $AUC_{0 \rightarrow \infty}$, and $Ke$, are listed in Table 6. As shown in the table, the $C_{\text{max}}$ values were 124.71 ± 16.08 ng/mL for tetrandrine, 84.56 ± 3.28 ng/mL for fangchinoline, and 57.61 ± 6.26 ng/mL for cyclanoline. This result may be due to the difference in the content of the three analytes.

### Table 4: Matrix effects and extraction recoveries for in rat plasma (n=6)

| Compounds       | Spiked concentration (ng/mL) | Matrix effect | Mean recovery |
|-----------------|-----------------------------|---------------|--------------|
| Tetrandrine     | 20.08                       | 98.3          | 106.3        |
|                 | 80.32                       | 96.4          | 110.5        |
|                 | 160.6                       | 98.9          | 113.5        |
| Fangchinoline   | 16.32                       | 95.8          | 107.4        |
|                 | 65.28                       | 101.1         | 102.2        |
|                 | 130.6                       | 97.9          | 107.1        |
| Cyclanoline     | 20.06                       | 95.8          | 105.3        |
|                 | 80.24                       | 94.4          | 100.9        |
|                 | 160.4                       | 97.8          | 101.4        |
| Matrix factor (%) | 0.97          | 6.2          | 8.7          |
| Normalized matrix factor | 0.91          | 5.2          | 9.1          |
| RSD (%)         | 8.7            | 7.5          | 5.2          |
| Mean (%)        | 8.7              | 7.5          | 5.2          |
| RSD (%)         | 7.5              | 5.2          | 4.2          |

### Table 5: Stabilities of the analytes in rat plasma (n=6)

| Analytes      | Concentration (ng/mL) | Stability (% RE) |
|---------------|-----------------------|------------------|
|               | Three Freeze-Thaw     | Short-term       | Long-term       | Postpreparation |
| Tetrandrine   | 20.08                 | 4.68             | 3.15            | 5.37            | 5.23            |
|               | 80.32                 | −1.10            | −1.65           | 0.87            | −3.31           |
|               | 160.6                 | −2.76            | −3.31           | −5.12           | −6.34           |
| Fangchinoline | 16.32                 | 11.7             | 5.35            | −6.73           | 4.22            |
|               | 65.28                 | 1.16             | 3.47            | 6.41            | 5.79            |
|               | 130.6                 | −5.11            | −4.15           | −7.12           | 5.35            |
| Cyclanoline   | 20.06                 | −1.96            | −2.37           | 3.25            | 4.12            |
|               | 80.24                 | −9.75            | −7.32           | 5.32            | 8.28            |
|               | 160.4                 | −0.49            | −3.92           | 4.41            | 5.62            |

RE: Relative error
Table 6: Pharmacokinetic parameters of the three constituents in rats after oral administration of fangji extracts (mean±standard deviation, n=12)

| Pharmacokinetic parameter     | Tetrandrine | Fangchinoline | Cyclanoline |
|-------------------------------|-------------|---------------|-------------|
| $C_{\text{max}}$ (ng/mL)      | 124.7±16.08 | 84.56±3.28    | 57.61±6.26  |
| $T_{\text{max}}$ (h)          | 10.39±3.04  | 10.17±3.04    | 6.40±3.16   |
| $T_{1/2}$ (h)                 | 20.83±6.89  | 24.29±6.89    | 11.94±4.47  |
| AUC$_{0\rightarrow t}$ (ng/h/L) | 2546.55±426.56 | 1551.05±156.83 | 724.36±70.19 |
| AUC$_{0\rightarrow \infty}$ (ng/h/L) | 2685.21±464.32 | 1712.53±159.44 | 783.04±73.36 |

$C_{\text{max}}$: Maximum plasma concentration; $T_{\text{max}}$: Time to reach $C_{\text{max}}$; AUC$_{0\rightarrow t}$: Area under the plasma concentration-time curve; AUC$_{0\rightarrow \infty}$: Area under the plasma concentration-time curve to time infinity.

Figure 3: Mean concentration-time profiles of tetrandrine, fangchinoline, and cyclanoline

in the Stephaniae Tetrandrae Radix extract. The $T_{\text{max}}$ vary from 6.40±3.16 min to 10.39±3.04 min, which showed that the difference in the absorption process may be due to the different structures of the three compounds. The $T_{1/2}$ values were 20.83±6.89 for tetrandrine, 24.29±6.89 for fangchinoline, and 11.94±4.47 for cyclanoline. The results suggested that the elimination of the three analytes was different in vivo. The AUC$_{0\rightarrow t}$ ranged from 724.36±70.19 to 2546.55±426.56 ng/h/L. The AUC$_{0\rightarrow \infty}$ ranged from 783.04±73.36 to 2685.21±464.32 ng/h/L. The results showed that the content of the analytes in vitro followed the order of tetrandrine>fangchinoline>cyclanoline. The values of $C_{\text{max}}$, AUC$_{0\rightarrow t}$, and AUC$_{0\rightarrow \infty}$ also followed the order of tetrandrine>fangchinoline>cyclanoline, showing the same trend as the content. Taken together, the above data showed that tetrandrine and fangchinoline (dibenzylisoquinoline alkaloids) can maintain a higher drug concentration longer than cyclanoline (a quaternary ammonium alkaloid) in vivo. We think that such a result may be closely related to the structural differences of the compounds. Tetrandrine and fangchinoline are dibenzylisoquinoline alkaloids with high polarity and a water-soluble alkaloid. These differences could cause the difference in $T_{\text{max}}$ values among the three analytes.

Conclusions

For the first time, a simple and sensitive UPLC-MS/MS method was developed for the determination of tetrandrine, fangchinoline, and cyclanoline in rat plasma. The method was accurate and rapid, and it met all the requirements for biological analysis. The method was then applied to a pharmacokinetic study of tetrandrine, fangchinoline, and cyclanoline in rat plasma. It is hoped that the results of this study can provide useful information for the future pharmacological studies of the prescription.

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Conflicts of interest
There are no conflicts of interest.

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