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Development and validation of a LC-ESI–MS/MS method for the determination of swertiamarin in rat plasma and its application in pharmacokinetics

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ABSTRACT

A new LC-ESI–MS/MS assay method has been developed and validated for the quantification of swertiamarin, a representative bioactive substance of *Swertia* plants, in rat plasma using gentiopicroside, an analog of swertiamarin on chemical structure and chromatographic action, as the internal standard (IS). The swertiamarin and IS were extracted from rat plasma using solid-phase extraction (SPE) as the sample clean-up procedure, and they were chromatographed on a narrow internal diameter column (Agilent ZORBAX ECLIPSE XDB-C_{18} 100 mm × 2.1 mm, 1.8 μm) with the mobile phase consisting of methanol and water containing 0.1% acetic acid (25:75, v/v) at a flow rate of 0.2 mL/min. The detection was performed on an Agilent G6410B tandem mass spectrometer by negative ion electrospray ionisation in multiple-reaction monitoring mode while monitoring the transitions of *m*/*z* 433 $[M+CH_3COO]^- \rightarrow 179$ and *m*/*z* 415 $[M+CH_3COO]^- \rightarrow 179$ for swertiamarin and IS, respectively. The lower limit of quantification (LLOQ) was 5 ng/mL within a linear range of 5–1000 ng/mL ($n = 7$, $r^2 \geq 0.994$), and the limit of detection (LOD) was demonstrated as 1.25 ng/mL (S/N ≥ 3). The method also afforded satisfactory results in terms of sensitivity, specificity, precision (*intra*- and *inter*-day), accuracy, recovery, freeze/thaw, long-time stability and dilution integrity. This method was successfully applied to determination of the pharmacokinetic properties of swertiamarin in rats after oral administration at a dose of 20 mg/kg. The following pharmacokinetic parameters were obtained (mean): maximum plasma concentration, 1920.1 ng/mL; time to reach maximum plasma concentration, 0.945 h; elimination half-time, 1.10 h; apparent total clearance, 5.638 L/h/kg; and apparent volume of distribution, 9.637 L/kg.

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1. Introduction

The *Swertia* plants, as the most important genus in the Gentianceae family, are widely used in traditional medicine in China and other Asian countries. They have a variety of proposed biological actions including anticholinergic, antimicrobial, stomachic, hepatoprotective and central nervous system effects [1,2]. Swertiamarin (Fig. 1A), an iridoid glycoside found in the plants of the *Swertia* genus, was a representative and abundant constituent of many *Swertia* species, such as *S. japonica*, *S. mileensis* and *S. chirata* [1,3,4], and was proven to have important and extensive pharmacological activities including hepatoprotective, antiedematogenic, antihypolidemic, antispastic, anticholinergic, antinociceptive, antibacterial, anti-inflammatory and antioxidant activities based on *in vitro* or *in vivo* pharmacodynamic experiments [5–10]. However, until now, even with the comprehensive research on bioactivity, there was little knowledge about its pharmacokinetics. Therefore, to research and describe the pharmacokinetic properties of swertiamarin thoroughly is necessary.

Several analytical methods for the quantification of swertiamarin have been reported, such as high-performance liquid chromatography with ultraviolet detection (HPLC–UV) [11,12], capillary electrophoresis (CE) [13] and high-performance liquid chromatography-tandem mass spectrometry (LC–MS/MS) [4,14,15]. Nevertheless, due to the lack of analytical sensitivity and low selectivity, HPLC/UV and CE methods mainly focus on the quantification of swertiamarin in raw materials or pharmaceutical preparations and are not suitable for the determination of swertiamarin in biological fluids.

LC–MS/MS is a well-established method in bioanalysis due to its inherent specificity, sensitivity and speed. However, to the best of our knowledge, previous LC–MS/MS methods were not fully validated for quantification of swertiamarin in plasma [4,14].

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Concerning the pharmacokinetic data of swertiamarin, the literature has only reported a half-life time after the intravenous administration of swertiamarin in rats ($n = 1$ at each time point) using arbitrary units derived from the peak area ratio of the analyte to the internal standard (IS) [15]. Thus far, the analytical methods of swertiamarin in biological fluids and the pharmacokinetic data of swertiamarin are still extremely limited.

In addition, swertiamarin was administered orally in all *in vivo* pharmacodynamic experiments [6–10], but there was no information related to the pharmacokinetic study after oral administration. Therefore, it is valuable to describe the pharmacokinetic properties of swertiamarin to support *in vivo* rat pharmacokinetic studies after an oral dose. In the present study, we have established and validated a new LC–MS/MS method for the quantification of swertiamarin and successfully applied the newly developed method to the pharmacokinetic study of swertiamarin in rats after oral administration. The method is appropriate for routine analysis in pharmacokinetic studies of swertiamarin.

2. Experimental

2.1. Chemicals and reagents

Swertiamarin (purity 98.5%) and gentiopicroside (Fig. 1B) (purity 99.7%) (IS) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol was purchased from Merck KGaAS (Darmstadt, Germany). Acetic acid, HPLC grade, was purchased from the Tedia Company (Ohio, USA). Distilled, deionised water was freshly generated by a Milli-Q reagent water system (Millipore, USA).

The blood samples used for the development and validation of the analytical method were obtained from Sprague–Dawley rats provided by the Central Animals House of Kunming Medical College. Heparin (Liquemine, 125,000 IU, The First Biochemical Pharmaceutical Co. Ltd., Shanghai, China) was used as an anticoagulant in all samples. Plasma was obtained by centrifugation and stored at −80 °C until the time for use.

2.2. Instrumentation and analytical conditions

The Agilent 1200 series HPLC system (Agilent, USA) was equipped with an autosampler, a degasser and two SL bin-pumps. Swertiamarin and gentiopicroside (IS) were separated on an Agilent ZORBAX ECLIPSE XDB-C_{18} column (100 mm × 2.1 mm, 1.8 μm). The column was kept at 40 °C. The mobile phase consisting of methanol and water containing 0.1% acetic acid (25:75, v/v) was used in isocratic elution at a flow rate of 0.2 mL/min. The sample solution (10 μL) was injected through an Agilent autoinjector into the mass spectrometer.

Mass spectrometric detection was performed on a G6410B triple quadrupole mass spectrometer (Agilent, USA) equipped with an electrospray ionisation (ESI) source. The ESI source was operated with a capillary voltage of 3800 V; the nebuliser pressure was 30 psi, and the dry gas temperature was 350 °C with a gas flow of 10 L/min. The compound parameters, i.e., fragmentor potential and collision energy (CE), were 84 V/5 eV, 78 V/5 eV for swertiamarin and IS, respectively. The mass spectrometer was operated at the ESI negative ion mode, and detection of the ions was performed in the multiple-reaction monitoring (MRM) mode, monitoring the transition of the *m/z* 433 precursor ion $[M+CH_3COO]^-$ to the *m/z* 179 product ion for swertiamarin and the *m/z* 415 precursor ion $[M+CH_3COO]^-$ to the *m/z* 179 product ion for IS. The data acquisition and quantification were performed using the Agilent Mass-Hunter Quantitative Analyst software (version B.01.04, Agilent Technologies, USA).

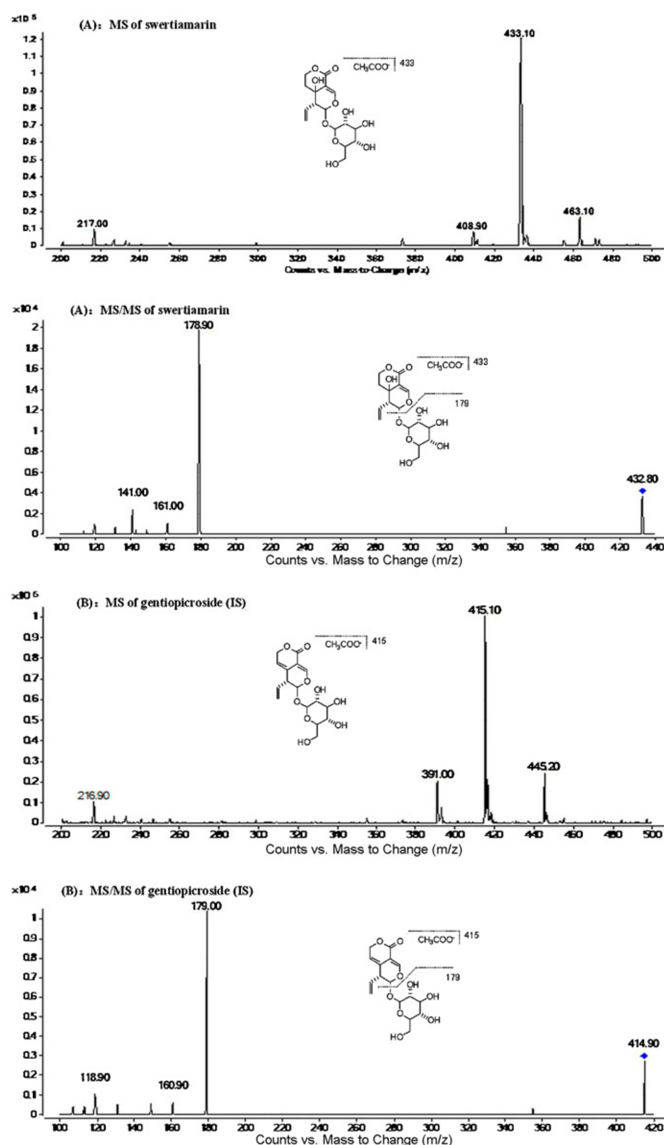

Fig. 1. MS and MS/MS spectra of (A) swertiamarin and (B) gentiopicroside (IS).

2.3. Preparation of standards and quality control (QC) samples

Stock solutions of swertiamarin and IS were prepared both in Milli-Q water at a concentration of 1 mg/mL and stored at 4 °C. A series of working standard solutions of swertiamarin ranging from 50 to 10,000 ng/mL and an IS solution at 1000 ng/mL were prepared by diluting their stock solutions with Milli-Q water. All the solutions were kept at 4 °C and were brought to room temperature before use. The plasma calibration standards of swertiamarin were prepared as follows: 10 μL of the working solution was evaporated to dryness by a gentle stream of nitrogen, and then 100 μL of blank rat plasma was added to obtain the concentrations of 5, 10, 20, 40, 100, 400 and 1000 ng/mL. Quality control (QC) samples were prepared in the same way as the calibration samples, representing low, middle and high concentrations of swertiamarin in plasma at 10, 100 and 800 ng/mL, respectively. All the spiked plasma samples were then treated according to the sample preparation procedure. Both the calibration standard samples and the QC samples

were applied in the method validation and the pharmacokinetic study.

2.4. Sample preparation

A simple solid-phase extraction (SPE) method was followed for the extraction of swertiamarin from rat plasma. A plasma sample (100 μL) was pipetted into a microcentrifuge tube, and then 10 μL of the IS working solution (1000 ng/mL) was added. The mixture was mixed for 15 s by a vortex. The SPE procedure involved conditioning the cartridges (Agilent C_{18}, 100 mg) with 1 mL of methanol, equilibrating with 1 mL of water, and then loading 100 μL of sample. After washing with 1 mL of water followed by vacuum drying, the analytes were then eluted with 1 mL of methanol. Subsequently, the collected eluent was concentrated to dryness by a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 100 μL of the mobile phase, and 10 μL of the sample solution was injected into the LC–MS/MS system for assay.

2.5. Validation procedures

A full validation according to the FDA guidelines was performed for the assay in rat plasma [16].

2.5.1. Specificity and selectivity

To investigate whether or not endogenous constituents interfered with the assay, six different blank rat plasma samples were analysed to detect the potential interferences at the LC peak region for the analytes and IS using the proposed extraction procedure and LC–MS conditions.

2.5.2. Matrix effect

The matrix effect was defined as the ion suppression/enhancement of the ionisation of the analytes, which was evaluated by comparing the corresponding peak areas of the postextraction-spiked samples to those of the standard solutions evaporated directly and reconstituted in the mobile phase. The matrix effect for swertiamarin was determined at QC of low, medium and high concentrations (10, 100 and 800 ng/mL, respectively), whereas the matrix effect over the IS was determined at a single concentration of 100 ng/mL in six replicates.

2.5.3. Calibration curve

The calibration curves were constructed by plotting the peak area ratio (analyte to IS) versus the spiked concentrations of swertiamarin in plasma. The final concentrations of the calibration standards obtained for plotting the calibration curve were 5, 10, 20, 40, 100, 400 and 1000 ng/mL. The results were fitted to the linear regression analysis using $1/x^2$ as the weight factor. The acceptance criteria for each back-calculated standard concentration were ±15% deviation from the nominal value, except at the lower limit of quantification, which was set at ±20%.

2.5.4. Precision and accuracy

Intra- and *inter*-day precision and accuracy were assessed from the results of six replicates of the low, medium and high QC samples (10, 100, 800 ng/mL, respectively) on three consecutive days. The criteria for the acceptability of the data included accuracy within ±15% of the relative error (RE) from the nominal values and a precision of within ±15% of the relative standard deviation (RSD), except for LLOQ, where accuracy and precision should not exceed ±20% of the deviation.

2.5.5. Limit of detection and lower limit of quantification

The lower limit of detection (LOD) of the MS analysis was defined as the analyte concentration in the plasma after the sample clean-up method that corresponds to three times the baseline noise ($S/N \geq 3$). The lower limit of quantification (LLOQ) of the assay was assessed as the lowest concentration on the calibration curve that could be quantitatively determined with an acceptable precision less than 20% and an accuracy within ±20%, which was established based on six replicates independent of the QC samples.

2.5.6. Recovery

The extraction recovery of the analytes, through the solid-phase extraction procedure, was determined by comparing the peak areas of extracted plasma from the QC samples ($n = 6$) with those obtained from the direct injection of the standard solutions without any preparation at the same concentrations. The recovery of swertiamarin was determined at three concentration levels of QC at low, medium and high concentrations, whereas the recovery of the IS was determined at a single concentration level.

2.5.7. Stability experiments

All plasma sample stability studies were conducted at QC of low, medium and high concentrations in triplicate. Plasma samples were stored according to the following four storage conditions: (1) the stability of swertiamarin in plasma during the sample preparation was assessed by detecting samples after storage for 4 h at room temperature; (2) for freeze/thaw stability, the plasma samples were determined through three freeze (−80 °C)–thaw (room temperature) cycles and were frozen for at least 12 h at −80 °C; (3) to evaluate the stability of the treated plasma samples in the autosampler, QC samples were prepared and placed in the autosampler for a period of 20 h, and then injected for analysis; (4) the long-term stability was performed by assaying the plasma samples after 20 days of storage at −80 °C. All the samples were analysed with the calibration curves that were freshly prepared. The analyte was considered stable when the percentage deviation was within ±15%.

2.5.8. Dilution integrity

Dilution of the biological matrix is required if some of the studied sample concentrations were expected to be higher than the upper limit of quantification. Dilution integrity experiments were carried out by a 5-fold dilution of the plasma samples with blank plasma for six replicates. The acceptable precision and accuracy were required to be within ±15%.

2.6. Pharmacokinetic study

The validated method was applied to a pharmacokinetic study of swertiamarin in rats. Six SD rats (200 ± 20 g), male and female, were purchased from the Central Animal House of Kunming Medical College. The study protocol was approved by the Institutional Animal Care and Use Committee of Kunming General Hospital of Chengdu Military Region. Before orally administering a single dose of swertiamarin (20 mg/kg), the rats were fasted for 12 h with access to water and then fasted for 2 h after administration. Blood samples were collected in heparin-containing tubes from the epicanthic veins of rats by capillary tubes before drug administration (0 h) and at 0.167, 0.333, 0.667, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after oral administration. Plasma was separated from the blood by centrifugation at 6000 × *g* for 5 min at room temperature. The plasma samples were stored at −80 °C until analysis. Plasma (100 μL) samples were spiked with IS and processed with the same sample preparation procedure. In addition to the plasma samples, QC samples were distributed among calibrators and unknown samples in the analytical run. The pharmacokinetic parameters were calculated for each subject by the DAS software (version 2.1.1, Drug and Statistics, Mathematical Pharmacology Professional Committee of China, Shanghai, China). A noncompartmental model was

employed to calculate the parameters. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly obtained from the experimental data. The elimination rate constant (K_e) was calculated by linear regression of the terminal semi-*log* plot of the plasma concentration versus time, and $t_{1/2}$ was calculated as 0.693/*K*e. The area under the curve (AUC) was calculated according to the logarithm of the linear trapezoidal method.

3. Results and discussion

3.1. Chromatographic conditions

We have investigated multiple chromatographic conditions, such as acetonitrile and methanol, using different buffers, such as ammonium acetate, acetic acid and formic acid, with a variable pH range and altered flow rates (in the range of 0.15–0.3 mL/min) to obtain the appropriate retention time, the best resolution and the highest sensitivity. The optimal mobile phase consisted of methanol and water containing 0.1% acetic acid (25:75, v/v) at a flow rate of 0.2 mL/min. Methanol, rather than acetonitrile, was chosen as the organic modifier because it led to lower background noise and resulted in the best resolution. The addition of 0.1% acetic acid helped to obtain better peak shape and to enhance the ionisation. Experiments were also performed with different C_{18} columns, such as Agilent ZORBAX SB-C_{18} (30 mm × 2.1 mm, 3.5 μm), Phenomenex Luna CN (150 mm × 2.0 mm, 3.0 μm), at low pH and in the presence of a high percentage of water in the mobile phase. A column with a narrow internal diameter (Agilent ZORBAX ECLIPSE XDB-C_{18}, 100 mm × 2.1 mm, 1.8 μm) was finally selected for the chromatographic separation because under the current LC conditions, the column provided excellent results in terms of response, retention time and peak shapes. The chromatographic conditions provided symmetric peak shapes, good sensitivity and suitable runtime for the swertiamarin and IS. Under the optimised conditions, no significant endogenous interference was found.

3.2. Mass spectrometric conditions

To find the most sensitive ionisation mode for the swertiamarin study, positive- and negative-ion ESI models were tested with various combinations of the mobile phase. In the positive LC-ESI–MS experiment, swertiamarin showed a very high tendency to form sodium adducts in methanol/water or in acetonitrile/water. The sodium adduct $[M+Na]^+$ was the main peak in the spectra. However, the signal intensity for the $[M+Na]^+$ ions in positive-ion ESI mode had low sensitivity and stability. We used an ammonium acetate buffer in combination with acetonitrile or methanol to find the most sensitive $[M+NH_4]^+$ ions in the positive-ion mode, but the ammonium adducts $[M+NH_4]^+$ of swertiamarin were not found. In a previous report, it was confirmed that swertiamarin showed a lower proportion of potassium and ammonium adducts [4]. In the negative-ion ESI mode, by investigating the full-scan mass spectra of swertiamarin, we found that when using the acetic acid buffer, the full-scan negative-ion mass spectrum showed that both swertiamarin and IS were acetate radical adducts of the molecular ion $[M+CH_3COO]^-$ of *m*/*z* 433 and *m*/*z* 415, respectively. After fragmentation in the collision cell, the most abundant and stable product ions were at *m*/*z* 179 and *m*/*z* 179 for swertiamarin (Fig. 1A) and IS (Fig. 1B), respectively. The CID parameters were optimised to enhance the highest response and specificity using the MRM mode comprising the precursor and product ions. The most suitable mass spectrometric conditions were determined by optimising all the parameters of the mass spectrometer, such as collision energy, fragmentor potential, source temperature, drying gas temperature, drying gas flow, nebuliser pressure and capillary voltage, to obtain a much higher and more stable response.

Table 1
Matrix effect and recovery (n = 6) of the assay method.

Concentration (ng/mL)	Matrix effect		Recovery	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)
10.0	103.8	2.6	89.3	4.4
100.0	97.9	4.4	87.1	6.4
800.0	96.5	2.2	85.2	5.7

3.3. Internal standard

For selecting the ideal internal standard, a similar chemical behaviour and a suitable retention time were of significant importance. Gentiopicroside, an iridoid glycoside, was selected as the IS because it was similar to the analyte in chemical structure, chromatographic behaviour, MS characteristics and recovery.

3.4. Sample preparation

Solid-phase extraction followed by liquid–liquid extraction and protein precipitation has been applied for the extraction of swertiamarin in biological fluids in our experiments. It is not advisable for the plasma samples consisting of swertiamarin to be pretreated by liquid–liquid extraction using organic solvents because the swertiamarin is a strongly hydrophilic compound, which renders it extremely difficult to extract from aqueous biological media and results in low recovery. The extraction recovery of swertiamarin was approximately 30% by liquid–liquid extraction using ethyl acetate. The protein precipitation was also not suitable for the extraction of swertiamarin in biological fluids due to severe matrix suppression; the matrix effect on swertiamarin was found to be approximately 60%. In this study, the SPE procedure was effective, succinct, consumed only small amounts of solvents or biological matrix, and had a short turn-around time for the analysis.

3.5. Method validation

3.5.1. Selectivity and specificity

In the present study, the specificity and selectivity were examined using independent plasma samples from six different rats. Fig. 2 shows a typical chromatogram for the drug-free plasma (Fig. 2A), drug-free plasma spiked with swertiamarin and IS (Fig. 2B) and an *in vivo* rat plasma sample after oral administration of swertiamarin (Fig. 2C). As shown in Fig. 2, there is no significant interference from plasma found at retention times of either the analyte or the IS. The retention time of swertiamarin and the IS were approximately 6.1 and 7.6 min, respectively. The results indicated that the method exhibited good specificity and selectivity and was applicable to plasma samples for the pharmacokinetic study.

3.5.2. Matrix effect

In this study, the matrix effect was evaluated by analysing the low (10 ng/mL), medium (100 ng/mL) and high (800 ng/mL) QC samples. The results are summarised in Table 1. The average matrix effect values were 103.8%, 97.9% and 96.5% for swertiamarin at low, medium and high QC, respectively. The matrix effect on IS was found to be 95.6% at the tested concentration of 100 ng/mL. The matrix effect on the ionisation of the analyte was not obvious under these conditions.

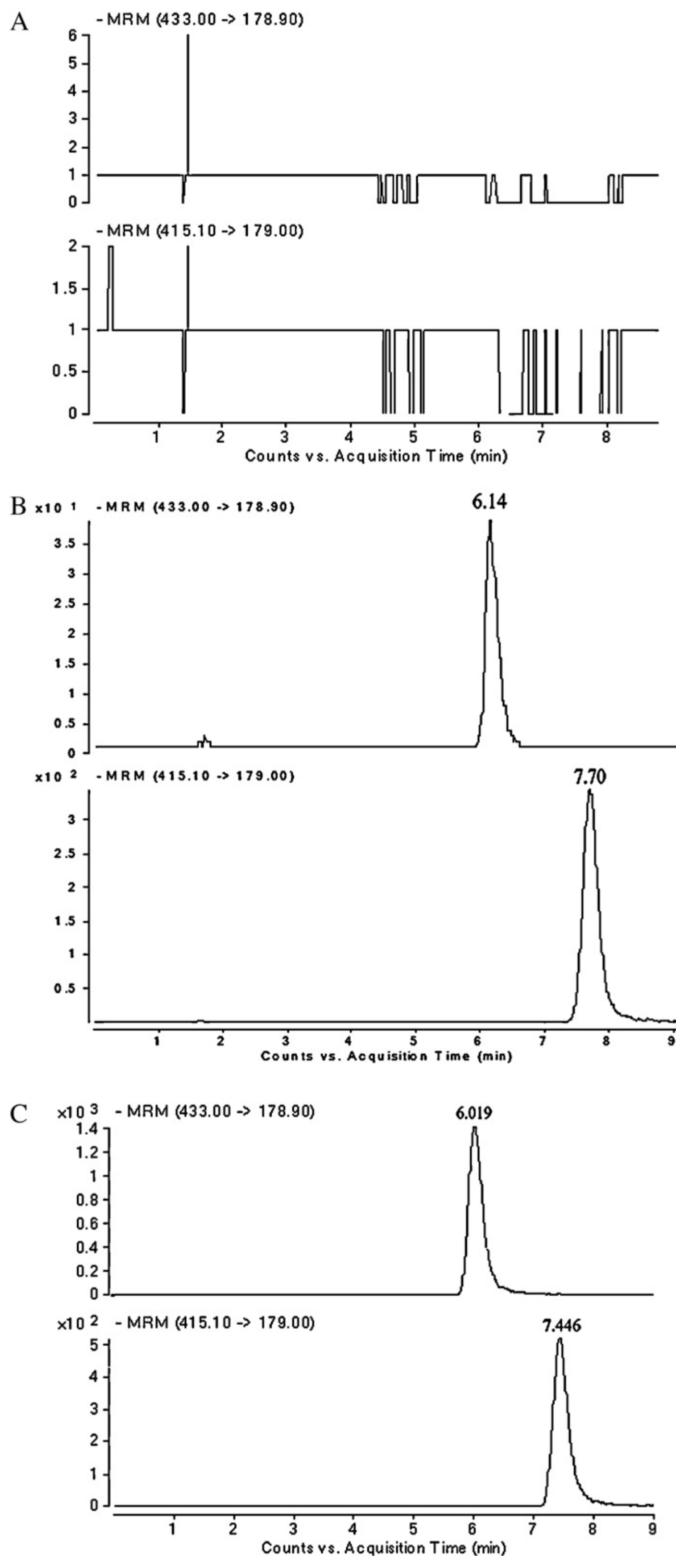

Fig. 2. Typical multiple-reaction monitoring (MRM) chromatograms of swertiamarin and gentiopicroside (IS): (A) a blank rat plasma sample; (B) a blank plasma sample spiked with swertiamarin (at the LLOQ of 5 ng/mL, Rt. 6.14 min) and IS (100 ng/mL, Rt. 7.70 min); (C) a rat plasma sample from 20 min after oral administration of 20 mg/kg swertiamarin spiked with IS.

Table 2
Precision and accuracy of swertiamarin in rat plasma ($n = 6$).

Concentration (ng/mL)		Precision (RSD, %)		Accuracy (RE, %)	
Spiked	Measured (mean ± SD)	*Intra*-day	*Inter*-day	*Intra*-day	*Inter*-day
10.0	10.2 ± 0.5	4.0	5.2	1.8	1.6
100.0	98.6 ± 7.0	4.0	7.1	6.4	−1.4
800.0	735.6 ± 36.1	2.9	4.9	−3.0	−8.1

3.5.3. Linearity, limit of detection and lower limit of quantification

The LOD of the swertiamarin assays was demonstrated as 1.25 ng/mL (S/N ≥ 3), and the LLOQ was 5 ng/mL. The calibration curves ranged from 5 to 1000 ng/mL, using seven calibration standards. The (mean ± S.E., $n = 5$) regression equation for calibration curves in plasma was $y = (1.273 \pm 0.021)x + (0.016 \pm 0.012)$, where y is the peak-area ratio [(peak area of analyte)/(peak area of IS)] versus concentration, and x is the concentration of the analyte. The correlation coefficient (r^2) was ≥0.994 for all the calibration curves, and the observed deviation was within ±15% for all the calibration concentrations. At LLOQ, the accuracy was within ±12.2%, and the precision was less than 6.2%. The method was found to be sufficiently sensitive for the determination of the pharmacokinetic analysis of swertiamarin in rats.

3.5.4. Precision and accuracy

The accuracy and precision data for *intra*- and *inter*-day plasma samples are presented in Table 2. The assay values for both occasions (*intra*- and *inter*-day) were found to be within the accepted variable limits. The data indicated that the present method has a satisfactory accuracy, precision and reproducibility.

3.5.5. Recovery

The extraction recovery was determined in six replicates by comparing the peak areas of the extracted plasma at 10, 100 and 800 ng/mL with those obtained from the direct injection of standard solutions without preparation at the same concentrations. The extraction recoveries of swertiamarin were 89.3%, 87.1% and 85.2% for QC samples at the concentrations of 10, 100 and 800 ng/mL, respectively. All the data are summarised in Table 1, and the extraction recovery of the IS was 84.4 ± 4.9%. The recovery of the determination of swertiamarin and IS in rat plasma was consistent, precise and reproducible.

Table 3
Stability of swertiamarin in rat plasma ($n = 3$).

Storage conditions	Concentration (ng/mL)		RE (%)
	Spiked	Measured (mean ± SD)	
0 h (for all)	10.0	9.6 ± 0.2	−4.0
	100.0	100.3 ± 1.1	−0.3
	800.0	703.7 ± 20.6	−12.0
At room temperature for 4 h	10.0	9.8 ± 0.2	−2.2
	100.0	99.6 ± 9.4	−0.4
	800.0	763.1 ± 6.4	−4.6
After three freeze/thaw cycles in plasma	10.0	8.7 ± 0.2	−12.5
	100.0	94.9 ± 1.8	−5.1
	800.0	749.9 ± 57.8	−6.3
In the auto-sampler for 20 h	10.0	10.0 ± 1.2	0.3
	100.0	92.8 ± 1.0	−7.2
	800.0	709.8 ± 3.8	−11.3
Long-term stability (at −80 °C for 20 days)	10.0	10.1 ± 0.3	0.5
	100.0	98.8 ± 2.7	−1.2
	800.0	778.1 ± 8.6	−2.7

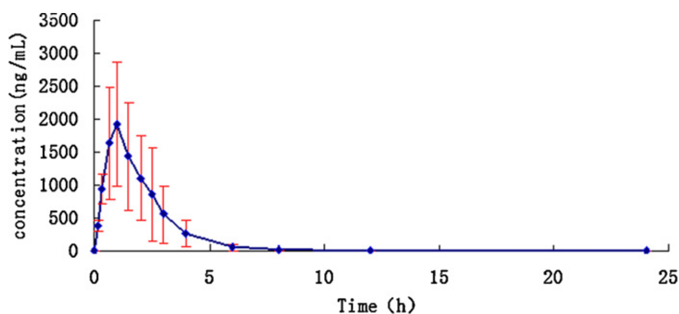

Fig. 3. Mean plasma concentration–time profile of swertiamarin after oral administration of 20 mg/kg to rats. Each point represents mean ± SD (n = 6).

Table 4
Main pharmacokinetic parameters of swertiamarin after oral administration of 20 mg/kg to rats (n = 6, mean ± SD).

Parameters	Mean ± SD
$t_{1/2z}$ (h)	1.104 ± 0.229
$V_{z/F}$ (L/kg)	9.637 ± 4.322
$CL_{z/F}$ (L/h/kg)	5.638 ± 2.151
$AUC_{0-\infty}$ (μg/L h)	3593.7 ± 985.4
$MRT_{0-\infty}$ (h)	1.929 ± 0.364
T_{max} (h)	0.945 ± 0.136
C_{max} (μg/L)	1920.1 ± 947.0

3.5.6. Stability

QC samples at three concentrations were analysed in three replicates for studying the possible conditions to which the samples might be exposed during storage and handling. It was found that swertiamarin was stable in rat plasma after being stored at room temperature for 4 h (BT-4 h), after repeated three freeze–thaw cycles (FT-3) and after being stored at −80 °C for 20 days. In addition, the treated samples were found to be stable in the autosampler for a period of 20 h, and the results were found to be within the assay variability limits during the entire process. All results of the stability tests are summarised in Table 3.

3.5.7. Dilution integrity

Dilution integrity experiments were carried out in six replicates by a 5-fold dilution with blank plasma, and assay precision and accuracy were tested using the same sample pretreatment method. For diluted samples, the precision was less than 4.3%, and the accuracy was within ±7.1%. The results suggested that samples whose concentrations exceeded the upper limit of the calibration curve could be re-analysed by an appropriate dilution.

3.6. Pharmacokinetic study

We applied the newly developed LC-ESI–MS/MS method to the pharmacokinetic study of swertiamarin and successfully obtained a series of the pharmacokinetic data of swertiamarin in six SD rats after oral administration of 20 mg/kg. The mean plasma concentration–time profiles of swertiamarin after oral administration is illustrated in Fig. 3, and the major pharmacokinetic parameters of swertiamarin after oral administration were calculated by a noncompartmental model and are presented in Table 4. The results indicated that swertiamarin was rapidly absorbed into the circulation system after oral administration. It reached its peak concentration at approximately 0.95 h and showed a short half-life that was almost less than 1.50 h.

4. Conclusions

A newly validated LC-ESI–MS/MS bioanalytical method for the quantification of swertiamarin in rat plasma had been developed systematically using solid-phase extraction as a sample clean-up procedure. This method afforded satisfactory results in terms of sensitivity, precision, accuracy, reproducibility and recovery. For the first time, this method was successfully applied to determination of the pharmacokinetic properties of swertiamarin in rats after oral administration at a dose of 20 mg/kg. In the past, swertiamarin was administrated as a daily dose in all pharmacodynamic experiments. The pharmacokinetic parameters determined in this study will help guide design of dosing in future studies.

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