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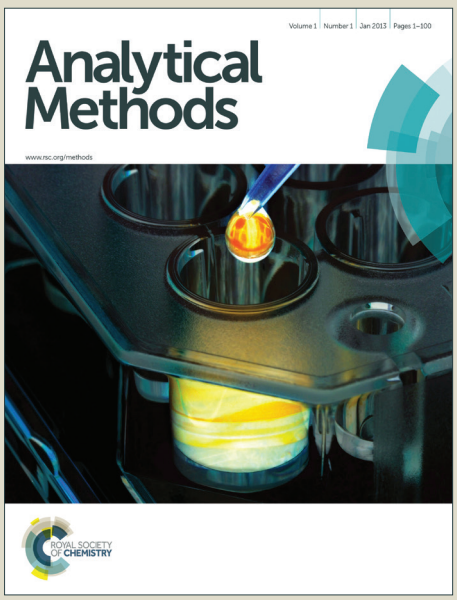

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Pharmacokinetics and brain distribution studies of ginsenoside Rd to rats via intranasal administration by LC-MS/MS

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ABSTRACT Ginsenoside Rd was shown to have the protective effects against several injuries and efficient for the treatment of acute ischemic stroke. Some researches of ginsenoside Rd in the past mainly focused on the pharmacokinetics after intravenous and oral administration. However, we still lack some basic knowledge about the plasma pharmacokinetics and brain distribution of ginsenoside Rd by any other route, such as intranasal administration. It was found that intranasal administration exhibited good brain-targeting. In this study, a sensitive LC–MS/MS method was developed and validated for the determination of ginsenoside Rd in rat plasma and brain tissue. Detection was performed on an ACQUITY UPLCTM BEH C18 column using gradient elution with a flow rate of 0.2 mL min^{-1}. Mass spectrometry was operated in selected reaction monitoring mode using a negative electrospray ionization interface. The method was linear over the concentration range of 1.0 – 1000 ng /mL, and the lower limit of quantification was 1.0 ng/mL for ginsenoside Rd. The method was validated in terms of specificity, linearity, intra- and inter-day precision (< 12.39%), accuracy (within ±10.1%), dilution integrity, recovery, matrix effect and stability, and has been successfully applied to the pharmacokinetic study of ginsenoside Rd in rats

after intranasal administration and evaluation of the brain targeting of ginsenoside Rd.

Keywords: Ginsenoside Rd, LC-MS/MS, Pharmacokinetics, Brain, Intranasal administration

1. Introduction

Ginsenoside Rd (Dammar-24[25]-ene-3,12,20[S]-triol-[20-O-β-d-glucopyranosyl]-3-O-β-d-glucop -yranosyl-(1→2)-β-d-glucopyranoside, Figure 1A), one of the major active components of ginsenosides, is used for the treatment of cardiovascular diseases, inflammation, different body pains, trauma, and internal and external bleeding due to injury [1]. Many studies have showed ginsenoside Rd possesses various bioactivities, such as antioxidant, anticancer, and immunological adjuvant [2]. Most importantly, ginsenoside Rd can also protect against neuronal oxidative damage induced by hydrogen peroxide and oxygen-glucose deprivation, and ameliorate histological and functional outcome after focal cerebral ischemia [3]. Moreover, a phase II randomized, double-blind, placebo-controlled, multicenter study was conducted and showed ginsenoside Rd may be of some benefit in acute ischaemic stroke [4].

The pharmacokinetics of plasma ginsenoside Rd have been studied in the literature. By using liquid chromatography–electrospray ionization–tandem mass spectrometry, Liu et al and Zeng et al determined the pharmacokinetics in humans after single intravenous (IV) administration (10, 45, or 75 mg) and multiple IV administration (10 mg) [5,6]. Wang et al described the pharmacokinetics in dogs after intravenous (IV) administration (0.2 mg/kg) and intragastric (IG) administration (2 mg/kg) [7]. In addition, only one study examined tissue distribution of ginsenoside Rd in rodents after IV administration (50 mg/kg) by radioactive tracer assay [8]. Till

now, LC-MS/MS methods have been reported for ginsenoside Rd quantification, but they were all for plasma samples and no LC-MS/MS method was established for measurement of brain tissue. So it is urgent to develop a novel LC-MS/MS method mainly for brain tissue.

Ginsenoside Rd, a dammarane-type steroid glycoside extracted from ginseng plants, has a high molecular weight, which limits it to cross the strict functional blood brain barrier (BBB) by IV or IG administration. Intranasal route has evolved as a non-invasive mode of drug delivery to brain as compared with the other routes of administration, which can delivers the drug directly to the brain by circumventing BBB and reduces drug delivery to non-targeted sites [9]. The acute ischemic stroke may be better treated by intranasal administration of ginsenoside Rd. Furthermore, the plasma pharmacokinetics and brain distribution of ginsenoside Rd after intranasal (IN) administration or by any other route in the rat is still unknown.

In this study, a sensitive and accurate LC-MS/MS method was developed and validated for ginsenoside Rd quantification in rat plasma and brain tissue. This method was successfully applied to explore the pharmacokinetic characteristics of ginsenoside Rd in plasma and brain via IN administration. This is the first report about pharmacokinetic characteristics of ginsenoside Rd in brain tissue.

2. Materials and methods

2.1. Chemicals and reagents

Ginsenoside Rd (batch no. 111573, purity＞98.0%) was obtained from Guizhou Xinbang

Pharmaceutical Company LTD (Guizhou, China). Saikosaponin A (internal standard, batch no. 111573, purity＞ 98.0%, Figure 1B) was purchased from the National Institute for Food and Drug Control (Beijing, China). HPLC-grade Methanol was obtained from Fisher (USA). HPLC-quality water was obtained using a Cascada™ IX-water Purification System (Pall Co., USA). C18 (SPE) cartridge (100 mg/mL) was purchased from Grace Company (USA). Other reagents were all of analytical grade.

2.2. Instrumentation

2.2.1. Liquid chromatography

Liquid chromatography was performed on a rapid resolution liquid chromatography system (Nexera UHPLC LC-30A, Shimadzu, Japan) equipped with two LC-30AD pumps, a SIL-30AC autosampler, a CTO-30A thermostatted column compartment and a DGU-20A5 degasser. The UHPLC separation was performed on an ACQUITY UPLC™ BEH C18 column (100 × 2.1 mm, 1.7 mm) with a flow rate of 0.2 mL·min^{-1} at 20 ℃。The mobile phase consisted of 0.1% (v/v) FA in water (A) and 0.1% FA in methanol (B). The following gradient elution was used: 0 - 3 min, 70 →75% B; 3 - 7 min, 75→80% B; 7 - 8 min, 80→100% B; 8 - 9 min, 100% B; 9 - 9.1 min, 100→ 70% B; 9.1 - 12 min, 70% B. The injection volume was 10 μL.

2.2.2. Mass spectrometer conditions

The detection was performed on an AB SCIEX Triple Quad™ 4500 (Applied Biosystems, Foster City, CA, USA) with an electrospray ionization source (Turbo Ionspray). The mass spectrometry detection was operated in negative electrospray ionization mode. The [M-H]$^-$ of each analyte was selected as the precursor ion. The quantification mode was selected reaction monitoring (SRM)

mode using the mass transitions (precursor ions → product ions).

The ESI ion source temperature was set at 700℃. Other mass spectrometric parameters were: curtain gas flow: 10 psi, collisionally activated dissociation (CAD) gas setting: medium, ionspray voltage: –4500 V, ion gas 1 and 2: 16 and 13 psi. Data acquisition and processing were performed using AB SCIEX Analyst 1.6 Software (Applied Biosystems).

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

The stock solutions of ginsenoside Rd and saikosaponin A were prepared separately in methanol at 1 mg/mL and aliquoted for storage. The stock solutions of ginsenoside Rd were serially diluted with mobile phase to provide standard solutions at desired concentrations. Calibration standards of ginsenoside Rd at eight concentration levels ranging from 1 to 1000 ng/mL were prepared daily by spiking blank plasma or brain homogenate with working solutions. QC working solutions were prepared in the same way. Low-, medium- and high-level QC samples were at concentrations of 2.5, 50 and 800 ng/mL for ginsenoside Rd. Working solution of saikosaponin A was prepared by diluting the stock solution with mobile phase (methonal and water, 70:30, v/v) to 500 ng/mL for plasma and 250 ng/mL for brain homogenate. All the stock and working solutions were stored at –20 ℃.

2.4 Sample preparation

2.4.1 Plasma

To each 100 μL plasma sample, 10 μL IS solution (500 ng/mL) was added. After being vortex mixed for 30 seconds, sample mixture was loaded on a C18 solid-phase extraction cartridge

pretreated with methanol (2 mL) and water (3 mL) under gentle vacuum. The loaded cartridge was washed with 0.5 mL water and then eluted with 0.5 mL methanol. The eluent was evaporated to dryness in a centrivap concentrator (Labconco, Kansas City, MO, USA). The residue was dissolved in 200 μL mobile phase, vortexed for 1 min and centrifuged at 14000 r/min for 20 min at room temperature. The supernatant transferred to a glass insert, and an injection volume of 10 μL was used in the LC-MS/MS system.

2.4.2 Brain

The whole brain was weighed and homogenized in 2 volumes of normal saline by using a homogenizer. To each 200 μL brain homogenate was added 10 μL IS solution (250 ng/mL). The mixture was vortex-mixed for approximately 30 seconds and loaded on a C18 solid-phase extraction cartridge pretreated with methanol (2 mL) and water (3 mL) under gentle vacuum. The loaded cartridge was washed with 1 mL water and then was eluted with 1 mL methanol. The eluent was evaporated to dryness in a centrivap concentrator. The residue was dissolved in 100 μL mobile phase. Subsequent steps were identical to those for plasma sample preparation.

2.5 Bioanalytical method validation

The validation method for ginsenoside Rd was conducted in two biological matrices of rat: plasma and brain tissue. The method was developed and conducted according to the U.S. Food and Drug Administration (FDA) guidelines for Bioanalytical Method Validation [10]. This analytical method was validated based on specificity, linearity, lower limit of quantification (LLOQ), accuracy, precision, sample dilution, recovery, matrix effect and stability.

2.5.1 Specificity

Specificity of the method was investigated by analyzing blank plasma and brain homogenate samples obtained from six different lots of rats to determine chromatographic interferences of the analyte and IS.

2.5.2 Linearity and LLOQ

Linearity of the method was evaluated with calibration standards over the different concentration range of 1 – 1000 ng/mL. Calibration standard samples were prepared by spiking different concentration of the analyte and IS to the matrices (drug free mouse plasma and brain homogenate). The linearity of each calibration curve was determined by plotting the peak area ratio (Y) of ginsenoside Rd /IS vs the nominal concentration (X) of the analyte. The linearity was evaluated by linear regression analysis and the minimally acceptable correlation coefficient (r^2) was 0.99. The LLOQ was determined at the lowest detectable concentration and a 1:10 base-line noise-calibration point ratio was taken into consideration. The precision and accuracy of LLOQ were less than 20%.

2.5.3 Precision, accuracy and dilution integrity

Precision and accuracy were determined by QC samples at 1.0 (LLOQ), 2.5, 50 and 800 ng/mL. The intra-day precision and accuracy were assessed during the same day by determining the concentrations of QC samples of plasma and brain using six replicates. Inter-day accuracy and precision were determined by repeating analysis of QC samples on three consecutive days. Precision was expressed by relative standard deviation (RSD) and accuracy was calculated as the

relative error (RE). The dilution integrity of the analyte was assessed only in plasma by pre-analytical dilution of five samples with high ginsenoside Rd in blank plasma at factors of ten and then analyzed.

2.5.4 Extraction Recovery and Matrix Effect

Extraction recovery and matrix effect were determined by assaying six replicates samples at concentrations of 2.5, 50 and 800 ng/mL and IS concentration of 25 ng/mL. The recovery was calculated by comparing the peak area of QC samples with the peak area of the analyte where the analyte was spiked after extraction of blank plasma and brain homogenate samples by using the same SPE procedure before evaporation. The matrix effect was measured by comparing the peak areas of each analyte (at three concentration levels of QC samples) and IS spiked into extracts from the blank plasma and brain homogenate versus those of the same analyte and IS presented in the mobile phase. The RSD of matrix effect should be less than 15%.

2.5.5 Stability

Stability of the analyte in rat plasma and brain homogenate under different conditions were assessed by determining six replicates of QC samples at 2.5, 50 and 800 ng/mL during the sample storing and processing procedure. Short-term stability samples were assessed at room temperature for 24 h and long-term storage stability was analyzed by analyzing the QC samples stored at -20°C for one month. The freeze–thaw stability was examined after three freeze and thaw circles. Stability of QC samples at room temperature in the autosampler tray for 12 h after reconstitution was also evaluated for both brain and plasma.

2.6 Experimental animals, pharmacokinetics and tissue distribution study

Sixty Sprague–Dawley rats, weighing 180 – 220 g each, were purchased from Beijing Vital River Laboratory Animal Technology (Beijing, China). They were raised with standard diet and water in temperature for acclimation. Then they were fasted overnight with access to water before dosing. All rat experiments were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Committee on Animal Care and Usage of the Beijing University of Chinese Medicine.

For intranasal administration, rats were placed on their back in slanted position and about 20 μL solution was administered using a polyurethane tube attached to a microliter syringe. The tube was inserted about 10 mm deep into one of the nostrils, enabling the delivery of the drug towards the roof of the nasal cavity. Each rat was given an intranasal administration of 10.0 mg/kg ginsenoside Rd dissolving in mixed solution (1% Tween80, 20% ethanol and 79% normal saline). Blood and brain tissue samples were collected at 8, 20 min and 1, 2, 6, 8, 10, 24, 48, 96 h after administration (six rats for one time point). Blood samples were taken into heparinized tubes and immediately centrifuged at 6000 rpm for 10 min and then 100 μL of plasma was separated and stored at −20 ℃ until preparation. The brain tissue samples were cleaned up of blood contamination from cerebral blood vessels and rinsed with ice-cold normal saline, wiped dry with filter paper, weighted and stored at −20 ℃ until use in “Section 2.4.2”.

2.7. Statistical analysis

All quantification and calibration data were calculated by using Excel spreadsheet software

(Microsoft Office 2013, Microsoft, Redmond, USA). All the results were expressed as means ± standard deviation (SD) of six replicates. The pharmacokinetic parameters were analyzed by using the Kinetica 4.4 software (Thermo Scientific, USA). For the concentration of brain, the calculation process is as below. Assuming that 1 g of brain tissue is equivalent to 1 mL, the concentration of brain gotten from the AB SCIEX Analyst 1.6 Software is C_{brain1} (ng/mL), the actual concentration of brain is C_{brain2} (ng/g). The whole brain was homogenized in 2 volumes of normal saline and each 200 μL brain homogenate was concentrated to 100 μL mobile phase after sample preparation. So $C_{brain2} = (C_{brain1} /2)*3$.

3. Results and discussion

3.1 Mass spectrometry

Under the optimized ESI conditions, both ginsenoside Rd and IS exhibited higher sensitivity in the negative mode than in the positive mode, and $[M-H]^-$ ions at m/z 945.6 and 779.4 were selected as the precursor ions, respectively (Fig. 1). The transitions m/z 945.6→783.4 and 779.4→617.3 were monitored for ginsenoside Rd and saikosaponin A, respectively, which represented the fragmentation at the glucosidic bond and a loss of the glucose structure; while the transitions of m/z 945.6→621.4 and 779.4→471.4 were used for confirmation (Fig. 2).

3.2 Optimization of chromatographic conditions

An ACQUITY UPLCTM BEH C18 column (100 × 2.1 mm, 1.7mm) was investigated to give an adequate separation of ginsenoside Rd and saikosaponin A. In order to improve the peak shape and obtain good sensitivity and selectivity, 0.1% formic acid was added to both the organic and

water phases. A mobile phase gradient was developed to separate ginsenoside Rd from other potentially interfering peaks. Due to the relatively equal recovery, similar polarity and retention time to ginsenoside Rd, saikosaponin A was chosen as internal standard [11]. Ultimately, these conditions enabled good separation, acceptable recovery to yield a highly sensitive and sufficiently robust assay.

3.3 Method validation

3.3.1. Specificity

The specificity of the method was accessed by comparing the chromatograms of ginsenoside Rd and IS in rat plasma and brain homogenate samples (standard and sample). Representative chromatograms are shown in Figure 3 and 4, including blank rat plasma and brain homogenate samples (Fig. 3A and 4A), blank plasma and brain homogenate spiked with ginsenoside Rd and IS (50ng/mL ginsenoside Rd, 25ng/mL saikosaponin A, Fig. 3B and 4B), plasma and brain homogenate samples collected at 2 h following intranasal administration of ginsenoside Rd (Fig. 3C and 4C). The retention times for ginsenoside Rd and IS were approximately 8.0 and 7.2 min, respectively. The method was specific and no significant interferences from endogenous substances of blank plasma and brain tissue were observed at the retention time of the analyte and IS.

3.3.2 Linearity and LLOQ

The plasma and brain homogenate calibration curve were constructed over an analytical measuring range of 1.0 – 1000 ng/mL with good reproducibility and linearity. The weighting

factor was 1/x for rat plasma and brain homogenate. Representative standard curves were Y=0.00578X + 0.00566 (r^2 = 0.998) for plasma and Y=0.0105X +0.0523 (r^2 = 0.999) for brain, respectively. The LLOQ for ginsenoside Rd in rat plasma and brain homogenate were both 1.0 ng/mL with the RSD < 20% at S/N ratios of 10.

3.3.3 Precision, accuracy and dilution integrity

The results for intra- and inter-day precision and accuracy were summarized in Table 1. For all QC sample concentrations of plasma and brain homogenate, the inter- and intra-day precision of the method was determined to be < 15%. For plasma, the intra- and inter-day accuracy ranged from -10.07 to 1.27% and -13.74 to 7.66%, respectively. For brain homogenates, the intra- and inter-day accuracy were from -8.08 to 1.40% and -10.10 to 1.19%, respectively. The results indicated all observed values were acceptable within the limits. Samples (5000 ng/mL) were diluted 10-fold with blank plasma prior to extraction for partial volume analysis. The dilution integrity data showed the RSD was 1.47% with accuracy of 96.92%. These results support sample dilution up to 10-fold for analysis.

3.3.4 Extraction Recovery and Matrix Effect

Table 2 showed the extraction recovery and matrix effect of three concentrations at 2.5, 50 and 800 ng/mL. Recoveries of ginsenoside Rd at three QC concentrations were 101.5 ± 4.83, 98.75 ± 10.15 and 93.82 ± 4.66% for plasma and 68.30 ± 8.13, 75.69 ± 5.22, 71.59 ± 1.89 for brain tissue (800, 50 and 2.5 ng/mL), respectively. The recovery of IS was 101.94 ± 5.56% and 72.67 ± 9.85% for plasma and brain tissue. The extraction recoveries of the method were consistent and

reproducible for ginsenoside Rd and IS. For the matrix effect, all of the ratios were in the range of 99.65 – 103.12% for plasma samples and 91.25 – 102.25% for brain tissue samples. As a result, there was no significant matrix effect observed for ginsenoside Rd.

3.3.5 Stability

Stability of ginsenoside Rd was investigated by determining the concentrations of QC plasma and brain homogenate samples under different storage conditions, including short-term temperature stability, long-term stability, freeze-thaw stability and post-preparative stability. The data were summarized in Table 3. The results were within the acceptable limits, which suggested that no significant loss of ginsenoside Rd in either plasma or brain homogenate was observed and the established method was suitable for large scale sample analysis.

3.4 Pharmacokinetics and tissue distribution study

The LC–MS/MS method was successfully applied to quantify drug in rat plasma and brain tissue samples obtained from SD rats after an intranasal administration of ginsenoside Rd solution. The plasma and tissue samples were collected at time points from 8 min to 96 h. Fig. 5 shows the mean plasma concentration–time curve and mean brain concentration–time curve. The pharmacokinetic parameters of plasma and brain tissue were displayed in Table 4. Ginsenoside Rd was quantifiable in all plasma samples but partial brain tissue samples, thus demonstrating the capability of the assay for characterizing ginsenoside Rd pharmacokinetics in plasma is within the 96 h and in brain tissue is within 24 h.

Ginsenoside Rd concentration in plasma reached a maximum at 2 h (T_{max}) with a maximum

concentration (C_{max}) of 4740.0 ng/mL. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) was 77713 (ng h/mL) and mean residence time (MRT) was 19.8 h. The $T_{1/2}$ was 15.9 h. Ginsenoside Rd concentration in brain tissue reached a maximum at 2 h with C_{max} of 69.8 ng/g. The $AUC_{0-\infty}$ was 677 (ng h/g) and MRT was 14.3 h. The $T_{1/2}$ was 10.8 h.

3.5 Discussion

Stroke remains one of the leading causes of death and adult disability worldwide [12, 13]. Ginsenoside Rd, a dammarane-type steroid glycoside extracted from ginseng plants, has exhibited an encouraging neuroprotective efficacy in both laboratory and clinical studies [8].With the progress in drug delivery, intranasal administration exhibited good brain-targeting. In this study, we explore the pharmacokinetic parameters of ginsenoside Rd in rat plasma and brain tissue via intranasal administration.

The pharmacokinetic parameters for ginsenoside Rd in rat plasma were compared with that of drug solution administered by oral and intravenous route. The mean plasma elimination half-life of intranasal administration was 15.9 h (100 mg/kg), while this number was approximately 14 h in mice (20, 50, 150 mg/kg, iv.), 39.4 h in dog (0.2 mg/kg, iv.) or 24.2 h in dog (2 mg/kg, ig.). In healthy humans, The $T_{1/2}$ was about 20 h (10, 40, 75 mg, iv. or 10 mg day 10, iv.). These parameters could explain the half-life of Rd is relatively long, which may avoid repeated dosing in patients. The half-life of intranasal administration was similar with the one of intravenous administration.

To our knowledge, this is the first report about pharmacokinetic parameters of ginsenoside Rd in brain tissue. Pharmacokinetic results showed that ginsenoside Rd can be detected immediately within 8 min and reached a maximum at about 2 h, indicated that they may be absorbed rapidly after intranasal administration, but the elimination of Rd may be slow in rats because $T_{1/2}$ was about 10.8 h. These may be useful for the further clinical use and the pharmacological studies of ginsenoside Rd.

The UHPLC separation and MRM detection gave the method high selectivity and consequently high accuracy. There were no interferences from endogenous substances for ginsenoside Rd. Compared with earlier published methods on plasma ginsenoside Rd, this method was found to make an improvement in sensitivity, which was 1 vs 3 or 5 ng /mL. Meanwhile, the extraction recovery of plasma was higher.

4. Conclusion

In this study, a sensitive LC–MS/MS method has been developed and validated for ginsenoside Rd quantification in rat plasma and brain tissue. It has been successfully applied to the pharmacokinetic study of ginsenoside Rd in rats after intranasal administration and evaluation of the brain targeting of ginsenoside Rd.

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Fig. 1. Full-scan mass spectra of ginsenoside Rd (molecular weight 947.15) (A) and saikosaponin A (molecular weight 779.4) (B) as obtained in negative ion mode.

Fig. 2. Product ion spectra of $[M-H]^-$ ion of ginsenoside Rd (A) and saikosaponin A (B) as obtained in negative ion mode.

Fig. 3. Representative chromatograms after selected reaction monitoring of ginsenoside Rd and saikosaponin A in rat plasma. Blank plasma (A), blank plasma spiked with the ginsenoside Rd and saikosaponin A (B), and typical chromatograms of plasma sample collected at 2 h following intranasal administration (C). Channel 1 represents ginsenoside Rd for confirmation. Channel 2 represents ginsenoside Rd for quantification. Channel 3 represents saikosaponin A for confirmation. Channel 4 represents saikosaponin A for quantification.

Fig. 4. Representative chromatograms after selected reaction monitoring of ginsenoside Rd and saikosaponin A in rat brain homogenate. Blank brain homogenate (A), blank brain homogenate

spiked with the ginsenoside Rd and saikosaponin A (B), and typical chromatograms of brain homogenate sample collected at 2 h following intranasal administration (C). Channel 1 represents ginsenoside Rd for confirmation. Channel 2 represents ginsenoside Rd for quantification. Channel 3 represents saikosaponin A for confirmation. Channel 4 represents saikosaponin A for quantification.

Fig. 5. Mean plasma concentration–time curve of ginsenoside Rd in rats after intranasal administration of 10.0 mg/kg. Each point represents the mean ± SD (n = 6)

Table 1 Precision and accuracy for analysis of ginsenoside Rd in rat plasma and brain homogenate samples (n = 6)

		Intra-daya			Inter-day		
Matrix	Nominal (ng/mL)	Measured (ng/mL)	RSD (%)	Accuracy (%)	Measured (ng/mL)	RSD (%)	Accuracy (%)
Plasma	800	789.83 ± 26.36	3.34	-1.27	780.44 ± 66.05	8.46	-2.44
	50	48.98 ± 1.46	2.98	-2.03	53.83 ± 3.75	6.97	7.66
	2.5	2.53 ± 0.11	4.39	1.27	2.59 ± 0.18	6.85	3.64
	1.0	0.90 ± 0.06	6.50	-10.07	0.86 ± 0.06	7.51	-13.74
Brain	800	735.33±26.1	3.55	-8.08	719.22±25.04	3.48	-10.10
	50	50.17±1.19	2.38	0.33	50.59±2.16	4.26	1.19
	2.5	2.54±0.22	8.71	1.40	2.5±0.31	12.39	0.13
	1.0	0.99 ± 0.12	12.51	-1.16	0.97 ± 0.14	14.18	-4.37

Table 2 Extraction recovery and matrix effect for ginsenoside Rd in rat plasma and brain homogenate (n= 6)

Matrix	Nominal (ng/mL)	Recovery（%）	RSD (%)	Matrix effect (%)	RSD (%)
Plasma	800	101.5 ± 4.83	4.59	103.12 ± 5.86	5.68
	50	98.75 ± 10.15	10.28	100.78 ± 9.72	9.64
	2.5	93.82 ± 4.66	5.15	99.65 ± 5.55	5.57
Brain	800	68.30 ± 8.13	11.91	96.52 ± 6.33	6.56
	50	75.69 ± 5.22	6.9	102.25 ± 1.39	1.63
	2.5	71.59 ± 1.89	2.64	91.25 ± 0.98	1.08

Table 3 Stability of ginsenoside Rd in rat plasma and brain homogenate (n = 6).

Matrix	Nominal (ng/mL)	Short-term stability (24 h at room temperature)		Long-term stability (30 days at-20°C)		Freeze-thaw stability (3 cycles)		Post-preparative stability (12 h at room temperature)	
		Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)
Plasma	800	817.67 ± 13.32	1.63	874.00 ± 15.35	1.76	804.00 ± 18.28	2.27	753.50 ± 14.31	1.90
	50	55.48 ± 1.19	2.14	57.38 ± 1.71	2.98	54.82 ± 0.89	1.63	58.00 ± 1.94	3.35
	2.5	2.24 ±0.20	9.11	2.08 ± 0.17	8.21	2.49 ± 0.18	7.08	2.47 ± 0.21	8.42
Brain	800	762.17 ± 29.59	3.88	729.17 ± 44.24	6.07	751.83 ± 27.29	3.63	706.83 ± 29.44	4.16
	50	52.28 ± 0.43	0.82	47.97 ± 1.17	2.43	50.87 ± 1.95	3.83	50.97 ± 1.85	3.63
	2.5	2.54 ± 0.35	13.67	2.50 ± 0.35	14.17	2.56 ± 0.36	14.15	2.66 ± 0.30	11.36

Table 4 Pharmacokinetic parameters of ginsenoside Rd in plasma and brain tissue after intranasal administration (n = 6)

	plasma		brain	
Paramaters	Unit	ginsenoside Rd	Unit	ginsenoside Rd
T_{max}	h	2	h	2
C_{max}	ng/mL	4740.0	ng/g	69.8
AUC_{0-t}	ng h/mL	76545.8	ng h/g	547.2
$AUC_{0-\infty}$	ng h/mL	77713	ng h/g	677
$T_{1/2}$	h	15.9	h	10.8
MRT	h	19.8	h	14.3

Parameters were estimated using the mean concentration-time profiles obtained from six different rats per time point (n = 6).

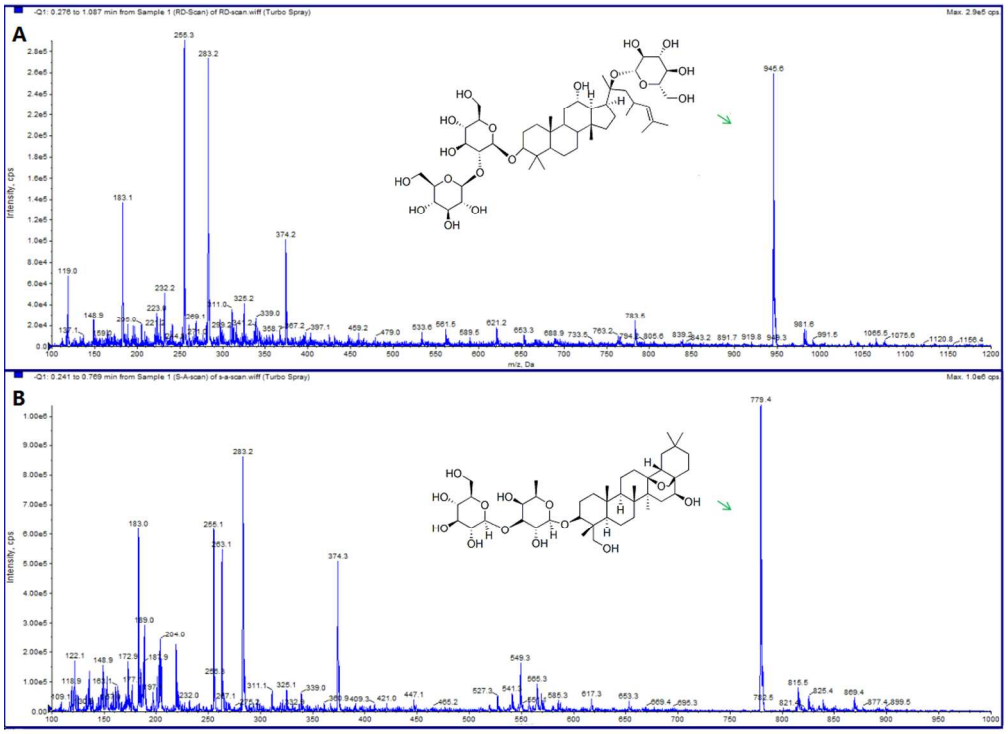


Fig. 1. Full-scan mass spectra of ginsenoside Rd (molecular weight 947.15) (A) and saikosaponin A (molecular weight 779.4) (B) as obtained in negative ion mode.

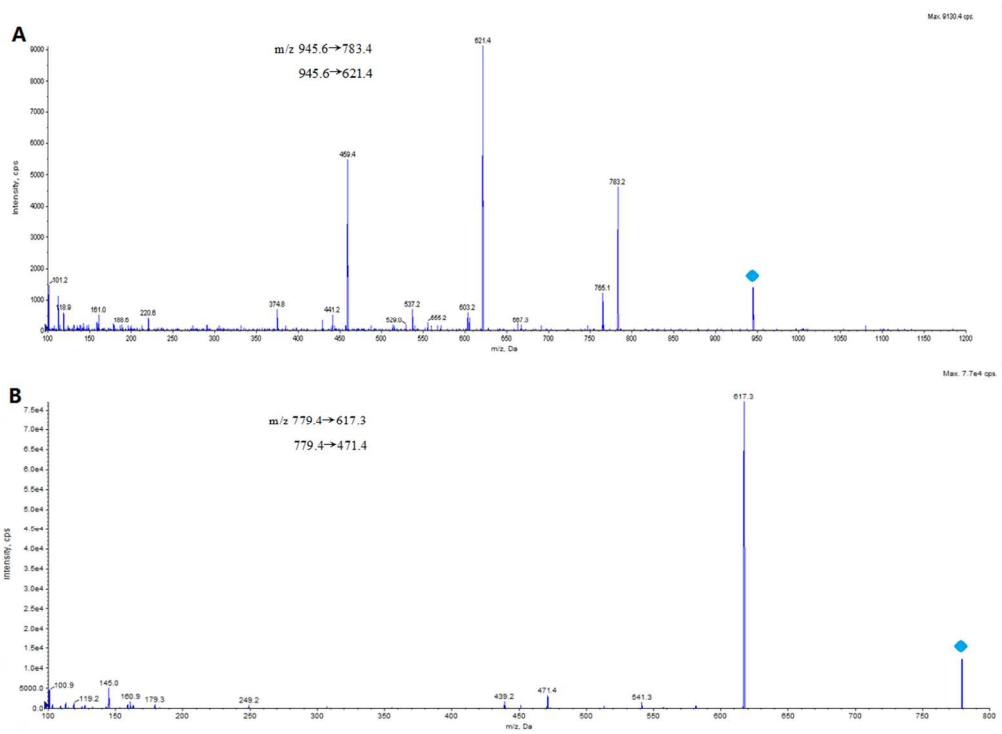


Fig. 2. Product ion spectra of [M-H]- ion of ginsenoside Rd (A) and saikosaponin A (B) as obtained in negative ion mode.

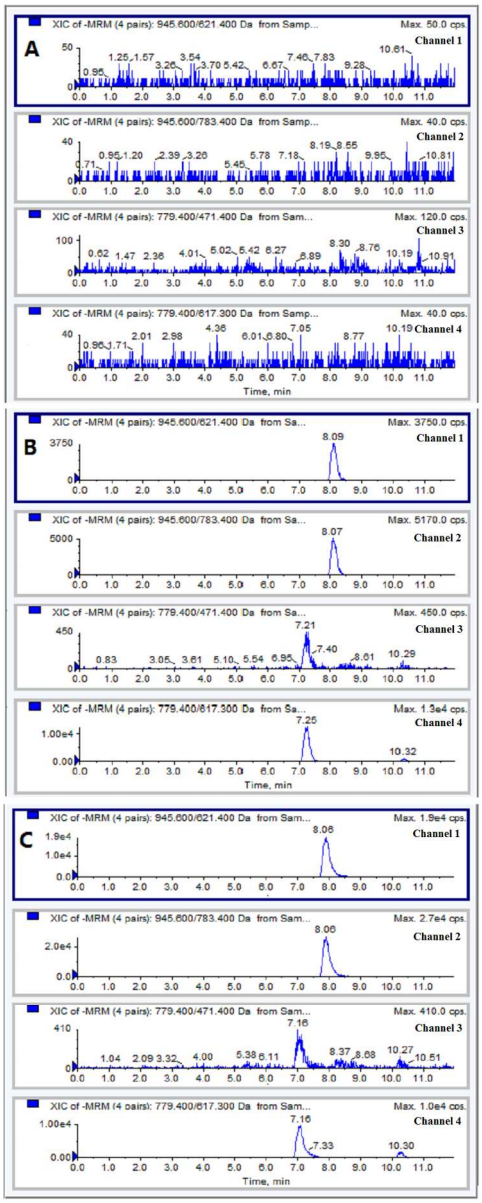


Fig. 3. Representative chromatograms after selected reaction monitoring of ginsenoside Rd and saikosaponin A in rat plasma. Blank plasma (A), blank plasma spiked with the ginsenoside Rd and saikosaponin A (B), and typical chromatograms of plasma sample collected at 2 h following intranasal administration (C). Channel 1 represents ginsenoside Rd for confirmation. Channel 2 represents ginsenoside Rd for quantification. Channel 3 represents saikosaponin A for confirmation. Channel 4 represents saikosaponin A for quantification.

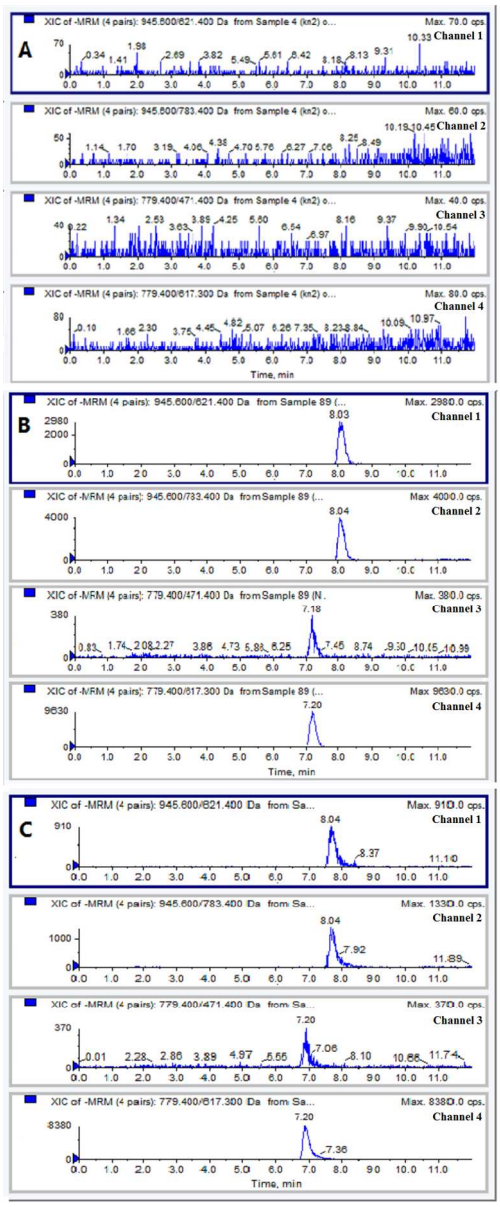


Fig. 4. Representative chromatograms after selected reaction monitoring of ginsenoside Rd and saikosaponin A in rat brain homogenate. Blank brain homogenate (A), blank brain homogenate spiked with the ginsenoside Rd and saikosaponin A (B), and typical chromatograms of brain homogenate sample collected at 2 h following intranasal administration (C). Channel 1 represents ginsenoside Rd for confirmation. Channel 2 represents ginsenoside Rd for quantification. Channel 3 represents saikosaponin A for confirmation. Channel 4 represents saikosaponin A for quantification.

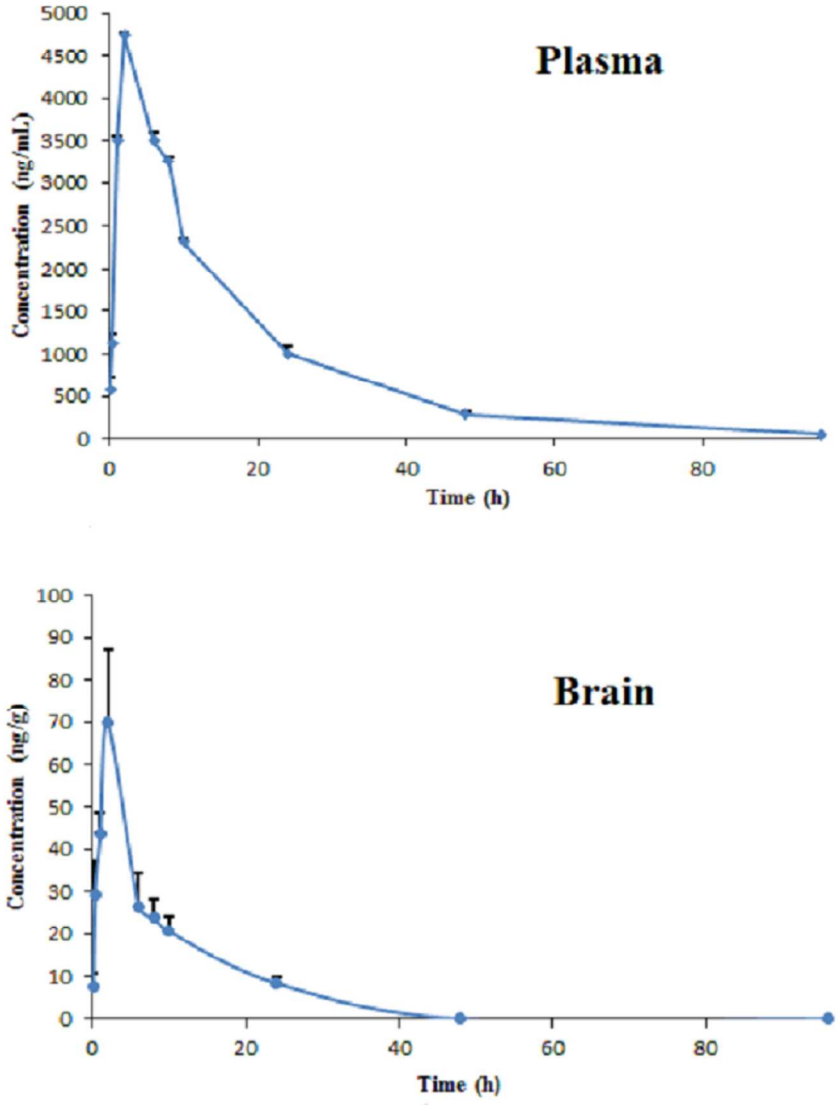
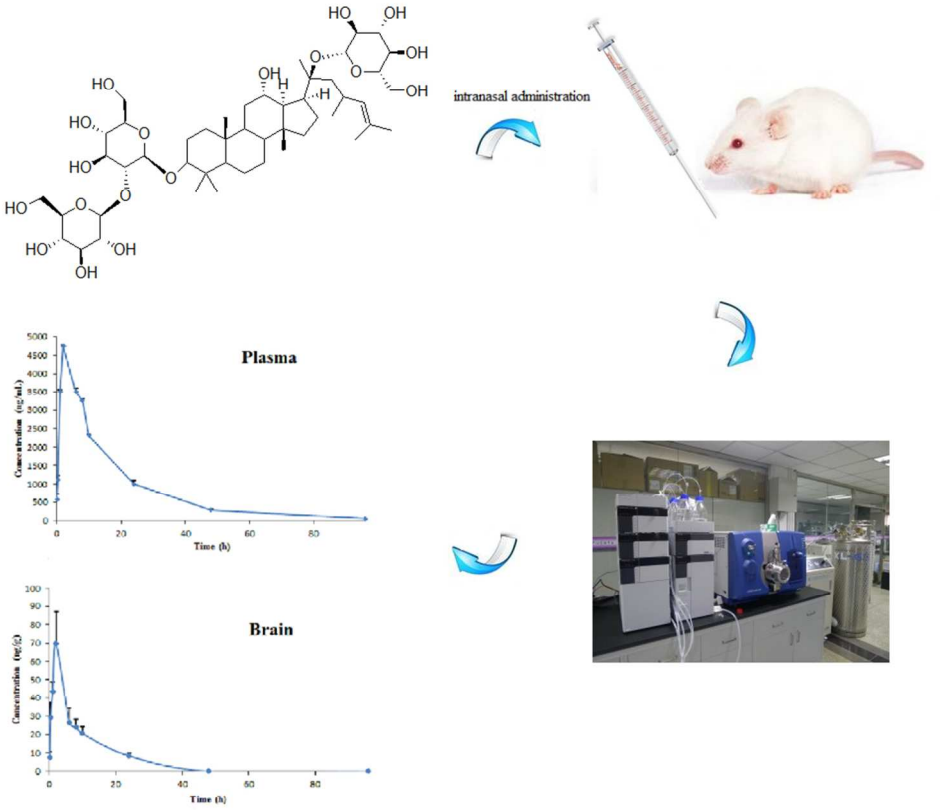


Fig. 5. Mean plasma concentration–time curve of ginsenoside Rd in rats after intranasal administration of 10.0 mg/kg. Each point represents the mean ± SD (n = 6)



Graphic abstract