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Simultaneous determination of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin in rat plasma by ultra-performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

An ultra-high pressure liquid chromatography-tandem mass spectrometry method has been developed and validated for identification and quantification of five major bioactive components in rat plasma after oral administration of Qihuotongqiao tablets. The analysis was performed on an Acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm; Waters, USA) utilizing a gradient elution profile and a mobile phase consisting of (A) water containing 0.5 mM ammonium chloride and (B) acetonitrile. Electrospray ionization (ESI) tandem interface was employed prior to mass spectrometric detection. The calibration curve was linear over the range of 4.2–416.0 ng/mL for notoginsenoside R1, 38.4–3840.0 ng/mL for ginsenoside Rg_1, 3.7–368.0 ng/mL for ginsenoside Re, 37.6–5640.0 ng/mL ginsenoside Rb_1 and 4.5–448.0 ng/mL for icariin, respectively. The average accuracies ranged from 87.2 to 109.3% with RSD $\leq$ 13.7%. The results indicated that ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS) provided improved chromatographic parameters resulting in significantly increased sample throughput including lower solvent consumption and lower limits of quantitation (LLOQ) for most of target analytes compared to previous method employing conventional high-performance liquid chromatography (HPLC) separation. So, the established method was validated, sensitive and reliable for the determination of five major bioactive components in rat plasma.

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1. Introduction

Panax notoginseng has been used for thousands of years as an important folk medicine in China for promoting blood circulation and removing blood stasis. *Herba epimedii* is traditionally used as a tonic, aphrodisiac and antirheumatic agent in China [1]. In recent years, many studies revealed that *Panax notoginseng sapoinis* (PNS) possess protection effects on coronary heart disease, cardiac angina, apoplexy and atherosclerosis in clinics [2]. PNS mainly contained four active saponins, which are ginsenosides Re, Rg_1, Rb_1 and notoginsenoside R1, respectively (Fig. 1) (their total content ≥60% in PNS). Icariin (Fig. 1) is major active constituent of *H. epimedii* which could improve cardiovascular function, hormone regulation, immunological function modulation, and anti-tumor activity. Some studies indicated that *H. epimedii*, in combination with other herbal drugs, has been used clinically as oriental traditional medicines against stroke, Alzheimer's disease or vascular dementia [3,4]. Owing to limitations of single chemistry, combinatorial multi-component therapies (CMCT) of medicines are accepted for their more specific and fewer side effects [5]. Chinese herbal medicines (CHMs) consisting of multi-components are abound naturally occurring resources for CMCT. Qihuotongqiao tablets composed of *P. notoginseng* and *H. epimedii* extracts are aimed to prevent and treat Alzheimer's disease (AD) [6,7]. So it is very important to develop a sensitive method to detect the five components in body fluid.

There are a number of published reports describing the quantification of ginsenosides and icariin based on high performance liquid chromatography with ultraviolet detection (HPLC–UV) [8,9], high performance liquid chromatography with fluorescence detection (HPLC–FLD) [10], liquid chromatography mass spectrometry (LC–MS) [11–14] and liquid chromatography-tandem mass spectrometry (LC–MS/MS) [15,16]. However, these methods were mainly focused on the quantification of some ginsenosides or icariin in the raw materials or final ginseng products or one of the components in plasma, and few of them were reported for the simultaneous determination of multiple ginsenosides in biological fluids. Up to now, there was no single assay for simultaneous quantification of the five active components mentioned above in biological samples by UPLC–ESI–MS. Being a common analytical tool for various compounds; UPLC–MS has several advantages over HPLC in terms of speed, sensitivity, selectivity, etc. Therefore, this

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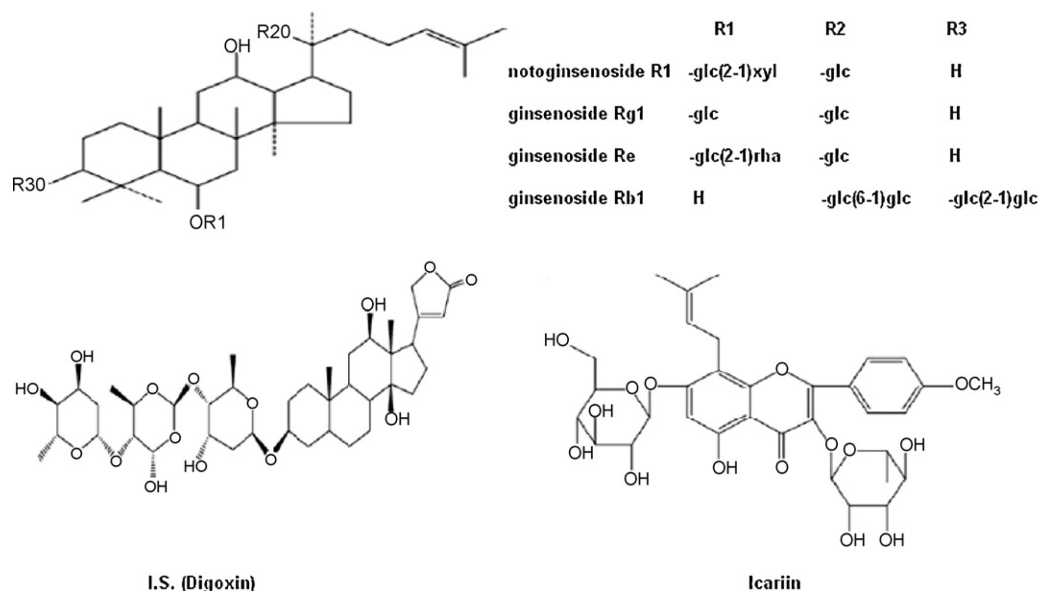

Fig. 1. Chemical structures of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1, icariin and the I.S. compound.

study was aimed at developing a rapid, sensitive and validated UPLC–MS method for simultaneous determination of five major compounds, i.e. notoginsenoside R1, ginsenoside Rg_1, ginsenoside Re, ginsenoside Rb_1 and icariin in rat plasma.

2. Experimental

2.1. Chemicals (materials and reagents)

The tablets of *P. notoginseng* and *H. epimedii* were provided by the Institute of Pharmaceutics, Zhejiang University. The standards of notoginsenoside R1, ginsenoside Rg_1, ginsenoside Re, ginsenoside Rb_1, icariin and digoxin (internal standard) were obtained from the National Institute for Drug Control of China (Beijing, China). Their structures are given in Fig. 1. Acetonitrile (Tedia, USA) and methanol (Merck, Germany) were of HPLC grade, ammonium chloride was of analytical grade. Water used throughout the experiments was generated by an ELGA academic water purification system (High Wycombe, UK). The experiment animals were supplied by the Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, China).

2.2. Apparatus

UPLC analyses were performed using a Waters Acquity Ultra Performance LC (UPLC) system (Waters, Milford, MA, USA). UPLC separation was achieved using an Acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm particle size; Waters, Milford, MA, USA), with a mobile phase flow rate of 0.2 mL/min. The mobile phase consisted of (A) water containing 0.5 mM ammonium chloride and (B) acetonitrile. A gradient elution program was applied as follows: 0–2.0 min hold on 25% B, 2.0–2.2 min hold on 28% B, 2.2–3.5 min hold on 35% B, 3.5–6.0 min hold on 60% B, and 6.0–6.5 min hold on 90% B, 6.5–9.0 min decreased from 90 to 25% B, giving a total run time 9 min. 0–3.5 min was increased linearly while 3.5–9.0 min was increased with the line concave. The injection volume was 7 μL. The pressure limit was set as follows: low: 0 Pa, high: 100 MPa; during the elution process, the highest pressure was about 55 MPa. Determination was performed using a Waters Micromass Quattro Premier Tandem quadrupole mass spectrometer (Waters, Manchester, UK). The instrument was operated using an electrospray source in positive mode for icariin and negative mode for four saponins. The ionization source conditions were as follows: capillary voltage of 3.71 kV, source block temperature of 110 °C and desolvation temperature of 350 °C. The desolvation gas flow was 500 L/h, and was obtained from an in-house nitrogen source. Quantification was performed using SIR (selected ion recording) mode and its conditions were optimized for each component during infusion. The cone voltage was 57 V for all of them but 99 V for icariin. The retention times, SIR transitions selected for the analysis of five components are shown in Table 1. Data acquisition and processing were performed using MassLynx 4.1. Software (Micromass, Manchester, UK).

2.3. Preparation of standard solutions and quality samples

Accurately weighed solid portions of standards were dissolved in methanol to prepare stock solutions separately: 0.520 mg/mL for notoginsenoside R_1, 0.480 mg/mL for ginsenoside Rg_1, 0.460 mg/mL for ginsenoside Re, 0.470 mg/mL for ginsenoside Rb_1, 0.560 mg/mL for icariin and 0.102 mg/mL for IS. Then, each stock solution was diluted step by step with methanol. Transfer certain work solution to a 1.5 mL plastic test tube, evaporate to dryness, and then resolve with 100 μL incipient mobile phase, 7 μL was injected. The chromatogram of the six standards is shown in Fig. 2. The effective concentrations in plasma for calibration curve ranged from 3.2 to 416.0 ng/mL for notoginsenoside R1, 28.8 to 3840.0 ng/mL for ginsenoside Rg_1, 2.8 to 368.0 ng/mL for ginsenoside Re, 28.2 to 5640.0 ng/mL ginsenoside Rb_1 and 3.4 to 448.0 ng/mL for icariin, respectively. For the validation of the method, three concentration levels of QC plasma samples were prepared containing notoginsenoside R1 (4.2, 83.2, 208.0 ng/mL), ginsenoside Rg_1 (38.4,

Table 1
The retention times, SIR transitions selected for the analysis of the five components and I.S.

Analytes	SIR time (min)	*m/z*	Retention time (min)	ESI
Notoginsenoside R1	2.2–3.6	966.86	3.39	–
Ginsenoside Rg_1	3.65–4.5	835.37	3.95	–
Ginsenoside Re	3.65–4.5	980.78	3.95	–
Ginsenoside Rb_1	6.3–7.0	1144.06	6.46	–
Icariin	4.55–5.5	677.36	5.14	+
I.S.	5.55–6.3	815.49	5.94	–

768.0, 1920.0 ng/mL), Re (3.7, 73.6, 184.0 ng/mL), Rb_1 (37.6, 752.0, 1880.0 ng/mL) and icariin (4.5, 89.6, 224.0 ng/mL).

Matrix effects were also evaluated by comparing the ratio of the peak area of analyte to that of I.S. from spike-after-extraction samples to those obtained for the standards in mobile phase at equivalent concentrations.

2.4. Sample preparation

In our study, a conventional protein precipitation method was used to prepare plasma samples. After 100 μL of plasma sample was transferred into a 1.5 mL plastic test tube together with 10 μL of I.S. work solution (2.04 μg/mL), 0.5 mL methanol was added. The analytes and I.S. were extracted from plasma by vortex-mixing for 3 min. Then the sample was centrifuged at 16,000 rcf for 30 min and 500 μL of the supernatant was transferred into another test tube and evaporate to dryness with vacuum at room temperature, Finally, the residue was reconstituted in 100 μL incipient mobile phase by vortex-mixing for 2 min and centrifuged at 16,000 rcf for 30 min, 7 μL supernatant was injected into chromatographic system for analysis.

2.5. Method validation

The lower limit of quantification (LLOQ) was determined as the lowest concentration point of the standard curve. The lower limit of detection (LLOD) was defined as the amount that could be detected with a signal-to-noise ratio of 3.

Plasma samples were quantified by using the ratio of the peak area of analyte to that of I.S. as the assay parameter. Standard curves representing peak area ratios vs. analyte concentrations were described in the form of $y = a + bx$ (weighing factor $1/x$).

The accuracy and precision were calculated by determining QC samples at high, middle and low concentration levels on three different validation days. The accuracy was expressed by (mean measured concentration)/(spiked concentration) × 100% and the precision by relative standard deviation (RSD%).

The extraction recoveries of analytes at three QC levels were determined by comparing the peak areas obtained from QC samples with the samples where the extracted matrix was spiked at the same concentration.

The matrix effects of analytes at three QC levels were determined by comparing the peak areas obtained from samples where

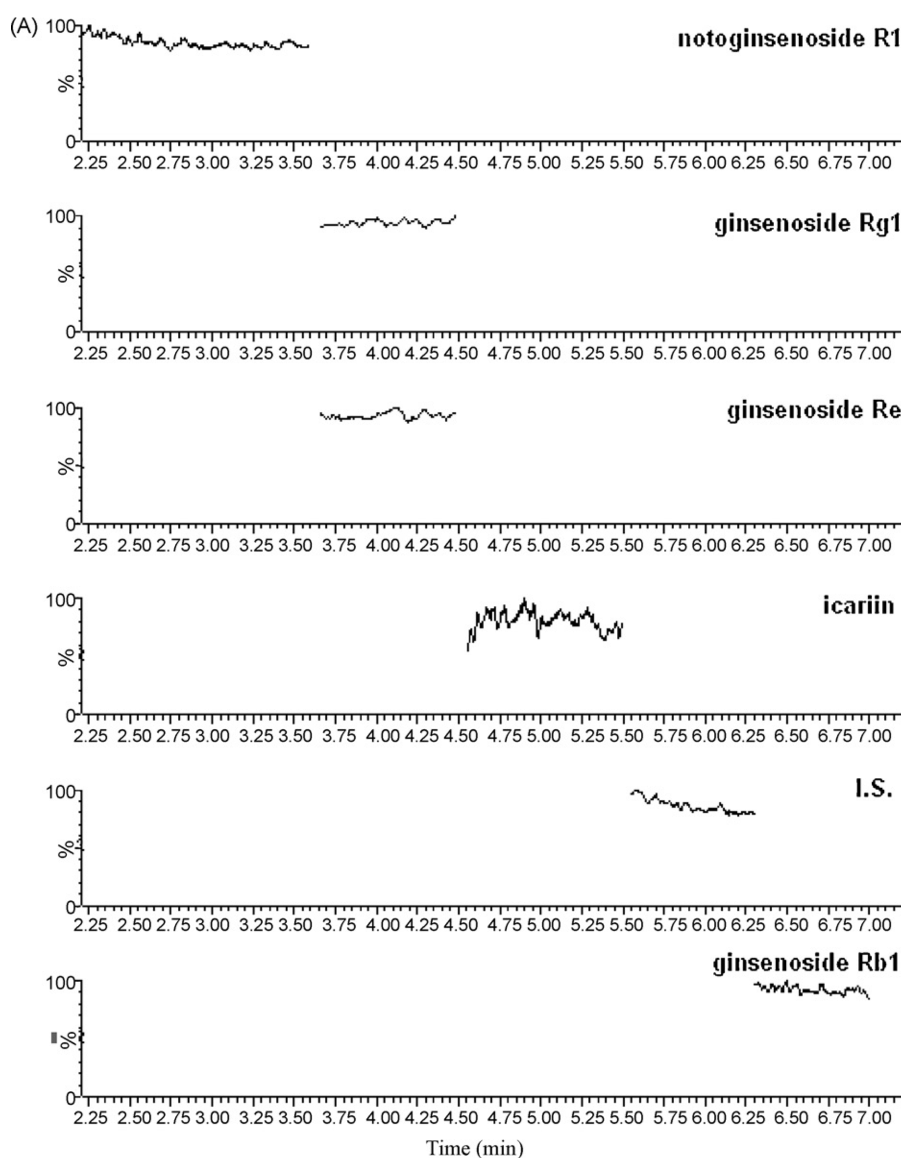

Fig. 2. SIR chromatograms of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1, icariin and I.S. with column 2 (A) blank plasma; (B) zero standard; (C) blank plasma spiked with notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1, icariin and the I.S.; (D) plasma sample at 9 min after administration of Qihuotongqiao tablet.

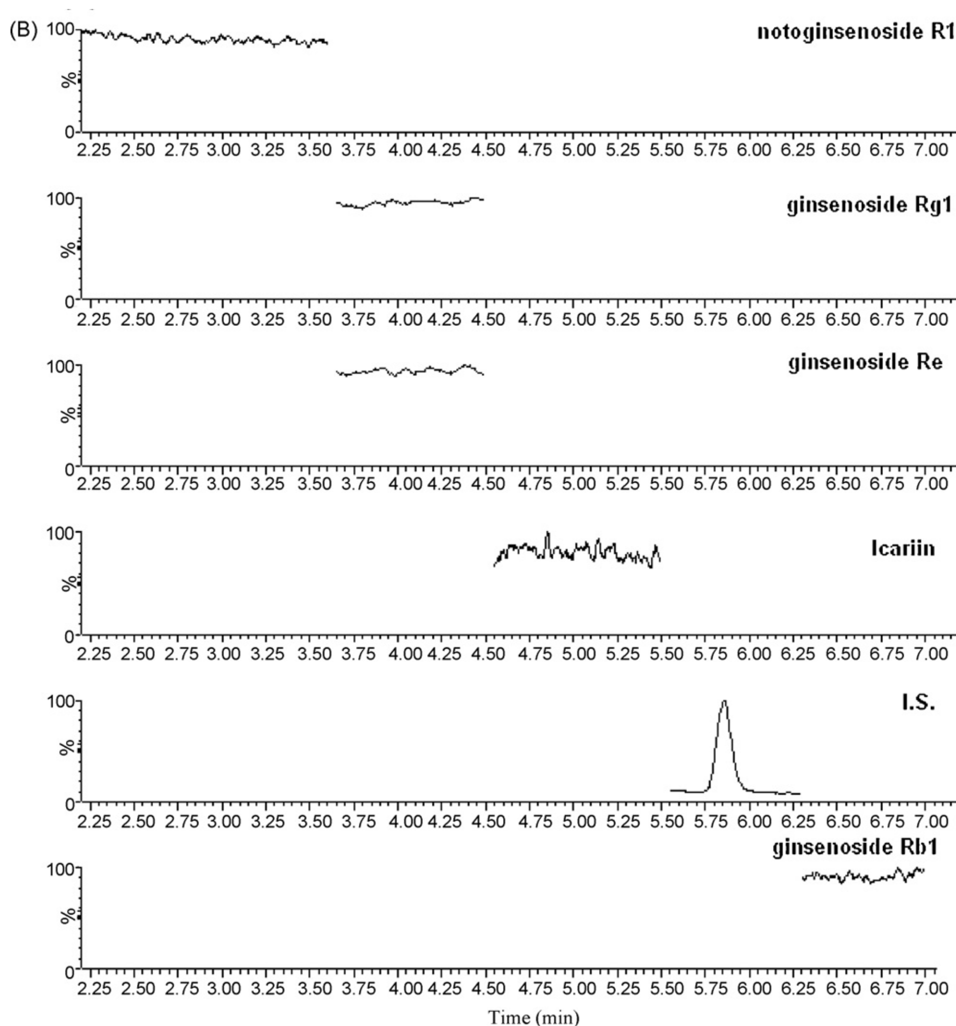

Fig. 2. (*Continued*)

the extracted matrix was spiked with standard working solutions at the same concentration.

The stability of analyte in rat plasma was assessed by analyzing QC samples at three concentrations exposed to different time and temperature conditions. The long-term stability was assessed after the QC samples had been stored at −80 °C for 15 days. The freeze–thaw stability was determined after three freeze–thaw cycles (−80 to 20 °C).

2.6. Application

The studies were proved by the Animal Ethics Committee of Zhejiang University. 5 Male Sprague–Dawley rats (180–200 g) were obtained from the Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, China). The rats were maintained in an air-conditioned animal quarter at a temperature of 22 ± 2 °C and a relative humidity of 50 ± 10%, having free access to water, and fed with a laboratory rodent chow (Hangzhou, China). The rats were fasted and given free access to water for 12 h prior to experiment. They were given oral administration of Qihuotongqiao tablets at a dosage of 1.06 g/kg, blood samples (about 400 μL) were drawn in heparinized polythene tubes at times 0, 4, 9, 25, 45, 60, 90, 120, 180, 240, 360, 480, 720, 1440, 2880, 4320 min and immediately centrifuged to separate 100 μL plasma. The obtained plasma samples were stored at −80 °C until analyses.

3. Results and discussion

3.1. Method development

3.1.1. Selection of UPLC–MS columns

The selection of UPLC–MS columns with high separation efficiency is a prerequisite. As the analytes were polar, two selective UPLC columns with different particle sizes and packing material, i.e. (1) Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm particle size; Waters, Milford. MA, USA), (2) Acquity UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm, Waters, Milford, MA, USA) were tested for their separation efficiencies. The separation efficiency and sensitivity of column 2 was better than column 1 (Figs. 2 and 3). Presume that the packing material of column 2 is the 1.8 μm high strength silica (HSS) particle with the high mechanical stability and appropriate morphology necessary to provide long column lifetimes and UPLC efficiencies at pressures up to 15,000 psi (1000 bar); an aqueous mobile phase compatible C18 bonded phase was designed to retain and separate polar organic compounds.

3.1.2. Optimization of MS conditions

The addition of ammonium chloride in mobile phase could enhance the formation of chloride adduct of the analyte molecule $[M+Cl]^-$ [17], but not for icariin. Since the response of icariin in $[M+Cl]^-$ form was quite low. In the opposite, icariin got a higher

Table 2
The extraction efficiency of four different solvents.

Analytes	Solvents	Ratio of the peak area	RSD (%)
Notoginsenoside R1	Methanol	0.6737	3.09
	Acetonitrile	0.5927	2.36
	Acetone	0.6467	4.03
	n-Butanol	0.6876	2.20
Ginsenoside Rg_1	Methanol	6.6998	1.03
	Acetonitrile	6.0877	2.02
	Acetone	6.4054	0.64
	n-Butanol	6.7685	5.98
Ginsenoside Re	Methanol	0.7805	0.76
	Acetonitrile	0.6800	3.95
	Acetone	0.7408	2.70
	n-Butanol	0.7841	5.74
Ginsenoside Rb_1	Methanol	3.6594	3.01
	Acetonitrile	1.5455	8.08
	Acetone	2.9576	4.88
	n-Butanol	3.4643	4.15
Icariin	Methanol	0.0752	4.21
	Acetonitrile	0.0583	3.40
	Acetone	0.0596	3.66
	n-Butanol	0.0689	1.86

sensitivity at an electrospray source in positive mode. In fact, the detective mode of multiple reactions monitoring (MRM) had been tried during the experiment because it was thought to be more specific and sensitive. But it was amazing that the responses were rather low for saponins. Guessing the reason is the adduct ion was hard to shiver.

At first, we used auto-tune to get initial optimum MS parameters but the sensitivities for all components are not good enough. Fine tuning was done to reach appropriate sensitivity and resolution. And the final parameters are as follows: 3 V for extractor, 0.1 V for RF lens and the dwell time is 0.2 s. Different ion source temperatures (100, 110, 120 °C) were compared, and at 110 °C, each component got a better response. Cone gas was set of 0, 5, 10, 15 L/h, no difference in sensitivity, so we turned off the cone gas.

3.1.3. Selection of the extraction method

Different organic solvents were tested to extract the analytes and I.S. from plasma: methanol, acetone, n-butanol and acetonitrile. Methanol got the best extracted efficiency for the five components generally. Although the protein sedimentation efficiency of acetonitrile is better than methanol, icariin dissolved worse in acetonitrile [18]. The other two organic solvents had the same problem. As it could be seen from Table 2, although the extraction efficiencies of n-butanol are close to methanol, the preparation process of n-butanol is a little different from others. Since n-butanol is less volatile, it was hard to evaporate to dryness and the preparation process was more troublesome.

3.2. Method validation

3.2.1. Specificity

The typical chromatograms of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin were presented in Fig. 2. Under the described chromatographic conditions, a good separation was achieved and no obvious interferences from endogenous plasma substances were

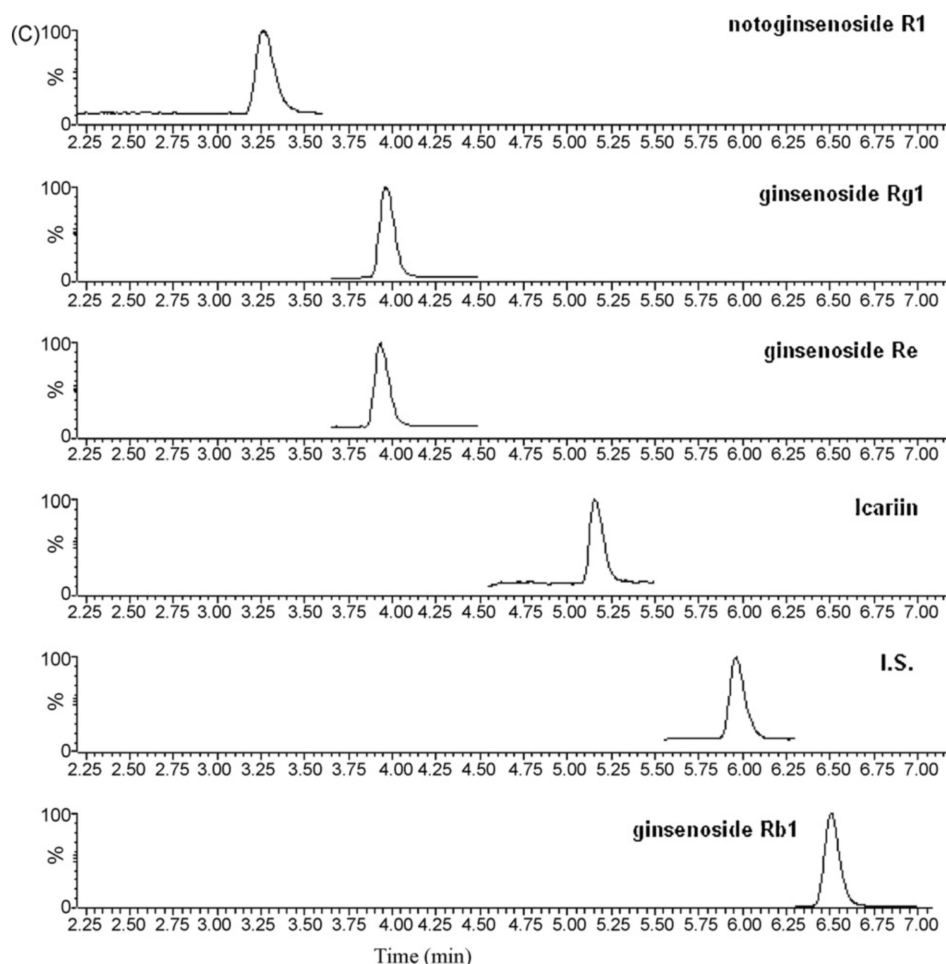

Fig. 2. (*Continued*)

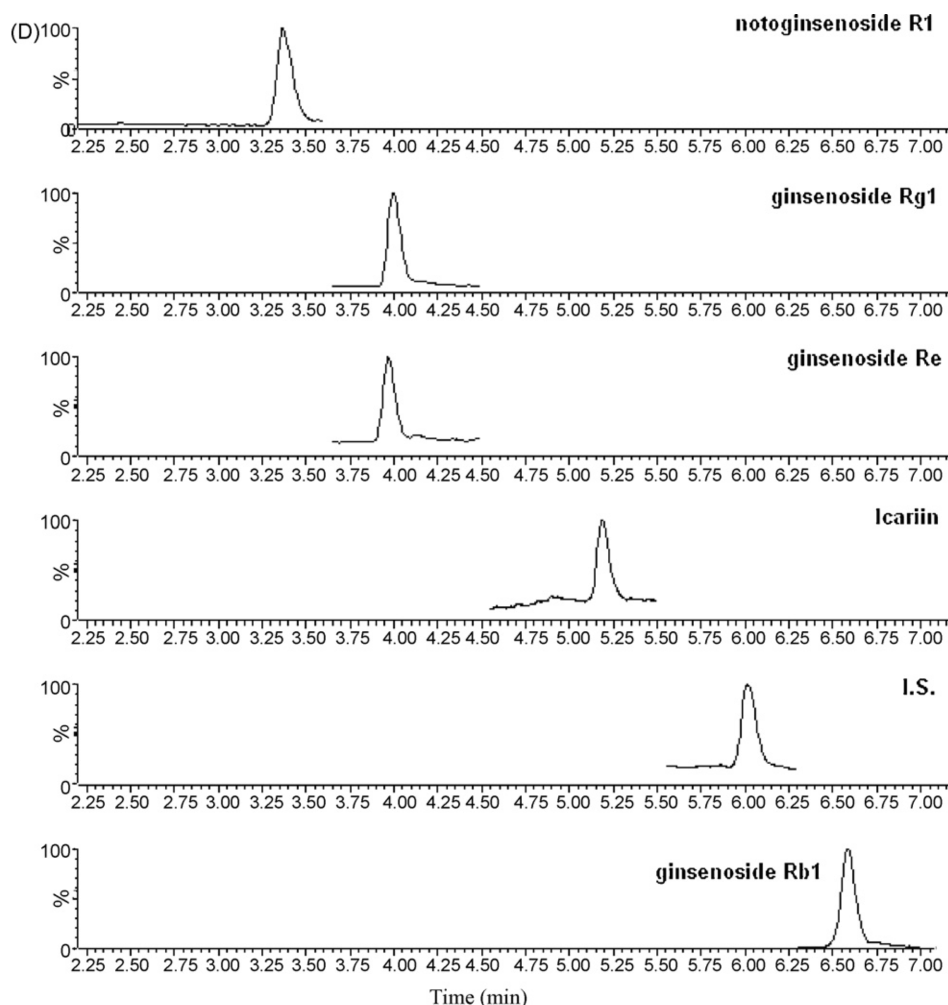

Fig. 2. (*Continued*).

observed. The retention time of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1, icariin and I.S. was 3.39, 3.95, 3.95, 6.46, 5.14 and 5.94 min, respectively. Although ginsenoside Rg_1 and Re have the same retention time, they still could be identified because of their different mass. Predominant chloridized adduct ions $[M+Cl]^-$ of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1, icariin and I.S. were formed with m/z 966.86, 836.37, 980.78, 1144.08, 677.36 and 815.49, respectively.

3.2.2. Linearity

The quantitative capability of the system employing UPLC–MS method was tested in the assay. Plasma samples were quantified by using the ratio of the peak area of analyte to that of I.S. as the assay parameter. Every calibration curve was performed with six different concentrations in quintuple (RSD < 13.3%). Table 3 showed the results of the standard calibration curves of integrated peak area ($n = 5$) and linearity (R^2). Calibration curves were linear and R^2 were all higher than 0.9981.

3.2.3. LLOD and LLOQ

The low limits of detection (LLOD) of these analytes using 100 μL plasma were 1.9–19.2 ng/mL. The lower limits of quantitation (LLOQ) were 2.8–28.8 ng/mL ($n = 5$, RSD ≤ 16.6%). Compared with some methods reported using MS detector [19], a higher sensitivity for Rg_1, Rb_1 and R1 was obtained in our study and made this method more advantageous to measure the trace concentration of these analytes in rat plasma. The results are shown in Table 3.

3.2.4. Extraction recovery, precision, accuracy and matrix effects

The extraction recoveries and matrix effects of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin in rat plasma were shown in Tables 4 and 5. At three concentration levels of these analytes, the extraction recoveries were all more than 78%. Table 6 summarized the intra- and inter-day precisions and accuracies of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin at three different concentration levels. As shown in Table 6, the intra- and inter-day

Table 3
The linearity, LLOD and LLOQ of the assay for notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin (weighing $1/x$).

Analytes	Calibration curves ($y = ax + b$)	Linear range (ng/mL)	R^2	LLOQ (ng/mL)	LLOD (ng/mL)
Notoginsenoside R1	$y = 0.004873 + 0.003968x$	3.2–416	0.9983	3.2	2.1
Ginsenoside Rg_1	$y = -0.004186 + 0.004519x$	28.8–3840	0.9997	28.8	19.2
Ginsenoside Re	$y = 0.01084 + 0.005301x$	2.8–368	0.9989	2.8	1.9
Ginsenoside Rb_1	$y = -0.01075 + 0.002272x$	28.2–5640	0.9981	28.2	18.8
Icariin	$y = 0.009827 + 0.0005593x$	3.4–448	0.9985	3.4	2.9

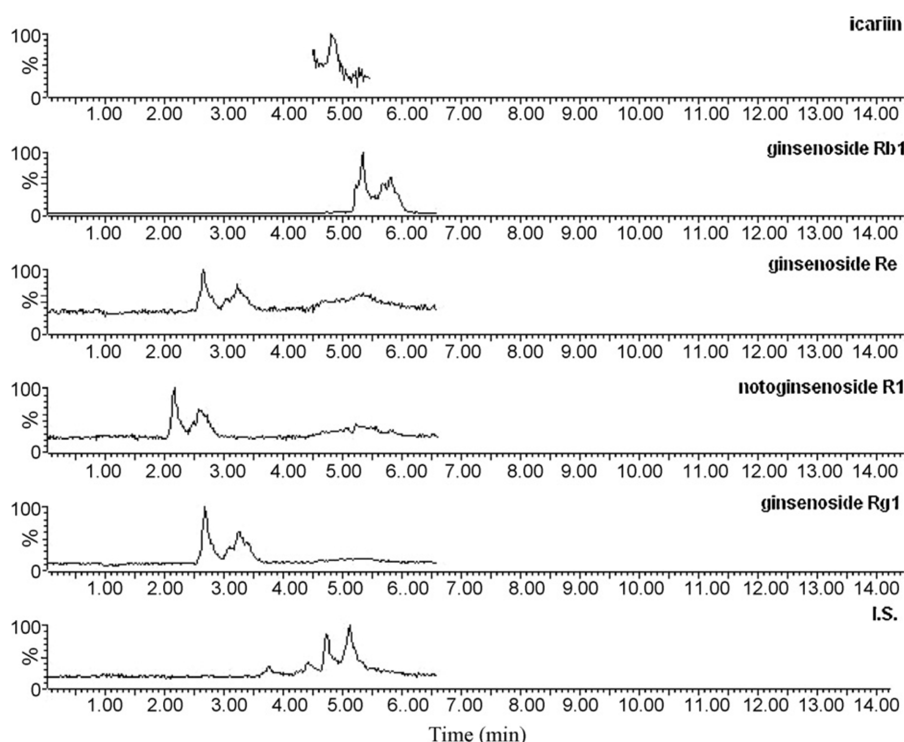

Fig. 3. SIR chromatograms of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1, icariin and I.S. with column 1.

accuracies of these analytes were within the range of 93.8–109.3% and 87.2–109.3%, respectively. The intra- and inter-day precisions (RSD) of these analytes were no more than 16.7% at lowest concentration and other concentrations were no more than 12.8%. The results demonstrated that the values were all within the acceptable range and the method was accurate and precise. In Table 4 there is a decrease of recovery% (and RSD %) with increased spiked concentration of some ginsenosides. Similarly, in Table 5 the matrix effect increased when spiked concentration increased in case of some solutes. Next, in Table 6 (and in Table 7) decreased intra-day accuracy for Rg_1 and Re compound following increased spiked concentration was described. We presume this phenomenon might be due to a limited amount of excess charge available on ESI droplets or to saturation of the ESI droplets with analyte at their surfaces at high analyte concentrations, thus, inhibiting ejection of ions trapped inside the droplets, cause the response lost at high concentration. And then it led the decreased of recoveries. At the same time, ion suppression is frequently accompanied by significant deterioration of the precision of the method [20].

3.2.5. Stability

The stability of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin was shown in Table 7. The results indicated that these analytes in rat plasma were all stable for three cycles of freeze–thaw, 4 h at room temperature, 15 days at −80 °C with accuracy in the range of 81.8–116.2%. Stock solutions of these analytes were all stable in ten days at −20 °C with the accuracy of 92.7–106.6% (Table 8).

Table 4
The extraction recoveries of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin in rat plasma ($n = 5$).

Analytes	Spiked concentration (ng/mL)	Recovery (%)	RSD (%)
Notoginsenoside R1	4.2	78.64	4.22
	83.2	92.40	8.67
	208.0	94.18	3.36
Ginsenoside Rg_1	38.4	89.75	3.53
	768.0	97.01	3.66
	1920.0	91.07	4.98
Ginsenoside Re	3.7	92.97	6.28
	73.6	98.31	5.86
	184.0	96.75	9.82
Ginsenoside Rb_1	37.6	91.56	8.17
	752.0	97.31	5.03
	1880.0	92.06	3.61
Icariin	4.5	95.16	13.65
	89.6	89.55	3.65
	224.0	94.56	5.49

Table 5
Matrix effects of the five components.

Analytes	Spiked concentration (ng/mL)	Matrix effects (%)	RSD (%)
Notoginsenoside R1	4.2	115.10	15.73
	83.2	113.51	10.67
	208.0	101.55	8.28
Ginsenoside Rg_1	38.4	106.75	3.57
	768.0	101.77	6.72
	1920.0	107.15	7.73
Ginsenoside Re	3.7	116.95	10.70
	73.6	102.10	12.49
	184.0	99.53	13.11
Ginsenoside Rb_1	37.6	112.73	6.34
	752.0	101.30	6.06
	1880.0	105.08	10.68
Icariin	4.5	137.95	11.82
	89.6	107.90	6.47
	224.0	118.57	12.25

Table 6
The intra- and inter-day accuracies and precisions of notoginsenoside R_1, ginsenoside Rg_1, Re, Rb_1 and icariin in rat plasma at high, middle and low concentration levels ($n = 5$).

Spiked concentration (ng/mL)	Intra-day			Inter-day		
	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)
Notoginsenoside R1						
4.2	4.6 ± 0.4	108.76	9.07	4.3 ± 0.3	102.61	7.25
83.2	80.5 ± 4.5	96.73	5.43	81.1 ± 2.1	97.43	2.58
208.0	201.8 ± 26.5	97.04	12.75	202.5 ± 6.1	97.35	3.00
Ginsenoside Rg_1						
38.4	40.2 ± 4.7	104.69	12.26	39.9 ± 0.5	103.92	1.16
768.0	780.3 ± 64.0	101.60	8.33	802.6 ± 20.3	104.51	2.53
1920.0	1876.4 ± 160.2	97.73	8.34	1926.0 ± 54.8	100.31	2.85
Ginsenoside Re						
3.7	3.9 ± 0.5	105.38	13.70	3.4 ± 0.6	91.31	16.70
73.6	78.3 ± 3.7	106.35	5.01	78.2 ± 1.8	106.22	2.23
184.0	175.0 ± 14.5	95.09	7.89	188.7 ± 11.9	102.54	6.30
Ginsenoside Rb_1						
37.6	35.5 ± 2.2	94.51	5.74	32.8 ± 2.7	87.16	8.36
752.0	705.9 ± 60.7	93.86	8.07	757.1 ± 44.4	100.68	5.86
1880.0	1785.8 ± 166.7	94.99	8.87	1946.6 ± 144.3	103.54	7.41
Icariin						
4.5	4.4 ± 0.5	97.55	10.61	4.6 ± 0.2	102.85	4.48
89.6	97.9 ± 5.1	109.26	5.72	97.9 ± 4.6	109.26	4.70
224.0	226.5 ± 20.7	101.13	9.23	232.2 ± 27.5	103.66	11.83

Table 7
The stability of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin in rat plasma ($n = 3$).

Spiked concentration (ng/mL)	At −80 °C for 15 days in plasma		At room temperature for 4 h in plasma		After three freeze–thaw cycles in plasma	
	Measured concentration (ng/mL)	Accuracy (%)	Measured concentration (ng/mL)	Accuracy (%)	Measured concentration (ng/mL)	Accuracy (%)
Notoginsenoside R1						
4.2	3.4	81.77	4.1	96.55	3.9	92.2
83.2	71.3	85.64	79.5	95.6	77.4	93.0
208.0	183.0	87.98	180.0	86.56	193.1	92.8
Ginsenoside Rg_1						
38.4	43.6	113.63	42.1	109.74	42.3	110.1
768.0	687.3	89.49	763.2	99.4	737.8	96.1
1920.0	1820.0	94.80	1784.1	92.92	1813.6	94.5
Ginsenoside Re						
3.7	3.6	96.96	4.2	113.04	4.0	107.9
73.6	64.8	87.98	73.8	100.3	71.5	97.1
184.0	163.3	88.74	173.2	94.14	1765.1	95.9
Ginsenoside Rb_1						
37.6	43.8	116.15	38.4	102.08	37.4	99.4
752.0	77.3	102.76	749.4	99.7	703.9	93.6
1880.0	2036.4	108.32	1773.2	94.32	1882.4	100.1
Icariin						
4.5	5.0	111.57	4.0	89.56	4.3	95.6
89.6	102.0	113.82	96.0	107.1	83.3	93.0
224.0	247.7	110.56	185.4	82.79	204.0	91.1

Table 8
Stability of the stock solution.

Analytes	Recovery (%, 0 day was reference)	
	0 day	10 days
Notoginsenoside R1	100	106.6
Ginsenoside Rg_1	100	92.7
Ginsenoside Re	100	95.3
Ginsenoside Rb_1	100	94.7
Icariin	100	101.2
I.S.	100	100.4

3.3. Plasma drug concentration–time curve

The method could be applied to the pharmacokinetic study of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin in rats following oral administration of Qihuotongqiao tablets. Concrete pharmacokinetic parameters can be seen from Table 9. The mean plasma concentration–time profiles were illustrated in Fig. 4. From the figure, it showed that icariin was eliminated quickly and nothing could be detected after 6 h. Both notoginsenoside R1 and ginsenoside Re were eliminated over after 12 h, while ginsenoside Rg_1 lasted longer in body. The pharmacokinetic behaviors of ginsenoside Rb_1 was markedly different from those of components mentioned above, it could be detected in plasma even after 72 h. The pharmacokinetic parameters of notoginsenoside R1, ginseno-

Table 9
Pharmacokinetic parameters of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin after oral administration of Qihuotongqiao tablet to 5 male SD rats (values are mean ± standard deviation).

Parameters	Notoginsenoside R1	Ginsenoside Rg_1	Ginsenoside Re	Ginsenoside Rb_1	Icariin
$AUC_{(0-t)}$ (mg/L min)	35.9 ± 5.2	97.8 ± 17.5	9.6 ± 1.2	4848.1 ± 2197.8	9.0 ± 0.7
$AUC_{(0-\infty)}$ (mg/L min)	39.6 ± 6.9	104.3 ± 16.8	12.4 ± 2.0	52,299.4 ± 2221.4	11.5 ± 3.3
Terminal half-time (min)	245.6 ± 118.5	418.8 ± 68.0	431.4 ± 149.5	1512.2 ± 1461.9	107.07 ± 15.4
C_{max} (mg/L)	279.2 ± 64.6	793.4 ± 272.7	84.2 ± 25.2	5269.0 ± 1483.2	88.8 ± 41.6
T_{max}[a] (min)	26.6 (9–45)	26.6 (9–45)	26.6 (9–45)	144 (60–240)	35.6 (4–60)

[a] Median (ranges).

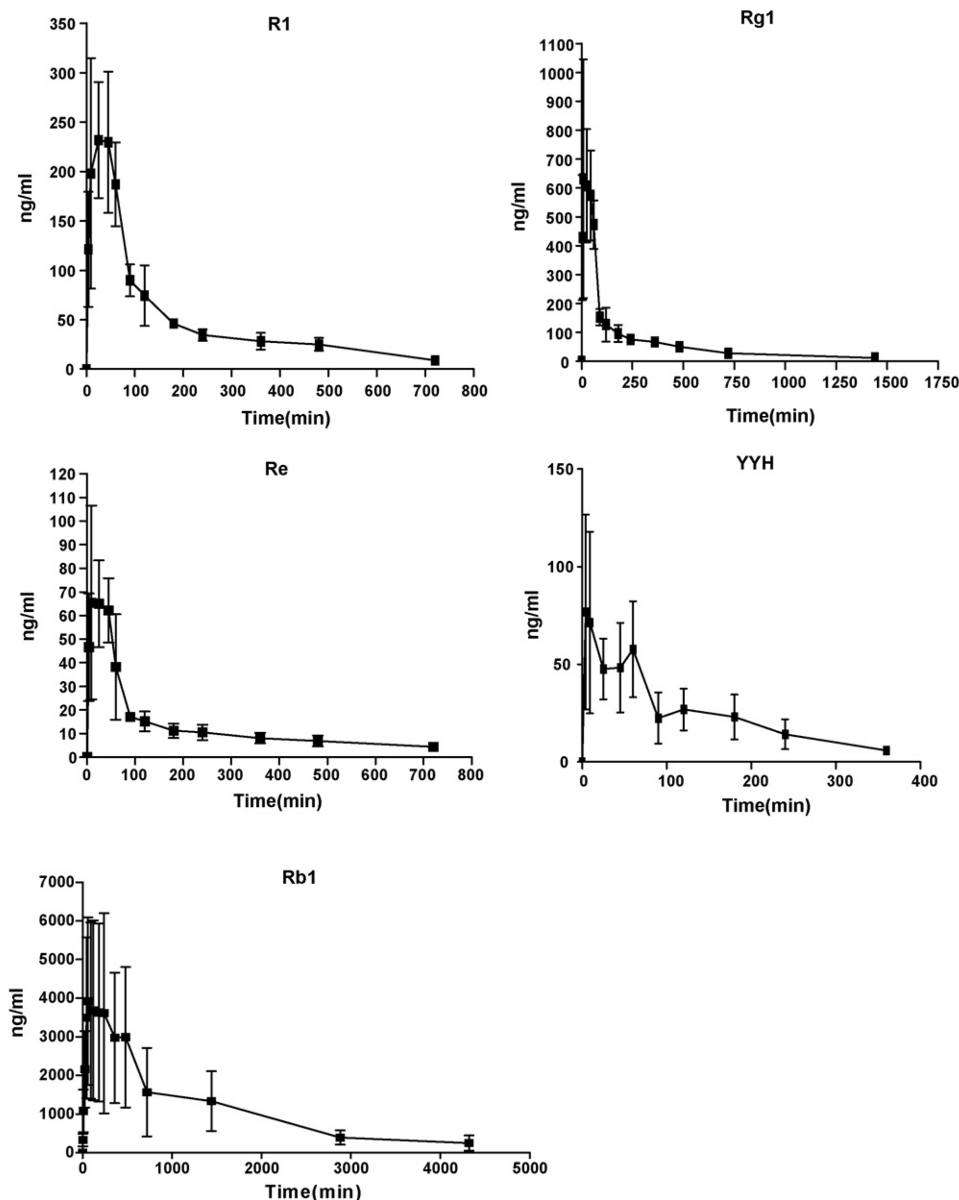

Fig. 4. The mean plasma concentration–time curves of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin in rat after administration of Qihuotongqiao tablet ($n = 5$).

side Rg_1, Re and Rb_1 were a little different from the literature reported [17,19], Conjecturing the reason is the difference of dosage, the compatibility and the administration way.

4. Conclusion

In the present study, a sensitive and rapid UPLC–ESI–MS method for the simultaneous determination of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin in rat plasma was developed and validated. A simple protein precipitation procedure was used to prepare the samples with high recoveries. The established assay was proved to be fast, precise, accurate, specific, and reproducible and could be applied to the pharmacokinetic study of Qihuotongqiao tablets. The drug–time curve indicated there was a significant difference in the elimination speed of notoginsenoside R1, ginsenoside Rg_1, Re, Rb_1 and icariin in Qihuotongqiao tablets.

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