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A new quantitation method of protodioscin by HPLC–ESI-MS/MS in rat plasma and its application to the pharmacokinetic study

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Abstract

A specific high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS method) was established for determining the concentration of protodioscin (PG) in rat plasma after intragastric administration of its standard form. Ginsenoside Rb₁ was selected as the internal standard (IS). The plasma sample was prepared using one-step deproteinization procedure by adding three parts of acetonitrile to precipitate proteins. The chromatographic separation was accomplished on an Inersil ODS-3 C₁₈ column (250 × 4.6 mm, 5 μm) with a mobile phase composed with acetonitrile and water containing 0.1% formic acid under a gradient elution mode at a flow rate of 1 mL min^{−1}. A 3:1 portion of the eluent after a microsplit was detected on a triple quadrupole tandem mass spectrometer coupled with electrospray ionization (ESI) in positive ion and multiple reaction monitoring (MRM) scanning modes. The mass transitions were selected as 888.1 → 1050.2 for PG and 948.2 → 1110.3 for IS, respectively. After careful validation, the plasma samples were always stable under different storage conditions. These analytical results rendered sensitive, selective, and reliable values by this established method which displayed high accuracy, adequate extracted recoveries, and almost negligible matrix effects. This method was applied to the pharmacokinetic studies on PG level in the rat plasma and its pharmacokinetic effect. The results of our studies suggest that the present method may be a useful tool for further clinical study of PG.

Keywords

Protodioscin; Ginsenoside Rb₁; HPLC-MS/MS; Pharmacokinetics; Rat plasma

1. Introduction

Protodioscin (PG, Fig. 1) is known as a typical furostanol steroid saponin with the chemical name of 25(R)-26-O-β-D-glucopyranosyl-furost-⁵⁽⁶⁾-en-3β, 22α, 26-triol-3-O-α-L-rhamnopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside [1].

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Although this compound exists abundantly in many oriental vegetables as well as medicinal plants, it is mainly isolated from the roots of three *Dioscorea* plants including *Dioscorea nipponica* Makino, *Dioscorea panthaica* Prain et Burkill, and *Dioscorea zingiberensis* C.H. Wright. This hydrophilic steroid saponin has been successfully developed into famous Chinese Herb Medicines, such as Dioscornin Tablet, Di'ao Xinxuekang Capsule, and Dunye Guanxinning Tablets, for treating cardiovascular diseases (CVD) since 1970s [2–4]. Modern pharmacological investigations revealed that PG has a cytotoxic activity against many cell lines from leukemia and solid tumors like HL-60 according to the accumulating data *in vitro* [5]. What's more, methyl protodioscin, an ester derivative of PG bearing a methoxy group (–OCH₃) in place of a hydroxyl group (–OH) at the C-22 position, reveals anti-proliferative effect against HepG2 and K562 cells through causing G2/M arrest as well as inducing apoptosis [6–7]. This evidence demonstrates that PG and its derivative are potential anticancer agents. In addition, its beneficial effect against ICVD (Ischemic Cerebrovascular Disease) has been studied in our laboratory.

Since few chromophores in the structure of PG cause low UV absorption, HPLC-ELSD has been often used to determine the PG in crude extracts of raw herbs, medicine or food *in vitro* [8]. However, due to the low detection limit of ELSD, this method is not suitable for detecting the trace amount of PG present in biological matrices such as blood plasma, urine, feces, and tissues *in vivo* [9]. So it is urgent to develop a new bio-analytical method to solve this intractable problem and further explore the pharmacokinetic profile of a single active constituent, which is crucial for understanding its pharmacological function and indispensable in drug development. With the recent advances in analytical techniques, high performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS method) has become a preferred tool in drug analysis due to its improved sensitivity, selectivity, and specificity [10–11]. This powerful technique has been considered as a promising method and applied in a wide range of fields like environmental and pharmaceutical research, especially for quantitating drugs or their metabolites from biological samples [12–13]. However, to the best of our knowledge, no report about determining the concentration of PG in rat plasma and its pharmacokinetic effects after oral administration has been published until now. Although the pharmacokinetics of PG in rat plasma has been reported after intravenous administration [14], the effects may be different according to the administration methods. When PG was orally administered, it should first pass through the digestive system, and then the lipid bilayer of cell membrane. And finally, its prototype could be found in the blood sample. On the contrary, its prototype could be determined in blood sample immediately after intravenous administration. Since the pharmacokinetic parameters must be different according to the above two administration methods, it was essential to carry out the present research.

Thus, the objective of the current study is to establish a reproducible, simple, efficient and fast HPLC–MS/MS method to measure the PG concentration in rat plasma through optimizing the extraction and separation conditions following its oral administration. After validation based on the USFDA guidelines, this new method was applied to pharmacokinetic studies on PG through calculating its parameters, and evaluating its pharmacokinetic effects in rats.

2. Materials and methods

2.1. Chemicals and reagents

PG was isolated from the rhizomes of *Dioscorea zingiberensis* C. H. Wright in our laboratory with the purity of over 98% determined by HPLC-ELSD, and its chemical structure was identified by MS and NMR. The ginsenoside Rb₁ (Fig. 1, purity >98%, NO. 0704-9508) was chosen as the internal standard (IS) and purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile (NO. MS1122-001) and methanol (NO. MS-1922) were chromatographic grade and offered by Sigma Aldrich (St. Louis, MO, USA) and Merck Company (Darmstadt, Germany), respectively. Formic acid (NO. XK13-2014316) was of MS grade and purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Distilled water used throughout the experiment was prepared from a Milli-Q purification reagent water system (18.2 Ω , Bedford, MA, USA). All other chemicals were higher than analytical grade and obtained from commercial sources.

2.2. Animals

The healthy adult male Sprague–Dawley (SD) rats, weighting 250–280 g and 8–10 weeks old, were supplied by the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China). They were raised single in polyethylene cage under a constant and friendly SPF environment (24 ± 1 °C, 55–65% relative humidity, and a 12 h light:12 h dark cycle). The animals were acclimated for this normal condition at least one week with free access to standard rodent chow and purified tap water. Twelve hours prior to this experiment, the food was withdrawn and the rats were fasted overnight but with water *ad libitum*. All experimental procedures and protocols were reviewed and approved by the Animal Ethics Committee of the Fourth Military Medical University (approved on September 10, 2014, No. FMMU 2014-09), which was in accordance with the guidance for the Care and Use of Laboratory Animals.

2.3. Instrument and HPLC–MS/MS

2.3.1. Chromatographic conditions—The chromatographic analysis was performed on a Waters 2695 HPLC system equipped with a quaternary pump, a vacuum degasser, a thermostatted column compartment, and an autosampler. The sample separation was accomplished on an Inersil ODS-3 column (250 $\times$ 4.6 mm, 5 μ m). The mobile phase consisted of acetonitrile (A) and water containing 0.1% formic acid (B), and was eluted at a flow rate of 1 mL min^{−1} in a linear gradient mode. The optimal gradient elution program was as follows: 0–5 min, 20–30% A; 5–8 min, 30–40% A; 8–15 min, 40–60% A. The injection volume was 10 μ L. A subsequent re-equilibration was performed before next injection, and the needle was rinsed with 50% methanol. The column and autosampler temperature was optioned at 25 °C and 4 °C, respectively.

2.3.2. Mass spectrometry conditions—Mass spectrometric analysis was carried out on a Varian triple quadrupole mass spectrometer (320-MS) coupled with electrospray ionization (ESI) interface which was connected to the HPLC system. One third of the eluent from the HPLC column was introduced into the inlet of the mass spectrometer after a three-

switch microsplit. The multiple reaction monitoring (MRM) mode and positive ionization conditions were finally chosen to monitor the PG and IS analytes. After optimization the detective parameters were operated as follows: heated capillary temperature and voltage were set 350 °C and 0.8 kV, while ion spray voltage and temperature were 4500 V and 300 °C, respectively. Nitrogen for desolvation and dried gas were used at flow rates of 8 and 11 L min⁻¹, respectively. Higher purity argon was used as the collision gas for collision-induced dissociation (CID) at approximate pressure of 1.5 mm Torr. The data acquisition and processing were executed on the Varian MS workstation software.

2.4. Preparation of stock solution and plasma sample

Appropriate amounts of PG (2.53 mg) and IS (2.54 mg) were each dissolved in methanol to make the stock solution. The working solutions for these two analytes were prepared at the concentration of 506 and 508 ng mL⁻¹, respectively. By successive dilution of these stock solutions with methanol the quality control (QC) samples and calibration standards were prepared.

A 200 µL aliquot of blank rat plasma sample was dispensed into 1.5 mL Eppendorf tube (EP tube), and 20 µL of IS work solution and 600 µL of acetonitrile were added. The combined solution was thoroughly mixed on a vortex shaker for 90 s and centrifuged at 5000 rpm for 15 min at 4 °C. The protein-free supernatant was collected and transferred into another clean EP tube, and evaporated to dryness under a gentle nitrogen stream in a water bath at 40 °C. The residues were reconstituted with 200 µL of methanol following vortexing for 90 s, and again centrifuged under the previous conditions to eliminate precipitation. Subsequently, the clear supernatant was pipetted into an auto-injector vial with a 200 µL polypropylene insert. A 10 µL aliquot of each sample solution was injected into the HPLC–MS/MS system for further analysis.

All the solutions were stored in a refrigerator at 4 °C for the subsequent use.

2.5. Preparation of calibration standards and QC samples

Appropriate amounts of working solution at the volume of 10, 20, 40, 80, 160, and 320 µL were each spiked into 200 µL aliquot of blank rat plasma to prepare the series of plasma calibration standard solutions. These mixed samples were processed according to the procedures described in Section 2.4 from the initial step “20 µL of IS work solution and...”. Then, the standard solutions at corresponding concentration of 25, 50, 100, 200, 400, and 800 ng mL⁻¹ were finally obtained, while the concentration of IS was always maintained at 50 ng mL⁻¹ throughout the study.

The QC samples were prepared from 20, 60, and 160 µL of working solution by spiking into a 200 µL aliquot of blank rat plasma in the same manner as the calibration standards to investigate the method validation. The levels of the QC samples were optioned at three different concentration of 50, 150, and 400 ng mL⁻¹ (low, medium, and high), respectively.

2.6. Method validation

The full validation for this HPLC–MS/MS method in the present research was performed in accordance to the requirements of US of Food and Drug Administration (FDA) for the analysis of biological samples [15].

2.6.1. Selectivity—Selectivity is ability that an analytical method could differentiate the target analytes from other unrelated components present in the sample. This analysis was carried out via evaluating six batches of blank rat plasma from different sources. The chromatograms of blank plasma samples were compared with those that spiked with analytes and plasma sample at 10 h after an oral dosage to investigate whether or not the target analytes had the same retention time with the endogenous interference compounds from plasma sample.

2.6.2. Precision and accuracy—The precision and accuracy of intra- and inter-day values were evaluated of QC samples at three concentration levels in six replicates within a day and three successive days. The precision was expressed as the relative standard deviation (RSD) to detect the distribution of measured concentration, while the accuracy as the relative error (RE) of the calculated mean values with the known one. For each QC, the values of RE and RSD did not exceed 15%.

2.6.3. Linearity of calibration curve, limit of detection (LOD) and lower limit of quantitation (LLOQ)—The calibration curve of PG was constructed by analyzing the standard plasma samples spiked with PG at gradient concentrations on three consecutive days. The linearity was calculated via plotting the peak area ratio of PG to IS (y) versus the nominal concentration of PG (x) with weighted factor ($1/x^2$) least square linear regression method. The LLOQ was defined as the lowest concentration of calibration curve which was regarded as the indicator to express the sensitivity of an assay. Its results were quantified based on a signal-to-noise ratio ($S/N < 10$) and accepted in a suitable precision ($RSD < 15\%$) and accuracy ($RE < 15\%$). The LOD was defined as the concentration that could be determined and measured based on the $S/N < 3$. This analysis was performed by evaluating six replicate samples.

2.6.4. Stability—The stability test was performed on QC samples in six replicates which were exposed to different conditions. The short-term stability was determined after exposure of the samples to ambient temperature for 4 h. The long-term stability was analyzed after storage of untreated samples at $-20\text{ }^{\circ}\text{C}$ for 30 days. The freeze–thaw stability was assessed after three complete freeze/thaw cycles on consecutive days (one cycle: freezing at $-20\text{ }^{\circ}\text{C}$ for 24 h and thawing at room temperature for 2 h). The post-preparation stability was investigated after storage of the extracted samples in the auto vial at $4\text{ }^{\circ}\text{C}$ for 24 h (Auto vial stability).

2.6.5. Extraction recovery and matrix effect—The corresponding amount of working solution was spiked into the blank rat plasma to obtain the QC samples which were processed as described in Section 2.4. Then denote the concentration of PG obtained from the freshly prepared calibration curve as A, the concentration when the analyte was added

into the pre-extraction and protein-free of QC rat plasma samples at equivalent concentrations as B, and the concentration from the pure standard solution without the biological fluid at the same level as C. The extraction recovery was defined as the ratio of A versus B (A/B), while the matrix effect as A/C. This analysis was executed in six replicates of QC samples.

2.7. Drug administration and application to a pharmacokinetic study

The SD rats were randomly allocated into three groups each containing 6 animals, and each group orally received the PG dissolved in purified water at the different doses of 50, 100, and 200 mg kg⁻¹. Then, 300 µL of blood sample was obtained from each rat under ether anesthesia through puncturing the retro orbital sinus at different time points of 0 h (prior to administration), 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h, 36 h, and 48 h after PG administration. The blood samples were collected into the EP tube containing an appropriate amount of sodium heparin. The plasma was immediately handled according to the method in Section 2.4. The curve of drug plasma concentration versus time was drawn using the plasma calibration curve. The primary pharmacokinetic parameters were calculated as follows: the peak plasma concentration (C_{max}); the time to peak concentration (T_{max}); the elimination half-life ($t_{1/2}$); the terminal elimination rate constant (k_e); mean residence time (MRT); clearance (CL); the area under the plasma concentration–time curve from time 0 to the time of the last measurable concentration (AUC_{0-t}); and from time 0 to infinity ($AUC_{0-\infty}$). These indicators were evaluated by non-compartmental analysis using the computer program *kinetica* (4.4.1).

2.8. Data analysis

The values were expressed as mean $\pm$ standard deviation (SD). All the data analyses were performed on SPASS software (19.0).

3. Results and discussion

3.1. Selection of internal standard (IS)

Using an appropriate IS is crucial for quantifying compound in plasma or tissues by the HPLC–MS/MS. The IS should display similar chromatographic behaviors (but with individual retention time different from endogenous interferences), structure, and ionization characteristics to PG. In addition, its extraction efficiency from the plasma sample and the response sensitivity in MS should be high under the same established methods. In order to satisfy these requirements, ginsenoside Rb₁ was finally chosen as the IS after investigating several compounds including ginsenoside Re, swertiamarin and gentiopicroside.

3.2. Method establishment and optimization

3.2.1. Chromatographic conditions—The use of an appropriate mobile phase is essential for successful analysis of the target compound. In order to achieve the satisfactory resolution of PG as well as IS, sensitive signal responses, and systemic peak shape, the solvent systems composed in acetonitrile–water and methanol–water in various proportions were investigated. After a series of attempts, the former produced more promising results with lower background noise and desired separation from the interfering peaks in plasma

matrix, as compared with the latter. Therefore, it was chosen as the mobile phase. Since the polarity of the two analytes differed greatly, a gradient elution mode was applied to shorten the run time of the entire analysis. It is generally known that ionization in the ESI mode often happens under solution conditions, and proper additives could generate higher responses in MS. By adding formic acid to the mobile phase, sharper peak shape and higher sensitivity were obtained. In addition, the flow rate and column temperature were also adjusted to receive acceptable outcomes. The best conditions to yield significant signal suppression and enhancement as described in Section 2.3.1 were finally attained after a series of trial and error experiments.

3.2.2. Mass spectrometric conditions—In order to obtain highly sensitive ionization pattern of PG and IS, both positive and negative modes were investigated under MRM as the scanning way in the current study. After comparison, the analytes was produced strongly intensive signal and more information in the positive mode where the precursor ions of $[M+H]^+$ were the most abundant in Q1 and ion fragments in Q3 of the full-scan spectra. Generally speaking, the collision energy in CID (Q2) exhibited obvious effect on the relative intensity of the product ions. Following several attempts, the energy for PG and IS in Q2 was selected as 24 and 26 EV, respectively. The corresponding full-scan product ion spectra and the postulated fragmentation pathways of PG and IS were illustrated in Fig. 2. The sensitive transition ions selected for quantification in MRM were ultimately determined as $741.9 \rightarrow 1050.2$ for PG and $786.0 \rightarrow 1110.3$ for IS. The fragmentation ion at m/z 741.9 of PG was produced from the protonated molecular ion $[M+H]^+$ (m/z 1050.2), which was attributed to the loss of one glucosyl and one rhamnosyl radicals in sequence, while the fragmentation ion at m/z 786.0 of IS was generated from the protonated molecular ion $[M+H]^+$ (m/z 1110.3) due to continuous loss of two glucosyls.

3.2.3. Plasma sample preparation—Establishing a simple method to remove the potential interference material such as plasma proteins was a primary step prior to the HPLC–MS/MS analysis, and there are several disposal ways including liquid–liquid extraction, Solid phase extraction, and direct protein precipitation were examined and compared. Considering simplicity and efficiency, protein precipitation with organic solvents were employed. Initially, two major solvents, i.e. methanol and acetonitrile, in their single or mixed form at various ratios were investigated. It demonstrated that reproducible and consistent recoveries of PG and IS were obtained with reduced endogenous-related substances when the acetonitrile alone was used as the precipitant. Also, the volume ratio between acetonitrile and plasma was explored. The results turned out that the 3:1 (v/v) ratio could minimize the matrix effects. Thus, the method described in Section 2.4 was eventually applied throughout this bio-analysis.

3.3. Method validation

3.3.1. Selectivity—The typical chromatograms of blank plasma, spiked plasma containing PG as well as IS, and a plasma sample collected at 10 h after oral administration of PG in MRM mode were displayed in Fig. 3. It was clear that there was no significant direct interference at the retention time of target analytes, which was caused by endogenous substances present in the drug-free rat blank plasma under the chromatographic conditions.

3.3.2. Precision and accuracy—The data to evaluate the precision and accuracy of inter- and intra-day variability for PG and IS levels carried out on QC samples at three concentration levels were listed in Table 1. The precision in RSD form was in the range of 0.26–2.74, while the accuracy in RE form in the range of –1.35–3.69. These values were accorded to the guidance of FDA in US for analysis of biological samples and located in the acceptable scope within 15%. These promising results demonstrate that this developed method can be successfully applied to the bio-analysis of PG in plasma with excellent precision and accuracy.

3.3.3. Calibration curves, LLOQ and LOD—A typical regression equation for PG was $y = 0.0324x - 0.809$ ($n = 6$), where y represented the ratio of PG peak area to that of IS, while x was its plasma concentration. In the investigated concentration range, this calibration curve revealed good linearity at the correlation coefficient (r^2) of 0.9959 within the scope ranging from 0.132 to 800 ng mL⁻¹. The LLOQ of PG in plasma was calculated as 0.132 ng mL⁻¹ in accordance with the S/N = 10 under which the precision and accuracy values didn't exceed 15% as mentioned in Section 2.6.3. The LOD was measured at the concentration of 0.05 ng mL⁻¹. Consequently this established method was appropriate to quantify the PG in plasma and could be further applied in pharmacokinetic analysis.

3.3.4. Stability—The data to investigate the stability of PG and IS in rat plasma under various aforementioned conditions were summarized in Table 2. The values of accuracy (RE) ranged from –7.75 to 2.30 in agreement with the required criteria. As to all the sample storing environment and processing procedures, the results implied that the detected analytes were stable throughout the period of the entire analysis.

3.3.5. Extraction recovery and matrix effect—The values of exploring the extraction recovery and matrix effect through analysis of QC samples at three levels were presented in Table 3. The average data of these two indicators for detecting PG in plasma were calculated within a range of 71.35–78.14% and 89.39–93.93 with RSD below 5.92, respectively. The results proved that a higher extraction rate via a one-step protein precipitation with acetonitrile organic solvent was eventually achieved, where the matrix effect could be negligible due to almost no endogenous interfering substances in plasma samples.

3.3.6. Pharmacokinetic analysis—This established and validated HPLC–ESI–MS/MS analysis was successfully applied to investigate pharmacokinetic bio-analysis of PG in rat plasma after oral administration at three doses. The corresponding pharmacokinetic parameters were calculated according to the non-compartment mode via kinetics software and illustrated in Table 4. The plasma concentrations were measured at different time intervals based on the calibration curve and the typical mean concentration versus time profile curves were presented in Fig. 4. As illustrated, PG could reach its C_{max} of 16.14, 29.74, and 50.62 ng mL⁻¹ in a relatively long T_{max} of 10.43 ± 2.15 , 10.87 ± 1.43 , and 11.25 ± 3.15 corresponding to three determined concentrations, respectively. These results indicated that PG was slowly absorbed into the circulatory blood system with low oral absolute bioavailability *in vivo*. It is well-known that drug absorption is a complicated

process having a close relationship with physicochemical as well as physiological properties [16]. The molecular mass of PG is 1049.2 Da, which is larger than the favorable value (<500 Da), leading to poor ability to cross the lipid bilayer cell membrane. In addition, this compound is a steroid saponin consisting of two parts, i.e. hydrophobic aglycone and polar sugar moieties, hence it gave low lipophilic solubility and this might be another reason for its poor cross membrane capacity. Other pharmacokinetic parameters like $t_{1/2}$ (over 11.47 ± 2.13), k_e (within 0.07 ± 0.01), MRT (within 19.23 ± 1.45), and CL (within 0.21 ± 0.02) were consisted with poor elimination and excretion characteristics of PG. Considering C_{max} , AUC_{0-t} and $AUC_{0-\infty}$, they increased and showed a perfect proportion within the dose range, demonstrating that these parameters of PG displayed a dose-dependent relationship and might exhibit linear pharmacokinetics. The concentration–time curve was indeed irregular in agreement with the previous report [17], i.e. the second peak was higher than the first peak, whose proper reason has also been discussed [17]. These pharmacokinetic parameters of pure PG exhibited a slight discrepancy as compared with its complex form extracted from the rhizome of *Dioscorea zingiberensis* C.H. Wright. This might be caused by the coexisting constituents in crude extracts and their synergistic or antagonistic interactions, finally causing individual pharmacokinetic characteristics. Of course, more detailed information should be further carried out to investigate the different mechanisms such as limited poor bioavailability and some other phenomena.

4. Conclusions

A reliable and reproducible HPLC–ESI-MS/MS method was developed and validated to quantify the PG in rat plasma after oral administration of its pure form for the first time. This proposed rapid and simple analysis can generate sensitive, accurate, and specific results. The pharmacokinetic parameters presented could provide valuable information for further investigation of PG and other relevant drug preparations, and laid the solid foundation for its application in pre-clinic and clinic.

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Fig. 1.
The chemical structure of protodioscin and ginsenoside Rb₁ (IS).

Fig. 2. The full scanning transition ions of protodioscin and ginsenoside Rb₁ (IS) in multiple reaction mode (MRM). (a) Protodioscin; (b) ginsenoside Rb₁.

Fig. 3. The typical HPLC–MS/MS chromatograms of protodioscin and ginsenoside Rb₁ (IS) in rat plasma determined in the multiple reaction mode (MRM). (1) Protodioscin; (2) ginsenoside Rb₁.

Fig. 4.

The average plasma concentration versus time curves of protodioscin in six rats after oral administration of its pure form at three levels. The data were presented as mean $\pm$ standard deviation. (a) 50 mg kg⁻¹; (b) 100 mg kg⁻¹; (c) 200 mg kg⁻¹.

Intra-day and inter-day precision and accuracy results of protodioscin in rat plasma at three different QC levels ($\bar{x} \pm SD$).

Table 1

Spiked concentration (ng mL ⁻¹)	Intra-day (<i>n</i> = 6)			Inter-day (<i>n</i> = 18)		
	Measured concentration (ng mL ⁻¹)	Precision (RSD, %)	Accuracy (RE, %)	Measured concentration (ng mL ⁻¹)	Precision (RSD, %)	Accuracy (RE, %)
50	49.63 ± 0.14	0.26	-0.74	49.33 ± 0.39	0.79	-1.35
150	152.68 ± 1.15	0.75	1.76	155.75 ± 2.41	1.55	3.69
400	409.01 ± 11.19	2.74	2.20	414.81 ± 1.59	0.38	1.16

Table 2

The stability of protodioscin in rat plasma at three different QC levels ($n=6, \bar{x} \pm s$).

Storage conditions	Spiked concentration (ng mL ⁻¹)	Measured concentration (ng mL ⁻¹)	Accuracy (RE, %)
Three freeze–thaw cycles stability	50	50.46 ± 1.32	0.92
	150	149.76 ± 1.02	−0.16
	400	409.41 ± 1.06	2.30
Short-term stability (room temperature)	50	46.41 ± 0.23	−7.75
	150	149.31 ± 2.73	−0.47
	400	400.26 ± 1.50	0.06
Long-term stability (−80 °C for 4 weeks)	50	46.93 ± 1.08	−6.55
	150	150.86 ± 1.62	0.57
	400	397.51 ± 0.73	−0.63
Auto vial (post-preparative)	50	50.25 ± 4.54	0.50
	150	150.36 ± 1.58	0.24
	400	409.30 ± 2.64	2.27

Table 3

The mean extraction recoveries and matrix effects of protodioscin in rat plasma at three different QC levels ($n = 6$).

Spiked concentration (ng mL ⁻¹)	Mean extraction recovery		Mean matrix effects	
	$\bar{x} \pm \text{SD}$	RSD (%)	$\bar{x} \pm \text{SD}$	RSD (%)
50	71.35 $\pm$ 1.78	2.49	93.93 $\pm$ 1.97	2.10
150	76.97 $\pm$ 1.71	5.92	89.39 $\pm$ 2.84	3.18
400	78.14 $\pm$ 1.61	2.06	91.35 $\pm$ 3.87	4.24

Table 4Pharmacokinetic parameters of protodioscin in rats ($n = 6$, mean $\pm$ SD).

Parameters	Unit	50 mg kg ⁻¹	100 mg kg ⁻¹	200 mg kg ⁻¹
C_{max}	ng mL ⁻¹	16.14 $\pm$ 0.45	29.74 $\pm$ 0.85	50.62 $\pm$ 1.44
T_{max}	h	10.43 $\pm$ 2.15	10.87 $\pm$ 1.43	11.25 $\pm$ 3.15
$t_{1/2}$	h	11.47 $\pm$ 2.13	12.13 $\pm$ 1.13	11.97 $\pm$ 3.05
k_e	h ⁻¹	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	0.06 $\pm$ 0.01
MRT	h	18.47 $\pm$ 1.82	19.23 $\pm$ 1.45	18.47 $\pm$ 2.39
CL	L h ⁻¹	0.21 $\pm$ 0.02	0.11 $\pm$ 0.01	0.06 $\pm$ 0.01
AUC _{0-t}	h ng mL ⁻¹	3580.85 $\pm$ 459.59	6595.38 $\pm$ 846.49	11224.46 $\pm$ 1440.617
AUC _{0-∞}	h ng mL ⁻¹	4480.08 $\pm$ 924.94	8251.613 $\pm$ 1703.60	14043.15 $\pm$ 2899.29