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Pharmacokinetics and tissue distribution study of two novel quinazoline PI3K/mTOR dual-inhibitors, potential anticancer agents

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Abstract: S_1 and S_2, two structurally similar quinazoline derivatives, are novel anticancer drugs targeting the PI3K/AKT/mTOR signaling pathway channel. However, their pharmacokinetic and tissue distribution characteristics are unknown, which has hindered further development and in-depth studies. In this study, a simple, rapid and sensitive method using high performance liquid chromatography was established and validated to quantitatively study the pharmacokinetics and tissue distribution profiles of S_1 and S_2 in rats following intravenous injection. The results indicated that after intravenous injection, the elimination of S_1 and S_2 fit the two-compartment model, and linear pharmacokinetics characteristics were observed. Furthermore, S_1 and S_2 were widely distributed and found in high concentrations in liver and kidney tissues, and a small proportion of S_1 and S_2 could cross the blood-brain barrier and be distributed in the brain. The current findings will contribute to interpretation and understanding the relationship between dosage and pharmacodynamic effects of S_1 and S_2.

Keywords: Quinazoline derivative, pharmacokinetics, tissue distribution, anticancer, HPLC.

INTRODUCTION

With the aging of the population, the growth in population, and the diversification of tumor pathogenic factors, the incidence and mortality of tumors have dramatically increased in recent years (Bi *et al.*, 2021). In 2020, there were 19.3 million new cancer cases and 10 million cancer-related deaths worldwide. The number of new cancer cases is anticipated to reach 28.4 million by 2040 (Sung *et al.*, 2021). Cancer is often considered both the leading cause of death and the key obstacle to making people live longer (Fidler *et al.*, 2016; Zhang *et al.*, 2021). Because of the high prevalence of cancer in modern society, research and development of anti-cancer drugs have received great attention.

It has been established that the PI3K/AKT/mTOR signaling pathway, which is closely associated with cell survival, growth and proliferation, plays a crucial role in physiological progression of cancer, including cell growth, invasion, and chemotherapy (Alzahrani, 2019). Dysregulation of this pathway can result in a variety of malignancies, such as including breast, prostate, colon, and malignant brain tumours (Blume-Jensen and Hunter, 2001; Xu *et al.*, 2020; Sun *et al.*, 2022). Therefore, it has been a research focus for many years to develop new anticancer medications that target this signal transduction pathway. In our previous study, we identified two quinazoline derivatives, S_1 and S_2, that can block this pathway and exhibit simultaneous inhibitory effects on the activity of PI3K and mTOR (Shao *et al.*, 2014; Chen *et al.*, 2023). Fig. 1 shows that the structures of S_1 and S_2 are very similar and the only difference is the 4'-substitutional group in the aryl sulfonamide moiety, which is a fluorine atom for S_1 and a methyl group for S_2. S_1 and S_2 were valuable and relevant for further study. They demonstrated comparable or greater inhibitory effects *in vitro* against a range of tumor cell lines and had excellent inhibitory effects on tumor growth in nude mice with U87MG xenografts compared to the positive control, BEZ235. However, the minor structural change may result in different pharmacokinetic properties *in vivo*.

During the pre-clinical and clinical phases of the pharmacologically active compounds, it is essential to investigate the pharmacokinetics of absorption, distribution, metabolism, and excretion (ADME) (Singh, 2006; Hussain *et al.*, 2011; Kim *et al.*, 2015; Leblanc *et al.*, 2018). Pharmacokinetic properties of new drugs greatly contribute to not only the selection of the administration route, design, and optimal dosage form, but also to the relationship between dose and effect (Leonov *et al.*, 2020). To some degree, the pharmacodynamics responses of the medication, such as toxicity and efficacy, are influenced by pharmacokinetics (De Smet and Brouwers, 1997; Sun *et al.*, 2019). However, for new compounds such as S_1 and S_2, the pharmacokinetic and tissue distribution characteristics are not yet available. Therefore, in this study, a simple, rapid, and sensitive method using high performance liquid chromatography (HPLC) was established and validated to quantitatively investigate the pharmacokinetic and tissue distribution profiles of S_1 and S_2 in a rat model. Investigating the S_1's and S_2's pharmacokinetic and tissue distribution behavior features of S_1 and S_2 at the early stages of drug development will be fundamental for understanding the relationship between dosage and pharmacodynamic responses.

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MATERIALS AND METHODS

Chemicals and regents

S_1 and S_2 were synthesized, and confirmation of the structure was conducted with nuclear magnetic resonance (NMR) and high resolution mass spectrometry (HRMS). 1, 2-proylene glycol, methanol, and ethyl acetate were purchased procured from Damao Chemical Reagent Factory (Tianjin, China). Polyethylene glycol 300 (PEG 300) was obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium hydroxide, potassium phosphate dibasic trihydrate, and potassium phosphate monobasic were supplied by Xi'an Chemical Reagent Factory (Xi'an, China). Saline was produced by Chenxin Pharmaceutical Co., Ltd. (Jining, China). Heparin sodium (150 UI/mg) was purchased secured from Baisha Biotechnology Co., Ltd. (Shanghai, China). HPLC grade methanol was procured from Concord Technology Co., Ltd. (Tianjin, China). Ultra-pure water was prepared by a Molecular 1850 water purification system (Shanghai, China). The chemicals used were analytically pure unless otherwise stated. All materials were obtained from commercial suppliers and were used without further purification.

Animals

Specific pathogen-free (SPF) Sprague-Dawley rats (220 ± 20 g), 8-10 weeks old, were purchased from the Laboratory Animal Center of Xi'an Jiaotong University (Xi'an, China). Half the number of rats were males, the other half were females. Prior to experiments, rats were housed in a breeding room under controlled experimental conditions (21 – 25 °C, 40 - 60% relative humidity, and a 12-h light-dark cycle), and were given free access to water and a standard diet for at least seven days to allow for adjustment to their new surroundings.

Chromatographic conditions

Pharmacokinetic and tissue distribution studies of S_1 and S_2 were conducted using a Shimadzu LC-10A HPLC system (Kyoto, Japan) with a Shimadzu SPD-10A detector (Kyoto, Japan). Samples were chromatographically separated on a Dikma Diamonsil C_{18} column (250mm × 4.6mm, 5μm; Beijing, China) using an isocratic mobile phase consisting of methanol and potassium phosphate buffer solution (pH 7.0, 20mM) (56:44, v/v) at a flow rate of 1.0mL/min at 35°C. The mobile phase was degassed using an ultrasonic cleaner for 15 min prior to use after being filtered using a 0.45μm microfiltration membrane produced by Keyilong Lab Equipment Co. Ltd, (Tianjin, China). The injection volume was 10μL and the detection wavelength was set to 242 nm.

Preparation of solutions

The different solutions were prepared as follows and kept at 4°C in the dark. Before use, all solutions were brought to room temperature.

pH 8.0 mixed solvent: 5.0mL of 1, 2-proylene glycol, 10.0mL of PEG 300, and 35.0mL of saline were mixed, and the pH of the solution was adjusted to 8.0 with a 1.0 mol/L sodium hydroxide aqueous solution.

S_1 and S_2 sample solutions: 0.3mL of 1, 2-proylene glycol, 0.6mL of PEG 300, and 0.3mL of 1.0 mol/L sodium hydroxide aqueous solution were added to 150.0mg of S_1 or S_2. After complete dissolution of the mixture using an ultrasonic approach, 1.8mL of water was added in portions and the solution was vortexed thoroughly to yield a 50.0mg/mL S_1 or S_2 solution, which was diluted with pH 8.0 mixed solvent to yield S_1 sample solutions at 1.7, 3.4, and 6.8mg/mL or S_2 sample solutions at 2.1, 4.2, and 8.4mg/mL.

S_1 and S_2 standard solutions: In a clean and dry 10mL volumetric flask, 10.0mg of S_1 or S_2 was weighed and dissolved in methanol to yield and a 1.0mg/mL stock solution, which was diluted in methanol to afford S_1 standard solutions at 0.1, 0.2, 0.5, 5.0, 10.0, 17.5, 25.0, 30.0, 32.0, 40.0μg/mL or S_2 standard solutions at 0.1, 0.2, 0.5, 5.0, 10.0, 15.0, 17.5, 22.5, 25.0, and 30.0μg/mL.

S_1 and S_2 internal standard (IS) solutions: In a clean and dry 25mL volumetric flask, 250μL of a 1.0mg/mL S_1 or S_2 stock solution was diluted in methanol to yield a 10.0μg/mL IS solution. S_1 and S_2 were used as IS for each other.

Preparation of calibration standards and quality control samples

According to the different concentrations of S_1 or S_2 in plasma or tissue of rats, an appropriate amount of standard solution was added to the clean tube, and most of the solvent was removed with a nitrogen stream at 37°C. To the obtained residue, 200μL of blank plasma or 1.0mL blank tissue homogenate was added and vortexed for 2 min to afford plasma samples or tissue samples at different concentrations, which were used for the determination of the linearity or as quality control (QC) samples. The calibration standards of S_1 and S_2 in plasma and tissues were prepared at the following concentrations: 0.1, 0.2, 0.5, 5.0, 10.0, 15.0, 20.0, and 25.0μg/mL of S_1 in plasma; 0.08, 0.8, 2.4, 4.8, 7.2, and 10.0μg/g of S_1 in heart; 0.2, 2.0, 4.0, 20.0, 40.0, and 60.0μg/g of S_1 in liver; 0.2, 0.8, 2.0, 4.0, 6.0, and 8.0μg/g of S_1 in spleen or lung; 0.4, 2.0, 6.0, 10.0, 14.0, and 20.0μg/g of S_1 in kidney; 0.04, 0.08, 0.2, 0.4, 0.6, and 0.8μg/g of S_1 in brain; 0.1, 0.2, 0.5, 10.0, 17.5, 25.0, 32.0, and 40.0μg/mL of S_2 in plasma; 0.2, 0.8, 2.4, 4.8, 7.2, and 10.0μg/g of S_2 in heart; 0.4, 2.0, 10.0, 20.0, 30.0, and 40.0μg/g of S_2 in liver; 0.2, 0.8, 2.0, 4.0, 6.0, and 8.0μg/g of S_2 in spleen or lung; 0.4, 2.0, 6.0, 10.0, 14.0, and 20.0μg/g of S_2 in kidney; 0.04, 0.08, 0.2, 0.4, 0.6, and 0.8μg/g of S_2 in brain. QC samples at the lower limit of quantification (LLOQ), low, medium, and high concentration were used to evaluate the precision,

accuracy, recovery, and stability of the chromatographic method. Furthermore, QC samples of S_1 and S_2 in plasma and various tissues were prepared at the concentrations as follows: 0.1, 0.2, 12.5, and 20.0μg/mL of S_1 in plasma; 0.08, 0.16, 3.2, and 8.0μg/g of S_1 in heart; 0.2, 0.4, 24.0, and 48.0μg/g of S_1 in liver; 0.08, 0.16, 3.2, and 6.0μg/g of S_1 in spleen or lung; 0.4, 0.8, 8.0, and 16.0μg/g of S_1 in kidney; 0.04, 0.08, 0.32, and 0.64μg/g of S_1 in brain; 0.1, 0.2, 17.5, and 30.0μg/mL of S_2 in plasma; 0.2, 0.4, 3.2, and 8.0μg/g of S_2 in heart; 0.4, 0.8, 16.0, and 32.0μg/g of S_2 in liver; 0.2, 0.4, 3.2, and 6.0μg/g of S_2 in spleen and lung; 0.4, 0.8, 8.0, and 16.0μg/g of S_2 in kidney; 0.04, 0.08, 0.32, and 0.64μg/g of S_2 in brain. Spiked samples were treated as the procedures indicated in section "Processing of bio-samples", and processed samples were kept at -20℃ in the dark.

Processing of bio-samples

Plasma samples and tissue samples were prepared as follows, and all samples were maintained at -20 ℃ in the dark until analysis.

Plasma samples: Blood was collected through the femoral artery and anticoagulated with heparin. Subsequently, plasma was prepared by centrifugation at 3500rpm and 4ºC for 10 min. To 200μL of plasma, 100μL of IS solution, and 800μL of methanol were added, and the mixture was vortexed for 2 min. After centrifugation at 12000rpm for 10 min at 4ºC, the supernatant was transferred to an Eppendorf tube and evaporated to dryness using a nitrogen stream at 37ºC to give a residue, which was redissolved with 100μL of the mobile phase and vortexed for 1 min. Then, the mixture was transferred to a new Eppendorf tube, and centrifuged at 12000rpm for 10 min at 4ºC, after which the supernatant was collected and used for HPLC analysis.

Tissue samples: Rats were sacrificed, and organs, including the heart, liver, spleen, lungs, kidney, and brain, were removed quickly and washed thoroughly with ice-cold saline to remove residual blood. Organs were weighed and homogenized using a glass tissue homogenizer in ice-cold potassium phosphate buffer (pH 7.0, 20mM) (1:4, w/v). Next, 1.0mL of tissue homogenate was transferred to a clean glass tube, then, 100μL of IS solution and 4.0mL of ethyl acetate were added. The mixture was vortexed for 2 min and centrifuged at 3000rpm and 4ºC for 5 min. The upper organic layer was transferred to a new glass tube and dried using a nitrogen stream at 37ºC to give a residue, which was vortexed with 200μL of methanol for 1 min and the solvent was evaporated using a nitrogen stream at 37ºC to afford another residue. The secondary residue was vortexed for 1 min with 100μL of the mobile phase, then transferred to a new Eppendorf tube and centrifuged at 12000rpm and 4ºC for 10 min. The supernatant was collected for HPLC analysis.

Method validation

The HPLC methods for the determination of S_1 and S_2 in plasma and tissues of rats were validated according to the validation guidelines of bioanalytical methods of the Food and Drug Administration (FDA) and European Medicines Agency (EMA) (EMA, 2012; FDA, 2018).

Specificity: The specificity of the assay was estimated by comparing six batches of blank plasma or tissue samples with corresponding spiked bio-samples at the LLOQ concentration to make sure that there were no interfering substances at the retention time of the analyte and IS.

Linearity and LLOQ: All calibration standard samples were analyzed and linear regression was employed to plot the peak area ratio of analyte to IS against the concentration of the analyte to generate the calibration curves of the plasma and tissue samples of S_1 and S_2 The LLOQ was defined as the lowest concentration on the calibration curve that could be determined quantitatively with an accuracy within 20% of the nominal concentration and a precision of no more than 20% of the relative standard deviation (RSD).

Accuracy and precision: The accuracy and precision in plasma and tissue samples were measured at four concentration levels (LLOQ, low, medium, and high concentration), and each concentration level included five replicates. The within-run accuracy and precision were measured in one analytical run, while the between-run accuracy and precision were determined in three analytical runs.

Recovery: The sample addition recovery was estimated by comparing the measured concentration value with the nominal concentration value. Three concentration levels (low, Low, medium, and high concentration) were set with five replicates at each concentration level.

Stability: The stability of S_1 and S_2 in plasma and tissue homogenates of rats was investigated by analyzing the QC samples at low, medium and high concentrations after storage under the following conditions: three complete freeze and thaw cycles, ambient temperature for 8 h, and -20℃ for 30 days. Experiments were performed in triplicate for each concentration.

Pharmacokinetics study

36 rats were randomly divided into six groups; half the number of rats were males, the other half were females. Prior to the experiment, rats were fasted for 12 h and allowed free access to water. **S_1** at doses of 3.4, 6.8, and 13.6mg/kg were administrated by intravenous injection. Using the same administration method, **S_2** was dosed at 4.2, 8.4, and 16.8mg/kg. After dosing, approximately about 0.3mL blood was collected from the femoral artery and transferred to heparinized tubes at 5, 10, 15, 20, 30,

40, 50, 60, 75, 90, 120, 150, and 180 min. For **S_2**, the sample points were 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, 210, and 240 min after dosage. After centrifugation at 3500rpm and 4ºC for 10 min, plasma was collected. Plasma samples were treated following the procedures indicated in section "Processing of bio-samples". During the experiment, to replenish their blood volume in a timely manner and maintain normal blood pressure, rats were permitted to drink water ad libitum. Data of the plasma drug concentration was fitted with the atrioventricular model and pharmacokinetic parameters, such as the area under the plasma concentration-time curve (*AUC*), clearance rates (*CL*), half-life ($t_{1/2}$), elimination rate constant (*k*), apparent volume of distribution (*V*) were calculated by the practical pharmacokinetics program 3P97 (version 97, Professional Committee on Mathematical Pharmacology of China, Shanghai, China).

Tissue distribution study

36 rats were randomly divided into six groups; half the number of rats were males, the other half were females. Before the experiment, the rats were fasted overnight and allowed to drink water *ad libitum*. After the injection of **S_1** at a dose of 6.8mg/kg or **S_2** at a dose of 8.4mg/kg through the tail vein, rats were euthanized at 5, 30, and 60 min. Heart, liver, spleen, lung, kidney, and brain And then desired tissues were harvested immediately and treated as described in section "Processing of bio-samples".

Ethical approval

All animal experiments were approved by the Biomedical Ethics Committee of Health Science Center of Xi'an Jiaotong University (No.XJTUAE2020-1405).

STATISTICAL ANALYSIS

SAS 9.4 software was utilized for the statistical analysis. All values of pharmacokinetic parameters and experimental results are expressed as the mean ± standard deviation.

RESULTS

Method validation

The HPLC methods for the determination of S_1 and S_2 in plasma and tissues of rats were validated successfully by evaluating the following parameters.

Specificity: S_1 and S_2, both as an analyte and IS for each other, had retention times of 8.62 min and 12.10 min, respectively, and no interfering peaks were detected at these retention times in blank plasma and tissue samples, which indicated that the method developed exhibited good specificity. Representative HPLC chromatograms for blank plasma samples and real plasma-samples containing analyte and IS are shown in fig 2. The representative HPLC chromatograms for blank tissue and real tissue-samples are shown in Supplemental figs. 1-6.

Linearity and LLOQ: Good linearity for S_1 in plasma and various tissues was obtained as follows: 0.1-25.0μg/mL in plasma, 0.08-10.0μg/g in heart, 0.2-60.0μg/g in liver, 0.08-8.0μg/g in spleen or lung, 0.4-20.0μg/g in kidney, and 0.04-0.8μg/g in brain.

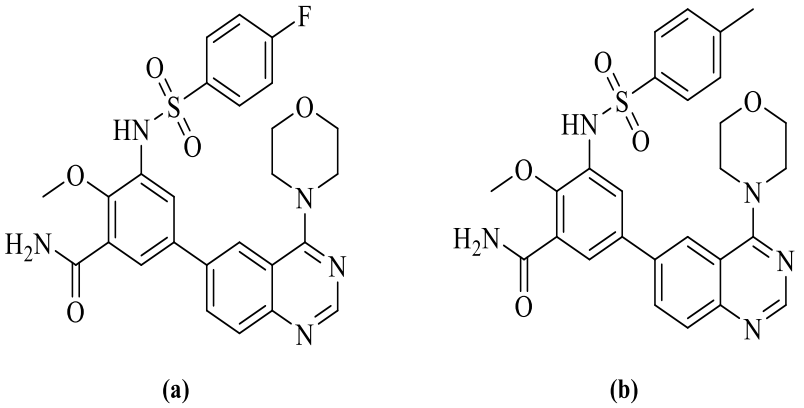
For S_2 in different biological matrices, good linearity was also exhibited as follows: 0.1-40.0μg/mL in plasma, 0.2-10.0μg/g in heart, 0.4-40.0μg/g in liver, 0.2-8.0μg/g in spleen or lung, 0.4-20μg/g in kidney, and 0.04-0.8μg/g in brain. The regression equations of S_1 and S_2 in various biological matrices are presented in. All correlation coefficients were greater than 0.99, which indicated that the linearity of the calibration curves was excellent. The LLOQ of both S_1 and S_2 in plasma was 0.1μg/mL. The LLOQ of S_1 and S_2 in tissues was as follows: 0.08 and 0.2μg/g in heart, 0.2 and 0.4μg/g in liver, 0.08 and 0.2μg/g in spleen, 0.08 and 0.2μg/g in lung, 0.4μg/g for both in kidney, 0.04μg/g for both in brain, respectively.

Accuracy and precision: The accuracy and precision of within-run and between-run for S_1 and S_2 in plasma and tissues at four concentration levels in five replicates are presented in Table 1.

The deviations of the measured concentration at LLOQ and the other three concentration levels were within 20% and 15% of the nominal concentration, respectively. However, the RSDs of the measured concentration at LLOQ and the other concentration levels were not higher than 20% and 15%. Both the accuracy and precision were within the acceptable range, thereby demonstrating the accuracy, reliability, and reproducibility of the method.

Recovery: The results of the sample addition recovery are listed in Table 2. The sample adding recovery of the analyte was within the range of 85-115% and the RSD of the relative recovery was no more than 15%, thus suggesting that the recoveries of the analyte and the IS in plasma and tissues were good.

Stability: The stability results are presented in Table 3. The deviations of the measured concentration of the QC samples at low, medium, and high concentration levels were less than 15% of the nominal concentration, and all RSDs were within 15%, thereby demonstrating that under the conditions of three freeze-thaw cycles, at ambient temperature for 8 h, and at -20ºC for 30 days, no significant degradation of any analyte or IS in plasma or tissue had occurred.



(a) **(b)**

Fig. 1: Structures of S_1 (a) and S_2 (b).

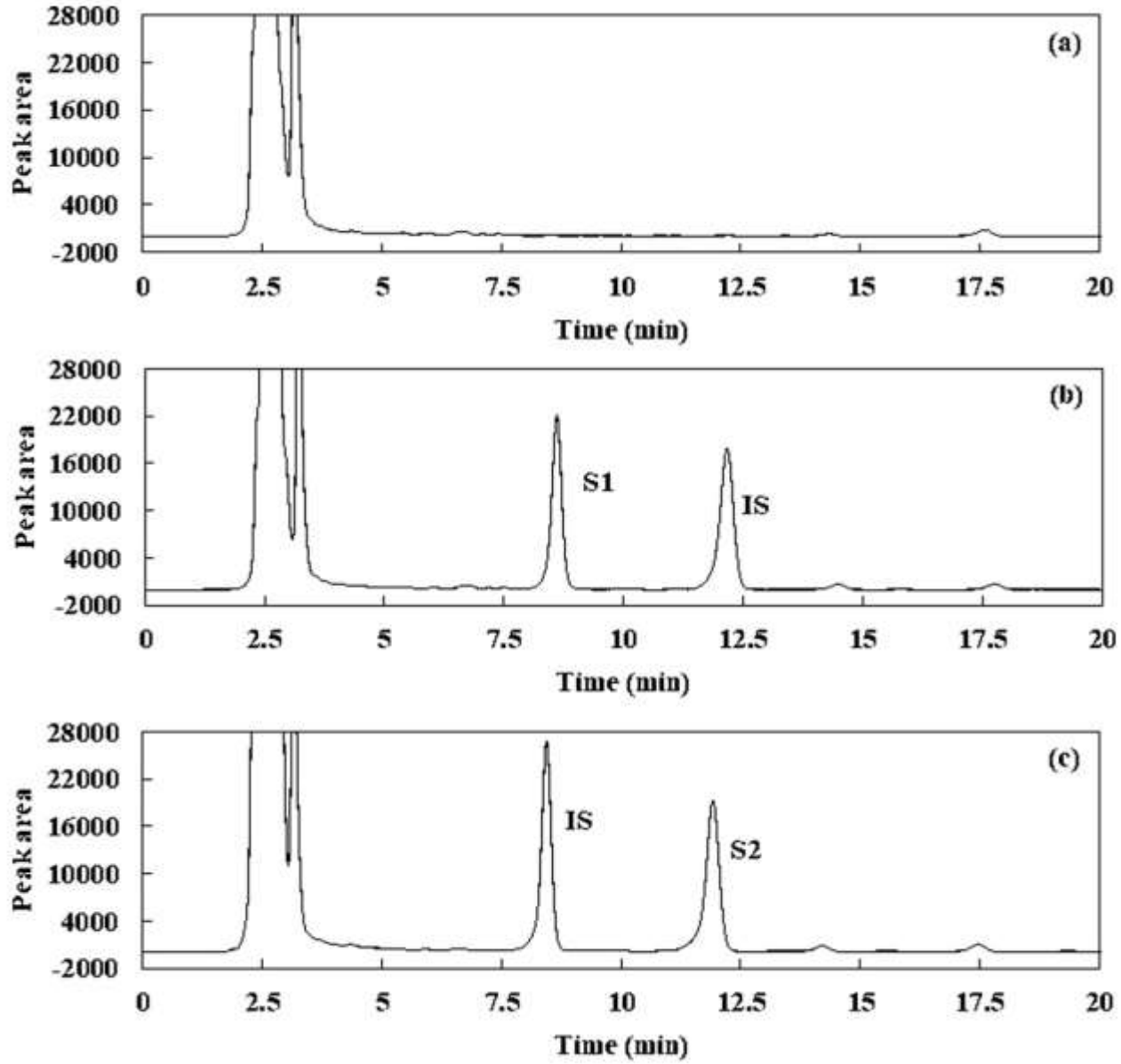


Fig. 2: Representative chromatograms of rat plasma. (a) Blank rat plasma; (b) **S1** and IS rat plasma; (c) **S2** and IS rat plasma.

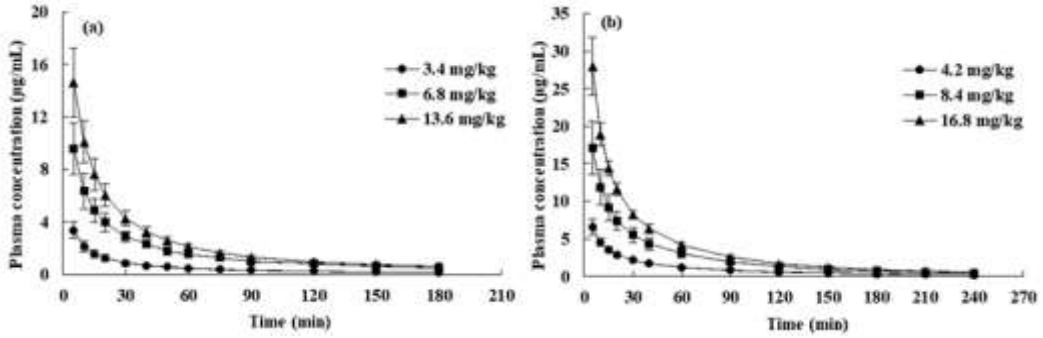


Fig. 3: Concentration-time profiles of **S₁** (a) or **S₂** (b) in rat plasma following intravenous injection of **S₁** or **S₂** at low, medium, and high doses (mean ± SD, n = 6).

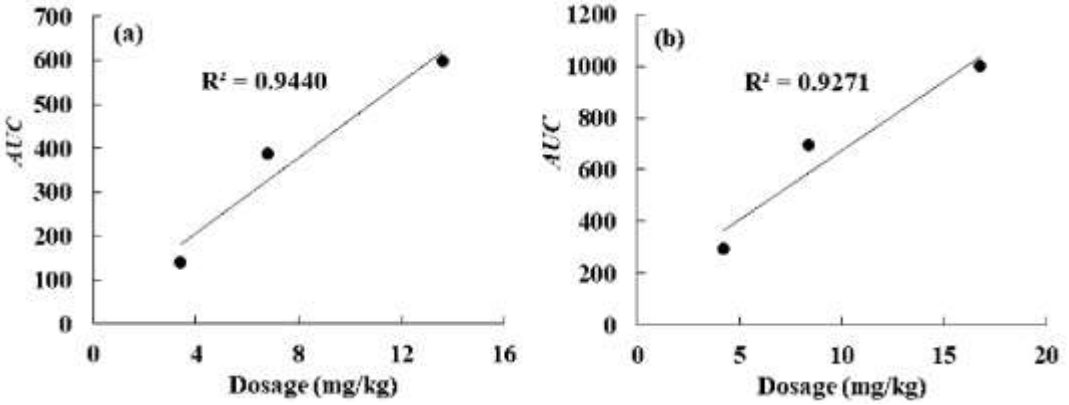


Fig. 4: Relationship between dosage of **S₁** (a) or **S₂** (b) with *AUC* for rat plasma after intravenous injection of **S₁** or **S₂** at low, medium, and high doses.

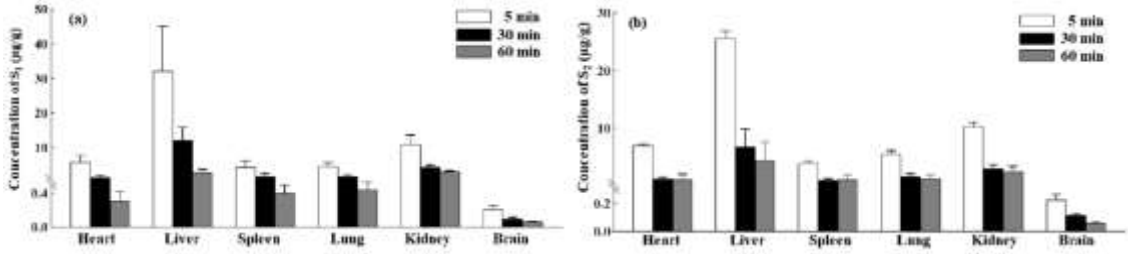


Fig. 5: Mean concentration of **S₁** (a) or **S₂** (b) in various tissues at 5, 30, and 60 min after intravenous injection in rats at a medium dose (Mean ± SD, n = 6).

Table 1: Linearity of S_1 and S_2 in rat plasma and tissues (n = 6).

Compound	Matrix	Equation	Linear range (μg/mL or μg/g)[a]	R^2
S₁	Plasma	$y = 0.1782x + 0.0031$	0.1 - 25.0	0.9994
	Heart	$y = 0.2391x + 0.0081$	0.08 - 10.0	0.9960
	Liver	$y = 0.2432x + 0.0409$	0.2 - 60.0	0.9952
	Spleen	$y = 0.2600x + 0.0026$	0.08 - 8.0	0.9981
	Lung	$y = 0.2732x + 0.0057$	0.08 - 8.0	09924
	Kidney	$y = 0.2690x - 0.0064$	0.4 - 20.0	0.9953
	Brain	$y = 0.2659x + 0.0046$	0.04 - 0.8	0.9940
S₂	Plasma	$y = 0.2013x + 0.0006$	0.1 - 40.0	0.9999
	Heart	$y = 0.2461x + 0.0006$	0.2 - 10.0	0.9954
	Liver	$y = 0.2632x - 0.0104$	0.4 - 40.0	0.9972
	Spleen	$y = 0.2540x - 0.0096$	0.2 - 8.0	0.9970
	Lung	$y = 0.2496x - 0.0076$	0.2 - 8.0	0.9964
	Kidney	$y = 0.2362x - 0.0084$	0.4 - 20.0	0.9983
	Brain	$y = 0.2568x - 0.0004$	0.04 - 0.8	0.9980

[a] The concentration unit in plasma is in μg/mL and in tissues is in μg/g.

Table 1: The accuracy and precision of **S_1** and **S_2** in rat plasma and tissues (n = 6).

Compound	Matrix	QCs (μg/mL or μg/g)[a]	Within-run			Between-run (%)		
			Measured concentration (μg/mL or μg/g)[a]	Accuracy (Mean, %)	Precision (RSD, %)	Measured concentration (μg/mL or μg/g)[a]	Accuracy (Mean, %)	Precision (RSD, %)
S_1	Plasma	0.1	0.092 ± 0.004	92.41	4.69	0.092 ± 0.004	91.82	4.65
		0.2	0.191 ± 0.006	95.57	3.22	0.190 ± 0.007	94.96	3.76
		12.5	13.956 ± 0.249	111.65	1.78	13.977 ± 0.281	111.81	2.01
		20.0	21.826 ± 0.352	109.13	1.61	21.871 ± 0.276	109.36	1.26
	Heart	0.08	0.065 ± 0.003	80.99	5.12	0.068 ± 0.005	85.62	6.74
		0.16	0.163 ± 0.014	101.78	8.82	0.164 ± 0.016	102.29	9.83
		3.2	3.608 ± 0.046	112.76	1.26	3.595 ± 0.048	112.34	1.33
		8.0	8.760 ± 0.127	109.50	1.45	8.757 ± 0.119	109.47	1.36
	Liver	0.2	0.196 ± 0.012	97.80	6.29	0.194 ± 0.015	97.13	7.65
		0.4	0.402 ± 0.014	100.50	3.49	0.391 ± 0.015	97.66	3.90
		24.0	24.118 ± 0.839	100.49	3.48	24.145 ± 0.763	100.60	3.16
		48.0	48.346 ± 0.535	100.72	1.11	48.401 ± 0.605	100.83	1.25
	Spleen	0.08	0.089 ± 0.005	111.15	5.43	0.089 ± 0.005	110.70	5.51
		0.16	0.174 ± 0.010	108.63	5.50	0.172 ± 0.012	107.52	7.07
		3.2	3.386 ± 0.142	105.81	4.19	3.376 ± 0.145	105.49	4.29
		6.0	6.163 ± 0.241	102.71	3.91	6.134 ± 0.201	102.24	3.28
	Lung	0.08	0.087 ± 0.002	109.25	2.72	0.090 ± 0.005	112.08	5.21
		0.16	0.172 ± 0.004	107.71	2.38	0.169 ± 0.010	105.34	5.74
		3.2	3.304 ± 0.052	103.25	1.58	3.311 ± 0.058	103.47	1.75
		6.0	6.517 ± 0.123	108.61	1.88	6.529 ± 0.139	108.82	2.13
	Kidney	0.4	0.446 ± 0.018	111.58	4.05	0.451 ± 0.023	112.78	5.12
		0.8	0.827 ± 0.023	103.41	2.79	0.819 ± 0.028	102.44	3.42
		8.0	8.309 ± 0.126	103.86	1.52	8.279 ± 0.158	103.49	1.91
		16.0	16.468 ± 0.215	102.93	1.31	16.625 ± 0.369	103.91	2.22
	Brain	0.04	0.043 ± 0.004	107.74	8.71	0.043 ± 0.003	108.19	7.58
		0.08	0.078 ± 0.007	97.43	8.35	0.078 ± 0.006	97.41	7.62
		0.32	0.295 ± 0.005	92.23	1.58	0.295 ± 0.005	92.26	1.64
		0.64	0.648 ± 0.062	101.27	9.50	0.651 ± 0.058	101.78	8.85
S_2	Plasma	0.1	0.116 ± 0.002	115.60	1.90	0.115 ± 0.003	115.00	2.93
		0.2	0.219 ± 0.008	109.26	3.72	0.218 ± 0.009	108.93	4.14
		17.5	19.644 ± 0.274	112.26	1.39	19.469 ± 0.313	111.25	1.61
		30.0	33.256 ± 0.534	110.21	1.62	33.430 ± 0.774	111.43	2.32
	Heart	0.2	0.201 ± 0.013	100.51	6.66	0.197 ± 0.015	98.69	7.71
		0.4	0.409 ± 0.019	102.28	4.53	0.417 ± 0.024	104.29	5.83
		3.2	3.532 ± 0.063	110.39	1.80	3.487 ± 0.061	108.98	1.76
		8.0	8.744 ± 0.165	109.30	1.88	8.659 ± 0.214	108.24	2.47
	Liver	0.4	0.472 ± 0.007	118.06	1.50	0.467 ± 0.014	116.65	2.98
		0.8	0.823 ± 0.024	102.92	2.90	0.827 ± 0.026	103.34	3.15
		16.0	15.806 ± 0.452	98.79	2.86	15.776 ± 0.403	98.60	2.56
		32.0	35.430 ± 0.981	110.72	2.77	34.868 ± 1.096	108.96	3.14
	Spleen	0.2	0.225 ± 0.009	112.67	4.11	0.221 ± 0.009	110.30	3.84
		0.4	0.446 ± 0.006	111.43	1.37	0.440 ± 0.008	109.91	1.90
		3.2	3.460 ± 0.085	108.12	2.45	3.443 ± 0.087	107.59	2.54
		6.0	6.056 ± 0.301	100.93	5.12	6.051 ± 0.306	100.85	5.06
	Lung	0.2	0.227 ± 0.006	113.52	2.64	0.224 ± 0.007	111.85	3.32
		0.4	0.427 ± 0.023	106.84	5.36	0.426 ± 0.019	106.48	4.34
		3.2	3.514 ± 0.120	109.81	3.40	3.464 ± 0.148	108.23	4.29
		6.0	6.717 ± 0.101	111.95	1.50	6.655 ± 0.174	110.92	2.61
	Kidney	0.4	0.449 ± 0.011	112.18	2.37	0.444 ± 0.016	111.07	3.70
		0.8	0.872 ± 0.029	109.03	3.33	0.856 ± 0.043	107.03	5.07
		8.0	8.881 ± 0.279	111.01	3.14	8.745 ± 0.268	109.31	3.06
		16.0	17.733 ± 0.217	110.83	1.22	17.378 ± 0.510	108.61	2.93
	Brain	0.04	0.046 ± 0.001	115.46	2.19	0.046 ± 0.001	114.02	2.31
		0.08	0.087 ± 0.004	108.60	4.99	0.087 ± 0.003	109.17	3.66
		0.32	0.347 ± 0.004	108.34	1.27	0.349 ± 0.009	108.99	2.43
		0.64	0.692 ± 0.012	108.05	1.74	0.691 ± 0014	107.96	1.96

[a] The concentration unit in plasma is in μg/mL and in tissues is in μg/g.

Table 2: The sample addition recovery of **S_1** and **S_2** in rat plasma and tissues (n = 6).

Compound	Matrix	QCs (μg/mL or μg/g)[a]	Measured concentration (μg/mL or μg/g)[a]	Recovery (Mean, %)	RSD (%)
S_1	Plasma	0.2	0.191 ± 0.006	95.57	3.22
		12.5	13.956 ± 0.249	111.65	1.78
		20.0	21.826 ± 0.352	109.13	1.61
	Heart	0.16	0.163 ± 0.014	101.78	8.82
		3.2	3.602 ± 0.048	112.57	1.33
		8.0	8.760 ± 0.127	109.50	1.45
	Liver	0.4	0.392 ± 0.025	97.96	6.25
		24.0	24.118 ± 0.839	100.49	3.48
		48.0	48.346 ± 0.535	100.72	1.11
	Spleen	0.16	0.174 ± 0.010	108.63	5.50
		3.2	3.386 ± 0.142	105.81	4.19
		6.0	6.163 ± 0.241	102.71	3.91
	Lung	0.16	0.172 ± 0.004	107.71	2.38
		3.2	3.304 ± 0.052	103.25	1.58
		6.0	6.517 ± 0.123	108.61	1.88
	Kidney	0.8	0.827 ± 0.023	103.41	2.79
		8.0	8.309 ± 0.126	103.86	1.52
		16.0	16.468 ± 0.215	102.93	1.31
	Brain	0.08	0.078 ± 0.007	97.43	8.35
		0.32	0.295 ± 0.005	92.23	1.58
		0.64	0.648 ± 0.062	101.27	9.50
S_2	Plasma	0.2	0.219 ± 0.008	109.26	3.72
		17.5	19.645 ± 0.274	112.26	1.39
		30.0	33.063 ± 0.534	110.21	1.62
	Heart	0.4	0.409 ± 0.019	102.28	4.53
		3.2	3.532 ± 0.063	110.39	1.80
		8.0	8.744 ± 0.164	109.30	1.88
	Liver	0.8	0.823 ± 0.024	102.92	2.90
		16.0	15.806 ± 0.452	98.79	2.86
		32.0	35.430 ± 0.981	110.72	2.77
	Spleen	0.4	0.446 ± 0.006	111.43	1.37
		3.2	3.460 ± 0.085	108.12	2.45
		6.0	6.056 ± 0.310	100.93	5.12
	Lung	0.4	0.427 ± 0.023	106.84	5.36
		3.2	3.514 ± 0.120	109.81	3.40
		6.0	6.717 ± 0.101	111.95	1.50
	Kidney	0.8	0.872 ± 0.029	109.03	3.33
		8.0	8.881 ± 0.279	111.01	3.14
		16.0	17.733 ± 0.217	110.83	1.22
	Brain	0.08	0.087 ± 0.004	108.60	4.99
		0.32	0.347 ± 0.004	108.34	1.27
		0.64	0.692 ± 0.012	108.05	1.74

[a] The concentration unit in plasma is in μg/mL and in tissues is in μg/g.

Pharmacokinetic study

The developed and validated HPLC method was utilized for the quantitative determination of S1 and S2 in rat plasma following tail intravenous injection at low, medium, and high doses. The mean arterial plasma concentrations of S1 and S2 at different time points are shown in table 5, and plasma concentration-time curves of S1 and S2 are displayed in fig. 3, which indicated that the plasma concentration of S1 and S2 decreased significantly over time. The main pharmacokinetic parameters are listed in table 6. When administered to rats using intravenous injection, the elimination of S1 and S2 followed the two-compartment model. Furthermore, after intravenous injection of S1 at doses of 3.4, 6.8, and 13.6mg/kg or S2 at doses of 4.2, 8.4, and 16.8mg/kg, the AUCs of S1 or S2 were dose-proportional as shown in Fig.

Table 3: The stability of **S_1** and **S_2** in rat plasma and tissues (n = 6).

Compound	Matrix	QCs (μg/mL or μg/g)[a]	Ambient temperature for 8 h			-20ºC for 30 days			Three freeze-thaw cycles		
			Measured concentration (μg/mL)[a]	Percentage of nominal concentration	RSD (%)	Measured concentration (μg/mL or μg/g)[a]	Percentage of nominal concentration	RSD (%)	Measured concentration (μg/mL or μg/g)[a]	Percentage of nominal concentration	RSD (%)
S_1	Plasma	0.2	0.194 ± 0.006	97.185	3.13	0.206 ± 0.011	103.23	5.38	0.200 ± 0.014	100.17	7.23
		12.5	12.656 ± 0.277	101.25	2.19	12.812 ± 0.561	102.50	4.38	12.675 ± 0.811	101.40	6.40
		20.0	21.113 ± 0.281	105.56	1.33	20.567 ± 0.362	102.83	1.76	20.392 ± 1.090	101.96	5.34
	Heart	0.16	0.152 ± 0.010	95.25	6.45	0.158 ± 0.009	98.93	5.52	0.151 ± 0.004	94.38	2.35
		3.2	3.269 ± 0.159	102.14	4.87	3.288 ± 0.214	102.75	6.51	3.270 ± 0.118	102.18	3.60
		8.0	8.553 ± 0.184	106.91	2.15	8.430 ± 0.269	105.38	3.20	8.275 ± 0.371	103.44	4.49
	Liver	0.4	0.396 ± 0.028	99.12	7.18	0.396 ± 0.010	98.94	2.54	0.374 ± 0.014	95.60	3.76
		24.0	23.678 ± 0.819	98.66	3.46	24.221 ± 0.394	100.92	1.63	22.147 ± 1.122	92.28	5.07
		48.0	48.238 ± 0.687	100.50	1.42	48.245 ± 0.687	100.51	1.42	44.034 ± 1.997	91.74	4.54
	Spleen	0.16	0.170 ± 0.011	106.31	6.66	0.170 ± 0.006	106.54	3.79	0.152 ± 0.007	94.83	4.69
		3.2	3.389 ± 0.110	105.90	3.25	3.356 ± 0.148	104.87	4.42	3.183 ± 0.114	99.46	3.58
		6.0	6.117 ± 0.300	101.94	4.90	6.112 ± 0.300	101.87	4.91	5.888 ± 0.216	98.13	3.66
	Lung	0.16	0.164 ± 0.009	102.55	5.68	0.167 ± 0.013	104.51	7.61	0.152 ± 0.007	95.04	4.86
		3.2	3.294 ± 0.070	102.92	2.13	3.286 ± 0.063	102.68	1.91	3.036 ± 0.075	94.88	2.48
		6.0	6.263 ± 0.238	104.38	3.80	6.541 ± 0.164	109.02	2.50	5.679 ± 0.082	94.65	1.44
	Kidney	0.8	0.821 ± 0.030	102.64	3.67	0.817 ± 0.024	102.13	2.94	0.764 ± 0.013	95.45	1.70
		8.0	8.267 ± 0.145	103.34	1.75	8.267 ± 0.145	103.34	1.75	7.959 ± 0.219	99.48	2.75
		16.0	16.379 ± 0.204	102.37	1.24	16.451 ± 0.300	102.82	1.82	15.101 ± 0.683	94.38	4.53
	Brain	0.08	0.082 ± 0.006	102.09	6.84	0.080± 0.004	100.54	5.15	0.082 ± 0.006	102.12	6.92
		0.32	0.312 ± 0.013	97.44	4.04	0.330 ± 0.009	103.04	2.80	0.306 ± 0.005	95.73	1.69
		0.64	0.631 ± 0.027	98.54	4.33	0.642 ± 0.034	100.32	5.22	0.601 ± 0.014	93.94	2.35
S_2	Plasma	0.2	0.207 ± 0.008	103.73	3.74	0.210 ± 0.004	105.04	1.69	0.202 ± 0.012	100.94	6.09
		17.5	17.704 ± 0.451	101.17	2.55	17.085 ± 0.267	97.63	1.56	17.719 ± 0.462	101.25	2.61
		30.0	30.895 ± 0.680	102.98	2.20	30.174 ± 0.397	100.58	1.31	30.001 ± 0.862	100.00	2.87
	Heart	0.4	0.416 ± 0.018	103.88	4.39	0.401 ± 0.013	100.26	3.15	0.390 ± 0.016	97.39	4.17
		3.2	3.364 ± 0.135	105.13	4.01	3.431 ± 0.104	107.23	3.02	3.204 ± 0.142	100.12	4.43
		8.0	8.133 ± 0.128	101.67	1.57	8.445 ± 0.226	105.56	2.68	8.186 ± 0.453	102.33	5.54
	Liver	0.8	0.833 ± 0.028	104.09	3.33	0.820 ± 0.016	102.48	2.00	0.805 ± 0.040	100.66	4.96
		16.0	16.383 ± 0.430	102.39	2.63	16.022 ± 0.468	100.13	2.92	15.129 ± 0.467	94.55	3.08
		32.0	32.407 ± 0.802	101.27	2.47	32.144 ± 0.530	100.45	1.65	30.704 ± 0.879	95.95	2.86
	Spleen	0.4	0.405 ± 0.010	101.20	2.47	0.413 ± 0.012	103.21	2.80	0.399 ± 0.022	99.85	5.62
		3.2	3.361 ± 0.129	105.04	3.85	3.355 ± 0.151	104.85	4.49	3.262 ± 0.172	101.92	5.27
		6.0	6.123 ± 0.218	102.05	3.56	5.960 ± 0.168	99.33	2.81	6.086 ± 0.378	101.43	6.20
	Lung	0.4	0.409 ± 0.009	102.18	2.26	0.425 ± 0.023	106.24	5.40	0.407 ± 0.034	101.86	8.32
		3.2	3.387 ± 0.139	105.83	4.12	3.364 ± 0.172	105.12	5.12	3.139 ± 0.155	98.08	4.93
		6.0	6.205 ± 0.176	103.42	2.83	6.057 ± 0.213	100.95	3.51	5.950 ± 0.307	99.17	5.15
	Kidney	0.8	0.837 ± 0.025	104.65	3.04	0.819 ± 0.015	102.35	1.81	0.802 ± 0.041	100.30	5.06
		8.0	8.426 ± 0.133	105.32	1.58	8.291 ± 0.132	103.64	1.59	8.027 ± 0.260	100.33	3.24
		16.0	16.359 ± 0.415	102.24	2.54	16.110 ± 0.336	100.69	2.09	15.471 ± 0.324	96.69	2.10
	Brain	0.08	0.085 ± 0.005	105.91	5.73	0.084 ± 0.006	104.51	6.64	0.080 ± 0.004	99.51	4.72
		0.32	0.334 ± 0.020	104.44	6.07	0.341 ± 0.008	106.64	2.37	0.321 ± 0.020	100.46	6.36
		0.64	0.688 ± 0.015	107.44	2.16	0.653 ± 0.018	101.96	2.73	0.634 ± 0.023	99.06	3.63

[a] The concentration unit in plasma is in μg/mL and in tissues is in μg/g.

Table 4: The mean arterial plasma concentration of **S₁** and **S₂** at different time points at low, medium, and high doses (n = 6).

Compound	Time	Plasma concentration(μg/mL) at different doses		
		low[a]	medium[a]	high[a]
S₁	5	3.364±0.640	9.588±1.945	14.617±2.637
	10	2.124±0.432	6.348±1.360	10.108±1.624
	15	1.563±0.288	4.879±0.893	7.640±1.222
	20	1.251±0.253	3.970±0.707	6.052±0.887
	30	0.881±0.155	2.877±0.406	4.273±0.588
	40	0.678±0.109	2.360±0.256	3.240±0.397
	50	0.588±0.102	1.782±0.339	2.601±0.288
	60	0.495±0.081	1.550±0.235	2.116±0.223
	75	0.405±0.061	1.288±0.174	1.627±0.150
	90	0.342±0.048	1.009±0.204	1.323±0.121
	120	0.260±0.031	0.837±0.073	0.957±0.083
	150	0.206±0.024	0.635±0.089	0.767±0.065
	180	0.172±0.018	0.534±0.085	0.659±0.058
S₂	5	6.629±1.061	17.093±3.534	28.018±3.846
	10	4.531±0.488	11.866±2.348	18.917±1.564
	15	3.545±0.384	9.168±1.715	14.384±1.009
	20	2.888±0.223	7.378±1.238	11.605±0.850
	30	2.183±0.170	5.524±0.975	8.265±0.560
	40	1.752±0.138	4.364±0.823	6.407±0.594
	60	1.242±0.094	3.105±0.590	4.206±0.346
	90	0.821±0.047	1.972±0.399	2.557±0.223
	120	0.587±0.027	1.360±0.286	1.692±0.148
	150	0.445±0.017	0.973±0.213	1.211±0.115
	180	0.348±0.010	0.735±0.153	0.906±0.079
	210	0.287±0.007	0.577±0.119	0.706±0.063
	240	0.247±0.004	0.474±0.092	0.559±0.047

[a] The low, medium, and high doses are 3.4, 6.8, and 13.6mg/kg for **S₁**, 4.2, 8.4, and 16.8mg/kg for **S₂**, respectively.

Table 5: Pharmacokinetic parameters in rats after intravenous injection of **S₁** and **S₂** at low, medium, and high doses (n = 6, mean ± SD).

Parameter	Values for **S₁**			Values for **S₂**		
	iv (3.4mg/kg)	iv (6.8mg/kg)	iv (13.6mg/kg)	iv (4.2mg/kg)	iv (8.4mg/kg)	iv (16.8mg/kg)
A (μg/mL)	3.69 ± 0.71	9.53 ± 1.88	14.54 ± 2.80	5.87 ± 0.99	15.54 ± 3.96	26.00 ± 3.60
α (1/min)	0.091 ± 0.002	0.077 ± 0.024	0.065 ± 0.004	0.062 ± 0.005	0.066 ± 0.008	0.062 ± 0.004
B (μg/mL)	0.80 ± 0.14	2.23 ± 0.82	2.89 ± 0.28	1.63 ± 0.18	4.54 ± 1.14	5.87 ± 0.75
β (1/min)	0.0089 ± 0.0005	0.0079 ± 0.0027	0.0087 ± 0.0003	0.0082 ± 0.0005	0.0098 ± 0.0003	0.0101 ± 0.0003
*V*c (mg/kg)/(μg/mL)	0.775 ± 0.142	0.589 ± 0.095	0.798 ± 0.156	0.570 ± 0.094	0.435 ± 0.098	0.532 ± 0.062
$t_{1/2(\alpha)}$ (min)	7.62 ± 0.14	7.94 ± 1.54	10.72 ± 0.07	11.29 ± 0.87	10.67 ± 1.22	11.26± 0.64
$t_{1/2(\beta)}$ (min)	78.13 ± 3.90	74.22 ± 8.22	80.04 ± 2.94	84.30 ± 4.82	70.95 ± 1.74	68.69 ± 2.17
k_{21} (1/min)	0.0235±0.0002	0.0221±0.0116	0.0180±0.0010	0.0199±0.0008	0.0224±0.0018	0.0196±0.0009
k_{10} (1/min)	0.0344±0.0012	0.0285±0.0033	0.0311±0.0017	0.0256±0.0023	0.0286±0.0021	0.0318±0.0013
k_{12} (1/min)	0.0420±0.0023	0.0346±0.0019	0.0242±0.0006	0.0244±0.0019	0.0243±0.0046	0.0204±0.0018
AUC (min·μg/mL)	139.71 ± 16.41	388.96 ± 70.06	599.32 ± 71.17	292.19 ± 20.57	697.63±137.22	1000.65±85.26
CL [mg/kg/min/(μg/mL)]	0.0265 ± 0.0040	0.0217 ± 0.0029	0.0246 ± 0.0034	0.0144 ± 0.0010	0.0123 ± 0.0024	0.0169 ± 0.0014

4, and the RSD of the dose normalized *AUC*s was less than 20%, thereby indicating a linear pharmacokinetic process of S_1 or S_2 in rats. Other pharmacokinetic parameters exhibited no obvious dosage dependency. S_1 and S_2 had an elimination half-life of about 77.5 and 73.9 min, respectively. The average dose-normalized *AUC*s of S_2 was 1.49-fold higher than that of S_1, and the *CL* of S_1 at different doses was approximately 1.68-fold higher compared to that of S_2.

Tissue distribution study

After intravenous administration of S_1 at a dose of 6.8mg/kg or S_2 at a dose of 8.4mg/kg to rats for 5, 30, and 60 min, the concentration of S_1 and S_2 in rat heart, liver, spleen, lung, kidney, and brain is shown in fig. 5. The data showed that S_1 and S_2 could be detected rapidly and had a wide distribution in the tested tissues. The concentration of S_1 and S_2 in various tissues decreased significantly ($P < 0.05$) over time. Higher distributions were observed detected in the liver and kidney, while the lowest distribution was observed found in the brain when compared with other tissues at the same time point. At 5 min after dosage, the concentrations of S_1 in various tissues were in the following order: liver > kidney > heart > lung ≈ spleen > brain, for S_2 the order was: liver > kidney > heart > lung > spleen > brain. While at 30 min, the concentrations of S_1 were in the following: liver > kidney > lung ≈ spleen > heart > brain. For S_2, the order was: liver > kidney > lung > heart > spleen > brain. One hour after administration, the concentrations of S_1 in tissues were in the following order: liver ≈ kidney > lung ≈ spleen > heart > brain, and the order of those for S_2 were as follows: liver > kidney >lung ≈ spleen ≈ heart > brain. Notably, both S_1 and S_2 can cross the blood-brain barrier, thereby suggesting the potential of for treating brain tumors.

DISCUSSION

The quantification of new drugs is a crucial part of drug development. One of the primary tasks of pharmaceutical analysis is to establish an assay method for the determination of new drugs. However, for new compounds, the method for determination is usually not yet available. Therefore, establishing a simple, rapid, and sensitive method is of utmost importance for obtaining a comprehensive pharmacokinetic profile. Due to the greater sensitivity, selectivity, cost-effectiveness, and accessibility, HPLC is widely used. To develop and validate a proper assay method for the analysis of the two quinazolines as potential anticancer drugs, S_1 and S_2, validation guidelines for bioanalytical methods by the FDA and EMA were used.

In the screening of the chromatographic mobile phase, it was found that the replacement of triple-distilled water with a pH 7.0 phosphate buffer solution can greatly improve the peak shape and the separation degree, which may be due to the fact that the pH 7.0 phosphate buffer solution can inhibit the dissociation of S_1 and S_2 and keep them in the molecular state. In the actual experiment, it was found that the effect of potassium salt was better than that of sodium salt. Therefore, the potassium phosphate buffer solution was chosen. Also, in the processing of tissue samples, the saline was replaced with a pH 7.0 potassium phosphate buffer solution, which can greatly improve the exaction rate. The previous solubility study showed that S_1 and S_2 had good solubility in both ethyl acetate and trichloromethane, but after extraction, ethyl acetate was located in the upper layer of the aqueous phase, while trichloromethane was located in the lower layer of the aqueous layer. Therefore, considering the convenience of transferring the organic phase, safety, environmental protection, and cost, ethyl acetate was selected as the extractant in the processing of tissue samples.

According to the results of the pharmacokinetic study and pharmacokinetic parameters, the elimination half-life of S_1 and S_2 was about 77.5 and 73.9 min, respectively, which indicated that both of them belong to fast-elimination drugs. These findings revealed that S_1 and S_2 would not accumulate in large quantities *in vivo* and suggested that in order to maintain a stable blood concentration and achieve a better therapeutic effect, it is necessary to increase the dosing frequency or administer through intravenous infusion.

For S_1 and S_2, the average dose-normalized *AUC*s were 47.45 and 70.73 min·g/mL, respectively, while the average *CL*s were 0.0243 and 0.0145 mg/kg/min/(μg/mL), respectively. Furthermore, compared with S_1, the elimination half-life of S_2 exhibited no significant difference with that of S_1 ($P > 0.05$), while S_2 had a higher *AUC*s and lower *CL*s, indicating a better therapeutic effect may be produced by S_2 *in vivo*.

According to the results of the tissue distribution study, 5 min after dosage, S_1 and S_2 could be detected in the heart, liver, spleen, lung, kidney and brain, which indicated that S_1 and S_2 could be distributed quickly in the above tissues.

The concentration of S_1 or S_2 in the liver and kidney was significantly higher than in other tissues, which suggested that S_1 and S_2 were probably metabolized in the liver and excreted by the kidney. The concentration of S_1 or S_2 in the heart decreased more rapidly than in the lungs and spleen, indicating S_1 and S_2 were eliminated more quickly in the heart. Both S_1 and S_2 can cross the blood-brain barrier, thereby suggesting the potential of for treating brain tumors. Compared with S_1, the elimination of S_2 in the heart, liver, spleen, and lung was much slower than that of S_1, which indicated that S_2 may have a longer duration time in above tissues and a better therapeutic effect would be produced by S_2 than S_1.

CONCLUSION

In this study, a simple, rapid, and sensitive HPLC method was developed and validated for the quantitation of the two novel quinazoline derivatives, S_1 and S_2, in rat plasma and rat tissues.

As novel PI3K/mTOR dual-inhibitors and potential anticancer agents, the pharmacokinetic and tissue distribution characteristics of S_1 and S_2 in normal rats were investigated and reported for the first time. The pharmacokinetics results indicated that S_1 and S_2 were eliminated in rats following the two-compartment model after intravenous injection and linear pharmacokinetics characteristics were observed. Both S_1 and S_2 belong to the group of fast-elimination drugs, and could be widely distributed in the liver and kidney, and a small proportion of S_1 and S_2 could cross the blood-brain barrier and be distributed in the brain. Pharmacokinetic and tissue distribution studies of S_1 and S_2 are extremely important and significant for investigating the relationship between dosage and pharmacodynamic effects.

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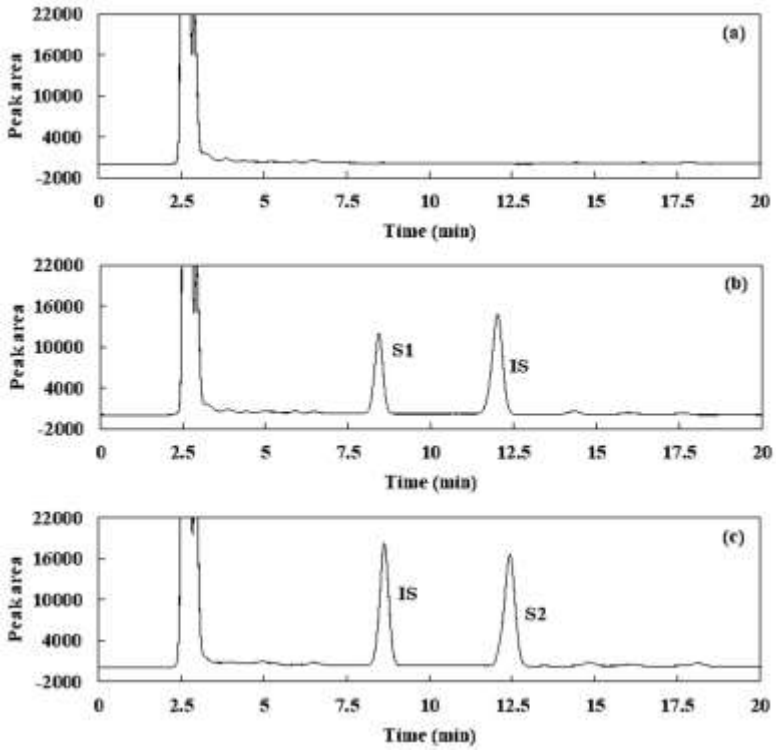
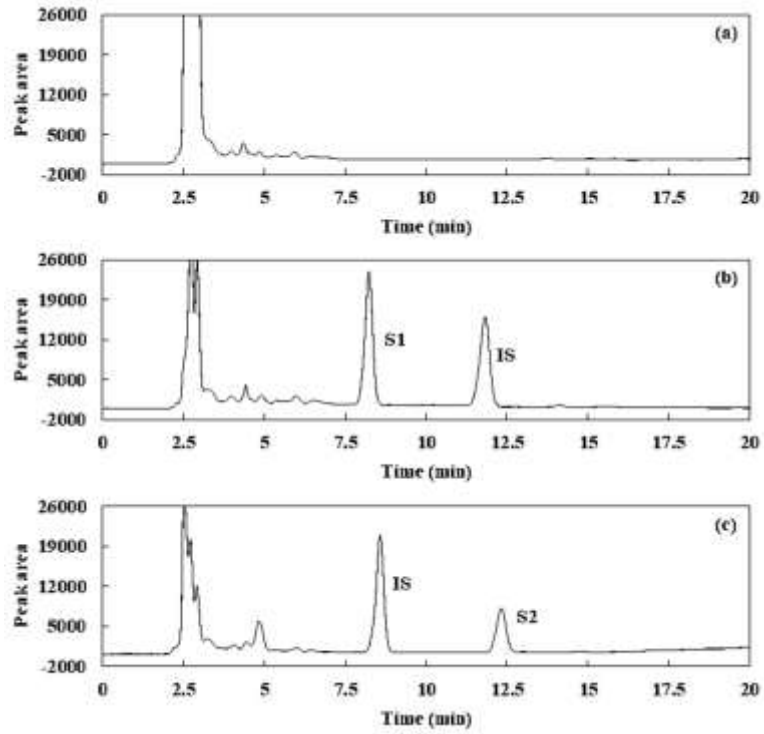
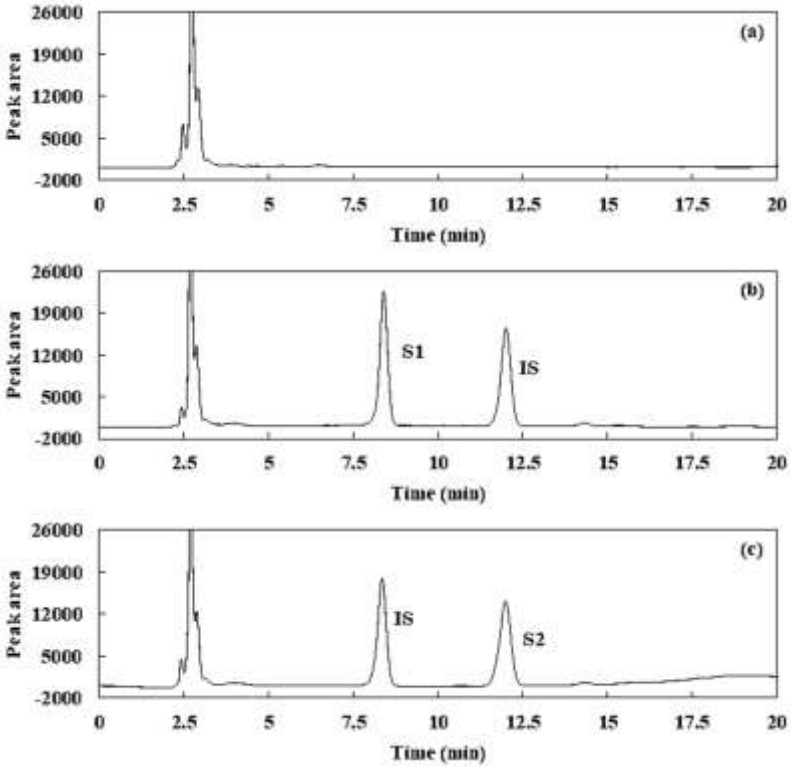
Supplemental figures



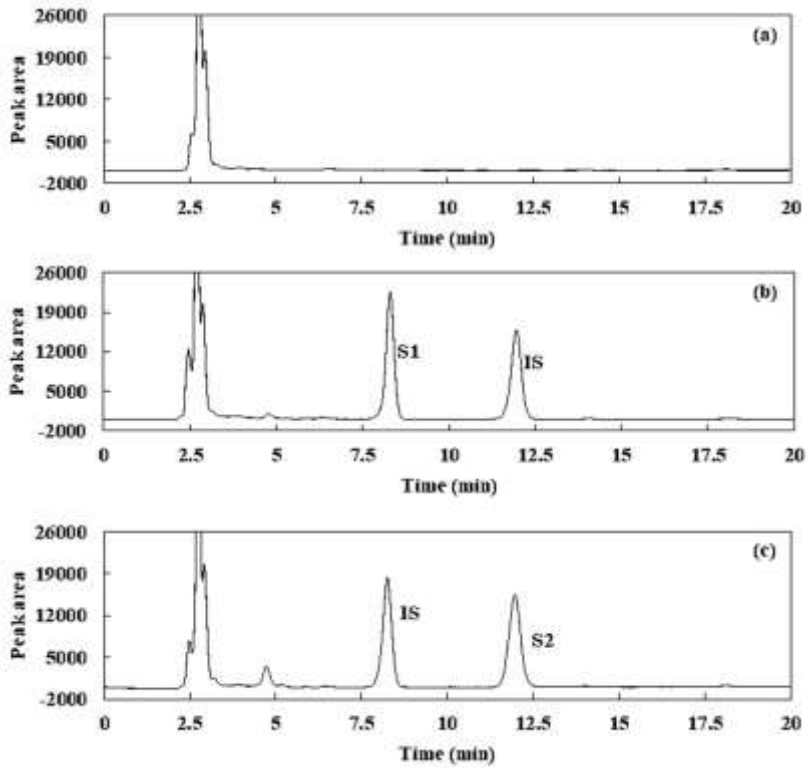
Supplemental fig. 1: Representative chromatograms of rat heart tissue. (a) Blank rat heart tissue; (b) S_1 and IS rat heart tissue; (c) S_2 and IS rat heart tissue.



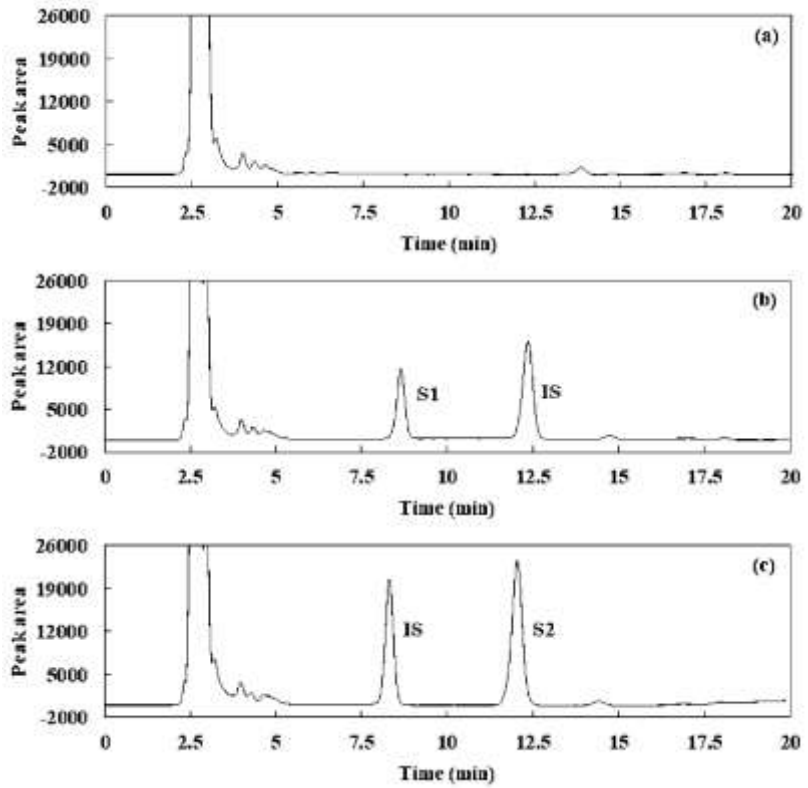
Supplemental fig. 2: Representative chromatograms of rat liver tissue. (a) Blank rat liver tissue; (b) S_1 and IS rat liver tissue; (c) S_2 and IS rat liver tissue.



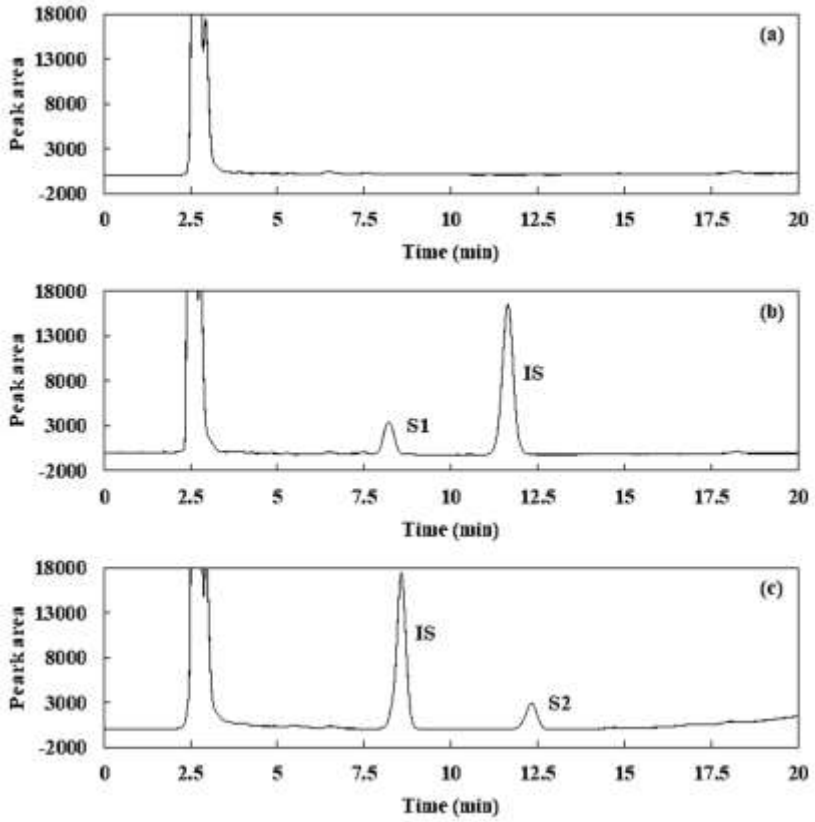
Supplemental fig. 3: Representative chromatograms of rat spleen tissue. (a) Blank rat spleen tissue; (b) S_1 and IS rat spleen tissue; (c) S_2 and IS rat spleen tissue.



Supplemental fig. 4: Representative chromatograms of rat lung tissue. (a) Blank rat lung tissue; (b) S_1 and IS rat lung tissue; (c) S_2 and IS rat lung tissue.



Supplemental fig. 5: Representative chromatograms of rat kidney tissue. (a) Blank rat kidney tissue; (b) S_1 and IS rat kidney tissue; (c) S_2 and IS rat kidney tissue.



Supplemental fig. 6: Representative chromatograms of rat brain tissue. (a) Blank rat brain tissue; (b) S_1 and IS rat brain tissue; (c) S_2 and IS rat brain tissue.

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