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Short communication

Simultaneous determination of safflor yellow A, puerarin, daidzein, ginsenosides (Rg_1, Rb_1, Rd), and notoginsenoside R_1 in rat plasma by liquid chromatography–mass spectrometry

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Abstract

A liquid chromatograph/mass spectrometry (LC/MS) method was developed for the simultaneous quantitation of seven compounds (safflor yellow A, puerarin, daidzein, ginsenosides (Rg_1, Rb_1, Rd), and notoginsenoside R_1) in rat plasma samples with sufficient sensitivity to facilitate analysis of samples collected after an intravenous injection of Naodesheng. The plasma samples were subjected to protein precipitation with acetone, and analyzed using negative atmospheric pressure chemical ionization mass spectrometry in selected ion monitoring (SIM) mode with baicalin as an internal standard. Good linearity for all the seven compounds was observed. The intra- and inter-day precision of analysis was <15.0% for each compound, and the accuracy ranged from 90.0% to 109.0%. This quantitation method was successfully applied to a pharmacokinetic study of following intravenous injection of rats with Naodesheng.
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Keywords: Safflor yellow A; Daidzein; Puerarin; Ginsenosides; Notoginsenoside R_1; HPLC–MS; Naodesheng injection; Plasma; Pharmacokinetic

1. Introduction

Naodesheng injection is a traditional Chinese medicine (TCM) preparation consisting of *Radix puerariae lobatae*, *Flos carthami*, *Radix et Rhizoma Notoginseng*, *Rhizoma chuanxiong*, and *Fructus crataegi*, which is effective in the treatment of cerebral arteriosclerosis, ischemic cerebral stroke, and apoplexy linger effect [1]. It has been reported that safflor yellow A, puerarin, daidzein, ginsenosides (Rg_1, Rb_1, Rd, Rg_2, Re, Rh_1), and notoginsenoside R_1 (structures are shown in Table 1) are the major bioactive components [2–5] of Naodesheng injection. Therefore, it is essential to simultaneously determine these compounds in rat plasma after intravenous injection of Naodesheng.

Earlier publications described methods for analysis of some of these compounds in biological samples using high-performance liquid chromatography (HPLC) with UV-detection [6–21] or MS-detection [22–24]. However, for the analysis of rat plasma samples obtained following injection of Naodesheng, it appears that there is, at present, no single assay for the simultaneous determination of all 10 constituents (flavones and saponins) using HPLC–MS.

Most TCM preparation are composed of a variety of herbs, which contain complicated chemical constituents. As far as the same component is concerned, it exhibits different effects in different formulations, so it is necessary to study the pharmacokinetic characteristics of the active constituent. So, the development of methods for the determination of active components of Naodesheng injection in plasma is essential for basic studies and the effective clinical use of this TCM.

A method has been previously published determining 10 components of Naodesheng injection using HPLC–MS/APCI [25]. Seven components were selected for a pharmacokinetic study because of the low concentrations of the other three components (ginsenoside Rh_1, Rg_2, Re) in botanical material [25–27] and the low sensitivities of the determination methods for these three components in rat plasma. In plasma samples, the peaks of safflor yellow A and puerarin could not be separated from those of endogenous substances eluted at the beginning of the previous gradient step used for determination of composition

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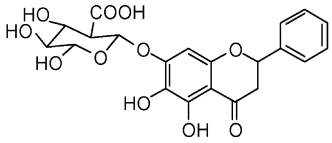
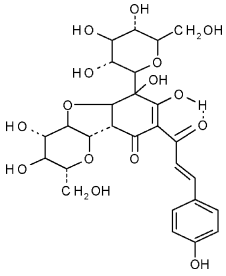
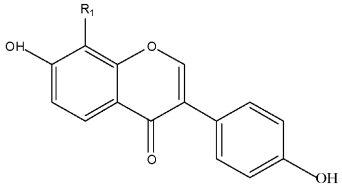
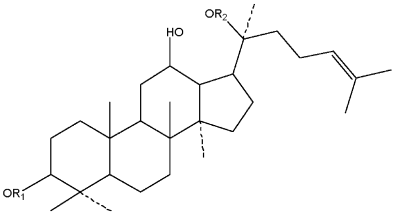
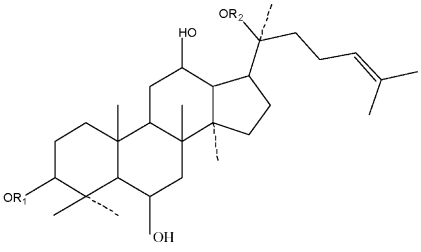
Table 1
Structure of the seven components in Naodesheng injection and Baicalin (I.S.)

Compounds	R_1	R_1	MW	Structures
Baicalin	–	–	446	
Safflor yellow A	–	–	594	
Puerarin	Glc	–	416	
Daidzein	H	–	254	
Ginsenoside Rb_1	$Glc^{2-1}Glc$	$Glc^{6-1}Glc$	1108	
Ginsenoside Rd	$Glc^{2-1}Glc$	Glc	946	
Ginsenoside Rg_1	Glc	Glc	800	
Notoginsenoside R_1	$Glc^{2-1}Xyl$	Glc	932	

of the injection. We modified the gradient process by making a small change at the beginning of the stepwise gradient.

A specific, reproducible, and accurate method for the simultaneous determination of several markers is necessary to comprehensively characterize the pharmacokinetic profile of this drug and to explore the relationship between the pharmacokinetic and pharmacodynamic effects of these compounds. Therefore, we describe here a method using HPLC–MS/APCI for the determination of safflor yellow A, puerarin, daidzein, ginsenosides (Rg_1, Rb_1, Rd), and notoginsenoside R_1 in rat plasma samples using baicalin (structures are listed in Table 1) as an internal standard (I.S.) with negative ionization after a single protein precipitation with acetone. This assay requires a short chromatographic separation and is sensitive, specific and fully validated. This method has been successfully applied to study the pharmacokinetics of Naodesheng in rats.

2. Experimental

2.1. Equipment and reagents

LCMS 2010 EV liquid chromatograph mass spectrometers (Shimadzu, Japan), equipped with an LC-10AD*vp* liquid chromatograph, DGU-14AM Degasser, SIL-HTC Auto sampler, an atmospheric pressure chemical ionization (APCI) interface, and a single quadrupole analyzer were employed. LCMS solution 3.0 software was used.

Safflor yellow A (>97.0%) was isolated in our laboratory (Department of Pharmaceutical Analysis, Shenyang Pharmaceutical University, Shenyang, PR China) and Ginsenoside Rd (>96.0%) in the Department of Phytochemistry (Shenyang Pharmaceutical University, Shenyang, PR China). Daidzein (>98.0%) was purchased from Sigma Company Inc. (USA)

and puerarin, baicalin, ginsenoside Rg_1, Rb_1, and notoginsenoside R_1 were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Puerariae extract (content of flavonoids: 70%, plot No. 20030301) and crataegus extract (content of flavonoids: 80%, plot No. 20030207) were purchased from Huizhou green source health food Co., Ltd. (Huizhou, PR China). *Notoginseng radix* extract (content of saponins: 95%, plot No. 20030215), *R. chuanxiong* extract (plot No. 20030223) and *F. carthami* extract (content of safflor yellows: 90%, plot No. 20020411) were purchased from Huxi Qianshan Bioengineering Co., Ltd., Chengdu Jintang Middle Product Co., Ltd. (Chengdu, PR China) and Tianjin Institutes for Safflor Yellows (Tianjin, PR China), respectively. Naodesheng injection, which was recently shown to be safe and stable, was prepared in our laboratory with five extracts. The contents of safflor yellow A, puerarin, daidzein, ginsenosides Rg_1, ginsenosides Rb_1, ginsenosides Rd, and notoginsenoside R_1 were 70, 1211, 33, 483, 614, 164, and 80 μg/ml, respectively. Heparin sodium injection was purchased from Tianjin Biochemical Pharmaceutical Corporation. HPLC-grade methanol was obtained from Dikma Company Inc. (USA). Analytical grade acetone and formic acid were obtained from the Chemical Reagent Factory of Shenyang (Shenyang, PR China). Water was purified using the Milli-Q purification system (Millipore Co., France).

2.2. Standard solutions

A stock solution of safflor yellow A (0.4 mg/ml), Puerarin (0.8 mg/ml), and ginsenoside Rg_1 (1.5 mg/ml) was prepared in one measuring flask by dissolving the reference substances in a mixture of methanol and water (1:1, v/v); Another concentrated stock solution of daidzein (0.05 mg/ml), ginsenoside Rb_1 (4.0 mg/ml), Rd (0.2 mg/ml), and notoginsenoside R_1 (0.2 mg/ml) was prepared by dissolving the reference substances in methanol. For the assay of plasma samples, working solutions were prepared by appropriate dilution of the above stock solutions with methanol. These working solutions were used to prepare the analytical standard solutions. The I.S. solution was prepared to give a final concentration of 0.9 mg/ml in a methanol–water (1:1, v/v) mixture. All solutions were stored at −20 °C and were found to be stable for at least 1 month.

The quality control (QC) samples at three concentration levels for the seven compounds, a low concentration level not higher than 3× LLOQ, a moderate level around the midrange and a high level close to the high end of the range, were prepared with blank plasma and suitable amounts of working solutions to determine the precision, accuracy, recovery, matrix effects, and stability of the method.

2.3. Chromatographic conditions

The separations were carried out using a Luna C_{18} column (5 μm, 150 mm × 4.6 mm, Phenomenex, USA) at 20 °C. The mobile phase was a stepwise gradient of water (0.1% of formic acid, v/v)–methanol (0 min, 70:30; 5 min, 60:40; 20 min, 20:80). Chromatography was performed at a flow rate of 0.8 ml/min and the injection volume was 10 μl.

2.4. MS/APCI detection conditions

The compounds were ionized in the negative atmospheric pressure chemical ionization ($APCI^-$) interface of the mass spectrometer. Selective ion monitoring (SIM) mode was used for quantitation by the $[M–C_6H_{11}O_5]^-$, $[M-H]^-$ or $[M+HCOO]^-$ molecular ions of the analytes (listed in Table 2). The detection conditions were as follows: interface temperature, 400 °C; curved desolvation line (CDL) temperature, 200 °C; CDL voltage, +10.0 kV; heat block temperature, 200 °C; detector voltage, 1.40 kV; nebulizing gas flow rate, 2.5 l/min.

2.5. Sample preparation

Plasma samples of 100 μl were transferred to tubes, and then 50 μl methanol and I.S. (baicalin) solution (0.9 mg/ml) were added. Protein precipitation was carried out with 400 μl acetone. After vortex mixing for 2 min and centrifuging at 4000 rpm for 15 min, the supernatant collected was evaporated to dryness under nitrogen gas at 40 °C. The residue was reconstituted in 100 μl of methanol-water (1:1, v/v) and centrifuged at 12,000 rpm for 3 min. A portion of the supernatant (10 μl) was injected into the HPLC system for analysis.

2.6. Validation of the method

2.6.1. Specificity

The specificity of the method was tested by screening analysis of blank plasma samples. There were no impurity peaks or contamination at the retention times corresponding to the analytes and I.S.

2.6.2. Linearity and lower limits of quantitation (LLOQs)

The linearity of the assay was assessed by preparing seven different concentrations of samples in plasma under the same conditions, in duplicate, as those of the test samples. Calibration was performed by least-squares linear regression of the peak-area ratios of the compounds to the I.S. versus the respective standard concentration with a weighting (1/square of concentra-

Table 2
The fragment information of the multi-components determined and Baicalin (I.S.)

Components	Fragment molecules	*m/z*	Retention time (min)
Baicalin	$[M-H]^-$	444.95	13.478
Safflor yellow A	$[M-C_6H_{11}O_5]^-$	431.15	7.229
Puerarin	$[M-H]^-$	415.05	6.379
Daidzein	$[M-H]^-$	253.05	13.559
Rg_1	$[M+HCOO]^-$	845.40	14.404
Rb_1	$[M+HCOO]^-$	1153.75	18.386
Rd	$[M+HCOO]^-$	991.45	20.034
R_1	$[M+HCOO]^-$	977.55	13.677

tion) factor. Quantitation was based on the ratio of the peak area of the analyte versus that of internal standard. The calibration equation was $y = a + bx$, where y is the peak area ratio of analyte to I.S., a is an intercept, b is a slope and x is the analyte concentration.

The lower limits of quantitation (LLOQ) were defined as the lowest concentration of analytes that can be determined with an acceptable accuracy and precision by a particular method. In our method the LLOQ values were established at the lowest concentration of the linear calibration range, while the upper limits of quantification (ULOQ) were established at the highest concentration of the linear ranges of the corresponding compounds.

2.6.3. Precision and accuracy

The intra-day precision, inter-day precision and accuracy were evaluated using three different concentrations QC samples over three consecutive days in our study. QC samples of three different concentrations were tested in six replicates and calculated with calibration curves obtained daily. The precision of the method at each QC concentration was expressed as the relative standard deviation (RSD) by calculating the standard deviation (S.D.) as a percentage of the mean calculated concentration. The accuracy of the assay is the closeness of the test value obtained to the nominal value.

2.6.4. Recovery and ion suppression

The extraction recovery were determined at three concentrations by comparing the analyte peak areas, to those obtained from the quality control samples ($n = 6$) after extraction, with those obtained from the corresponding unextracted reference standards prepared at the same concentrations.

The ion suppression effect may be caused by a number of factors such as the matrix, interference from metabolites or co-eluting compounds [28–30]. The matrix effect was evaluated by comparing the peak area ratio of post-extraction control plasma spiked with standard solutions of analytes and I.S. solution to that of the neat standard solutions at three QC levels in five different lots of plasma. Furthermore, a three-way crossover experiment was performed to assess interference effects from co-eluting compounds in this study. In these studies, subjects were treated with drug A in the first part (1), with drug B, that may potentially interact with A, in the second part (2) of the study and, finally, in the third part (3), with drugs A + B combined. The bioanalytical and PK data obtained in these drug interaction studies are usually obtained based on the analyses of biofluid samples generated in parts 1 and 3 from a number of subjects using a bioanalytical method developed for drug A in control plasma. When the slope of the standard curve constructed using a control plasma and in part 3 are practically the same, the matrix effect from B on the quantification of A may be considered as negligible.

2.6.5. Stability

The bench-top stability of the seven components was determined periodically by injecting replicate preparations of processed samples for up to 24 h. Their stability in frozen plasma was assessed by analyzing the QC samples stored at −20 °C for at least 1 month. Their freeze–thaw stability was evaluated after three freeze and thaw cycles. The QC samples were stored at −20 °C for 24 h and thawed at room temperature. When samples were completely thawed they were refrozen within 24 h. This cycle was repeated twice and then the samples were analyzed during the third cycle.

2.7. Application of the assay to pharmacokinetic studies

The present method was used to determine the plasma concentrations of the seven components after the intravenous injection of Naodesheng. All data were subsequently processed with the computer program 3p97 (Practical Pharmacokinetic Program, 1997, China) to determine the compartment models and pharmacokinetic parameters.

Male and female pathogen-free Wistar rats (200–220 g) were obtained from the Laboratorial Animal Center of Shenyang Pharmaceutical University (Shenyang, China). They were kept in our laboratory for at least 3 days before use and had access to the standard laboratory food and water ad libitum. Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University and the procedure was approved by the Animal Ethics Committee of this institution.

After having fasted for about 12 h, but allowed free access to water, the rats were given an injection via the caudal vein at a dose of 10 ml/kg. Then, 0.3 ml blood samples were collected from the suborbital veniplex at 0.033, 0.167, 0.333, 0.667, 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, 48.0, 72.0, and 96.0 h after intravenous dosing, transferred to heparinized tubes, and centrifuged to obtain plasma.

Table 3
Regression data and LLOQs of the multi-components determined

Components	Linear range (μg/ml)	Slope	Intercept	Correlation coefficient (r)	LLOQ (μg/ml)
Safflor yellow A	0.08–40	0.0103	0.0012	0.997	0.08
Puerarin	0.16–80	0.2484	0.0054	0.998	0.16
Daidzein	0.05–5.0	0.0492	−0.0008	0.997	0.05
Rg_1	0.3–150	0.1866	0.0067	0.998	0.3
Rb_1	4–400	0.0018	−0.0005	0.996	4.0
Rd	0.2–20	0.0331	−0.0020	0.997	0.2
R_1	0.2–20	0.1226	0.0041	0.998	0.2

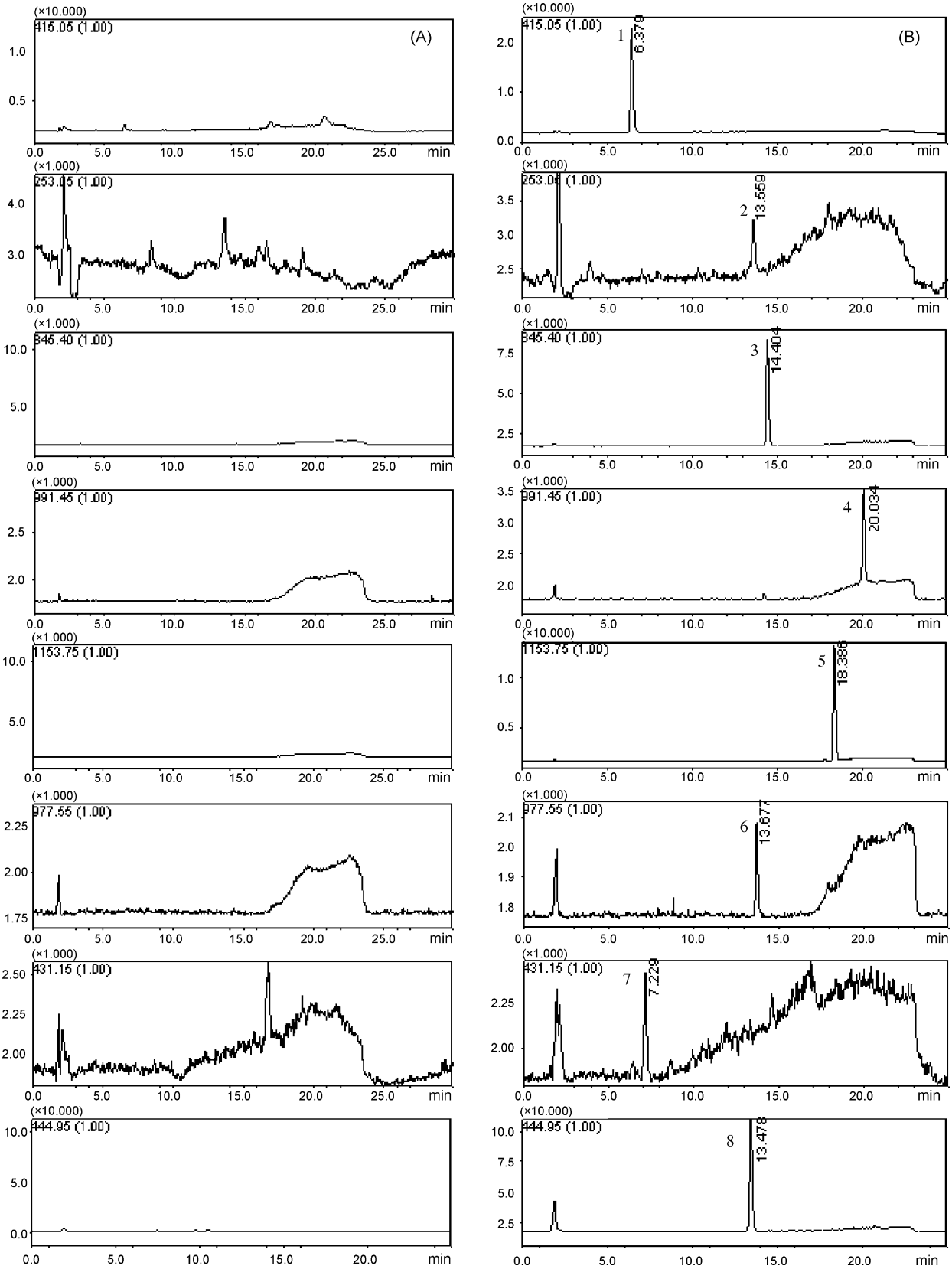


Fig. 1. Chromatograms of the seven compounds in plasma. (A) Blank plasma; (B) blank plasma spiked with safflor yellow A (0.4 μg/ml), puerarin (0.8 μg/ml), daidzein (0.1 μg/ml), ginsenoside Rg_1 (1.5 μg/ml), Rb_1 (8.0 μg/ml), Rd (0.4 μg/ml), notoginsenoside R_1 (0.4 μg/ml) and baicalin (900 μg/ml) (I.S.); (C) plasma sample obtained 4 h after intravenous administration of Naodesheng injection. Peak: 1, puerarin; 2, daidzein; 3, ginsenoside Rg1; 4, ginsenoside Rd; 5, ginsenoside Rb1; 6, notoginsenoside R1; 7, safflor yellow A; 8, baicalin. I.S.

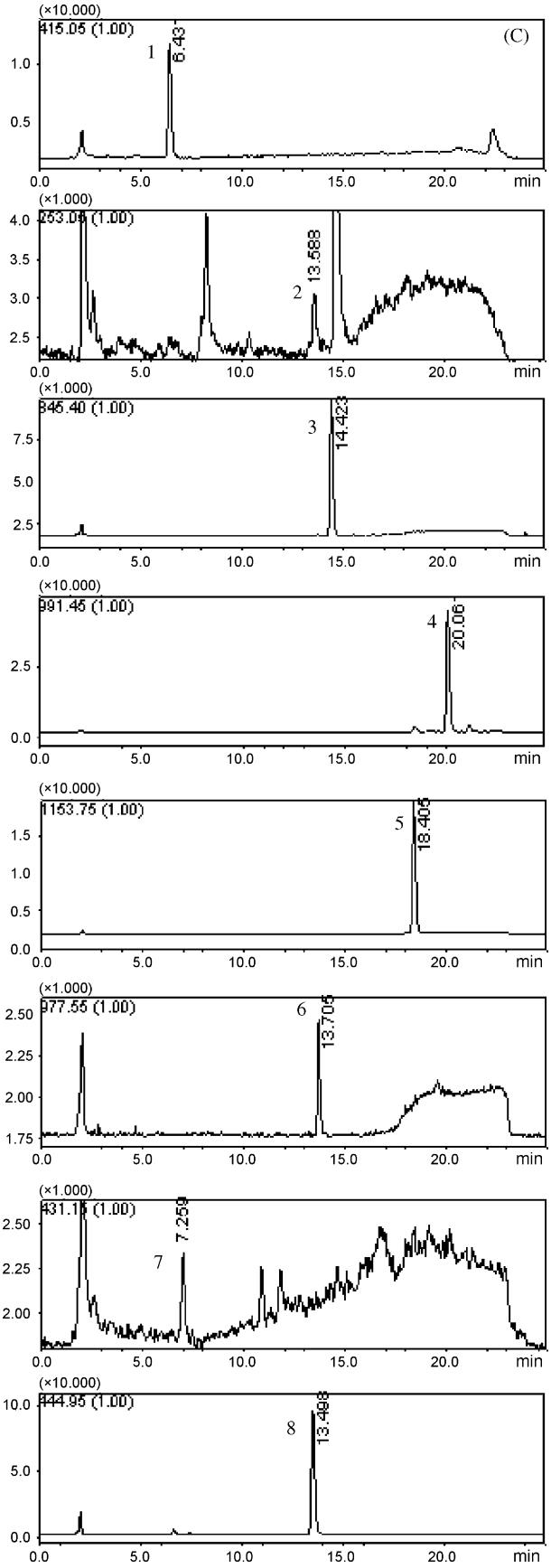


Fig. 1. (*Continued*).

3. Results and discussion

3.1. HPLC–MS/APCI

APCI was selected as the ionization method because it is generally less prone to be affected by matrix suppression compared with electronic spray ionization (ESI) [28–30]. Furthermore, peaks observed in our experiment with the APCI interface were far more symmetric and sharper than those with the ESI interface. In addition, we performed the assay under more efficient chromatographic conditions with gradient elution to separate the analytes of interest from undetected endogenous and exogenous compounds that may affect the efficiency of ionization of the analytes. Then, examination of the direct full scan mass spectra in the positive and negative ionization modes revealed that the signals obtained with the Turbo Ion Spray source in the positive

Table 4
Back calculated values and deviations from nominal values over the concentration ranges

Components	Nominal concentration (μg/ml)	Accuracy (%)	Concentration (μg/ml) mean ± SD
Safflor yellow A	0.08	104.4	0.08 ± 0.01
	0.16	91.4	0.15 ± 0.03
	0.4	98.2	0.39 ± 0.06
	1.6	105.6	1.69 ± 0.23
	4	94.7	3.79 ± 0.35
	8	109.0	8.72 ± 0.79
	40	96.8	38.72 ± 1.65
Puerarin	0.16	102.7	0.16 ± 0.01
	0.32	92.9	0.30 ± 0.02
	0.8	104.9	0.84 ± 0.09
	3.2	95.9	3.07 ± 0.21
	8	103.7	8.30 ± 0.37
	16	104.1	16.66 ± 0.93
	80	95.7	76.59 ± 2.14
Daidzein	0.05	95.8	0.05 ± 0.01
	0.1	109.5	0.11 ± 0.01
	0.25	96.2	0.24 ± 0.01
	0.5	105.4	0.53 ± 0.02
	1	93.9	0.94 ± 0.03
	2.5	94.4	2.36 ± 0.08
	5	104.8	5.24 ± 0.16
Rg_1	0.3	95.9	0.29 ± 0.01
	0.6	108.6	0.65 ± 0.03
	1.5	99.3	1.49 ± 0.14
	6	99.2	5.95 ± 0.33
	15	105.0	15.76 ± 0.63
	30	95.1	28.53 ± 1.28
	150	97.0	145.4 ± 4.5
Rb_1	4	95.5	3.82 ± 0.13
	8	107.8	8.63 ± 0.25
	20	107.7	20.53 ± 0.56
	40	90.1	36.05 ± 1.24
	80	104.7	83.79 ± 1.85
	200	90.8	181.7 ± 3.2
	400	103.4	413.4 ± 9.2
Rd	0.2	97.0	0.19 ± 0.01
	0.4	107.9	0.43 ± 0.01
	1	92.7	0.93 ± 0.03
	2	106.3	2.13 ± 0.05
	4	95.8	3.83 ± 0.06
	10	105.2	10.52 ± 0.18
	20	95.0	19.01 ± 0.37
R_1	0.2	96.9	0.19 ± 0.01
	0.4	107.8	0.43 ± 0.01
	1	95.3	0.95 ± 0.02
	2	103.3	2.07 ± 0.03
	4	97.0	3.88 ± 0.07
	10	94.4	9.44 ± 0.26
	20	105.3	21.06 ± 0.42

mode had too poor a resolution and too low an intensity to permit quantitative measurement. However, in the negative ion mode, the intensities were high enough for detection.

The standards of these components were analyzed by direct-flow injection to optimize the APCI–MS conditions. The seven analytes showed different fragmentation patterns and the most abundant fragment for each component was chosen for SIM quantitation (Table 2).

The addition of 0.1% of formic acid to the mobile phase was found to ensure the stability of production of the molecular ion $[M+HCOO]^-$ and chromatographic peak symmetry.

The determination of the seven constituents was completed in one chromatographic run, which lasted less than 20 min. Fig. 1 shows representative chromatograms for blank plasma, blank samples spiked with standard samples, and actual plasma samples after intravenous administration. With very little background noise, a stable baseline was maintained throughout. The retention time of safflor yellow A, puerarin, daidzein, ginsenoside Rg_1, Rb_1, Rd, notoginsenoside R_1 and baicalin (IS) was 7.229, 6.379, 13.559, 14.404, 18.386, 20.034, 13.677, and 13.478 min, respectively.

We tried to separate these compounds from endogenous interference by isocratic elution but, with so many endogenous analytes present, it was difficult to separate all the analytes simultaneously. Hence, gradient elution was applied for the separation and simultaneous determination of the seven compounds in rat plasma samples.

3.2. Method validation

3.2.1. Specificity

The specificity of the method was tested by analysis of blank plasma samples. There was no significant chromatographic interference around the retention times of the analytes and I.S. in drug-free specimens (Fig. 1).

3.2.2. Linearity and LLOQs

Standard curves exhibited good linearity with all coefficients of correlation (r) within the range 0.996–0.998. The LLOQs are appropriate for quantitative detection of analytes in the pharmacokinetic studies. Linear ranges, slopes, intercepts, LLOQs, and correlation coefficients obtained from typical calibration curves are listed in Table 3. Back calculated values and deviations from nominal values for each calibration standard are shown in Table 4.

3.2.3. Precision and accuracy

The precision of this analytical method was evaluated by calculating the RSD of three QC samples on the same day ($n=6$) and on different days ($n=3$). The resulting assay precision and accuracy data are listed in Table 5. The intra-day RSD of the assay was less than 12.4% and the inter-day RSD less than 14.7% for each concentration at three QC concentration levels, thereby indicating good assay precision. The accuracy ranged from 90.0% to 109.0% (Table 5).

3.2.4. Extraction recovery and ion suppression

The extraction recovery of the seven constituents were determined by comparing the peak area of each analyte in plasma samples spiked with the analyte prior to extraction with that in the reference standard plasma-free samples with no extraction. The results showed that the extraction recovery were in the range 70.7–87.1% at the three concentration levels. The extraction recovery of the I.S. was 86.1% (Table 5).

The results obtained while testing five different lots of drug-free rat plasma indicated that the evaluated method was free from any matrix effect. The ion suppressions from co-eluting compounds were negligible (data not shown).

3.2.5. Stability

The QC samples prepared in rat plasma, after undergoing three freeze–thaw cycles, showed no significant degradation. In the extracts, the seven compounds tested were stable for up to 24 h without any significant degradation. In addition, they were stable in plasma at −20 °C for up to 1 month. Stock solutions of these analytes in methanol were stable for up to 45 days. Experiments to assess long-term stability are in progress (data not shown).

3.3. Application of the assay to rat plasma

The assay developed was sensitive enough to measure all the seven compounds in rat plasma samples obtained following

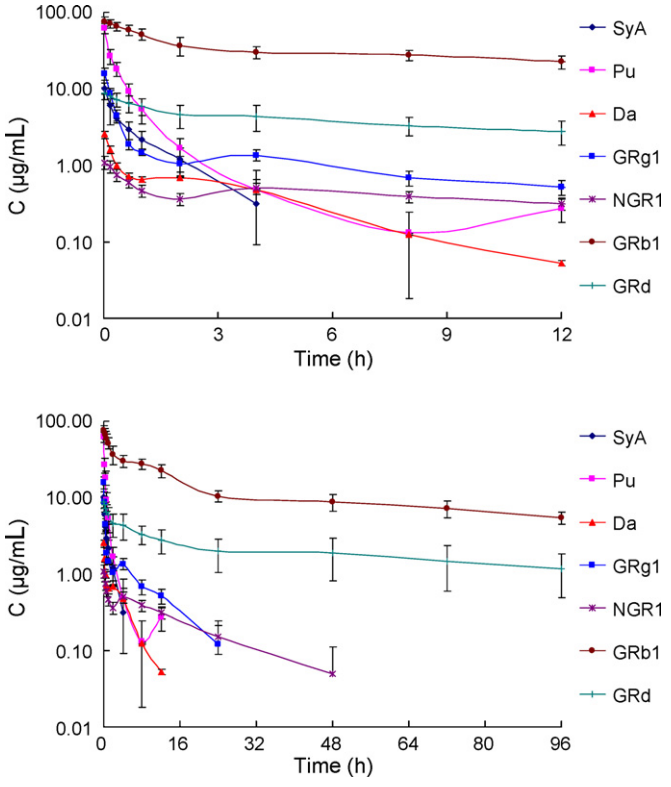


Fig. 2. Plasma concentration–time profiles of safflor yellow A, puerarin, daidzein, ginsenosides (Rg_1, Rb_1, Rd) and notoginsenoside R_1 after intravenous administration of Naodesheng injection.

Table 5
Precision, accuracy, and extraction recoveries of the LC-MS method

Components	Concentration (μg/ml)		Precision (%)		Extraction recovery (%)	Accuracy (%, RE)
	Spiked	Measured	Intra-day ($n=6$)	Inter-day ($n=3$)		
Safflor yellow A	0.16	0.16	7.5	11.5	70.7	100.0
	1.6	1.54	6.9	8.5	74.1	96.3
	32	32.05	4.0	4.7	73.9	100.2
Puerarin	0.32	0.3	6.4	7.6	75.5	93.8
	3.2	3.25	5.7	6.4	76.9	101.6
	64	66.29	1.3	3.8	80.2	103.6
Daidzein	0.1	0.09	12.4	14.7	76.2	90.0
	0.5	0.54	9.7	10.7	79.4	108.0
	4	3.92	4.6	5.4	83.7	98.0
Rg_1	0.6	0.55	11.4	13.1	82.6	91.7
	6	6.54	7.5	6.8	86.4	109.0
	120	122.6	2.4	1.9	87.1	102.2
Rb_1	8	7.48	7.6	8.5	81.3	93.5
	40	39.7	6.2	5.1	82.7	99.3
	320	325.7	2.4	3.7	82.5	101.8
Rd	0.4	0.36	7.3	7.8	74.2	90.0
	2	1.92	5.8	6.3	76.1	96.0
	16	16.24	2.6	3.4	79.5	101.5
R_1	0.4	0.42	6.9	7.3	82.8	105.0
	2	2.06	4.1	4.7	82.4	103.0
	16	15.28	2.4	2.9	86.5	95.5
Baicalin (I.S.)	900	–	–	–	86.1	–

administration of Naodesheng herbal injection. Fig. 2 shows the plasma concentration–time profiles of these components in rat plasma after administering the injection. According to 3P97 software, their disposition kinetics could be adequately described by a two-compartment model with first-order elimination. The estimates of the pharmacokinetic parameters are listed in Table 6.

4. Conclusion

The method reported here is the first HPLC–MS quantitative assay of safflor yellow A, puerarin, daidzein, ginsenosides (Rg_1, Rb_1, and Rd) and notoginsenoside R_1 in rat plasma samples following intravenous injection of Naodesheng. This method is rapid, sensitive, reproducible, and accurate. The proposed

Table 6
Mean pharmacokinetic parameters of the seven compounds after intravenous administration of Naodesheng injection (10 ml/kg) to rats ($n=6$)

Parameters[a]	Values (mean ± S.D.)						
	Safflor yellow A	Puerarin	Daidzein	Rg_1	Rb_1	R_1	Rd
A (μg/ml)	8.30 ± 1.45	61.65 ± 7.42	2.55 ± 0.21	17.01 ± 2.94	63.12 ± 7.06	0.59 ± 0.10	5.95 ± 0.65
B (μg/ml)	2.34 ± 1.21	1.73 ± 1.35	0.97 ± 0.04	1.31 ± 0.24	14.45 ± 4.71	0.68 ± 0.12	3.04 ± 1.74
α (1/h)	4.37 ± 0.66	4.25 ± 0.72	9.81 ± 0.70	4.98 ± 0.16	0.65 ± 0.08	5.44 ± 0.01	1.05 ± 0.19
β (1/h)	0.38 ± 0.15	0.32 ± 0.11	0.25 ± 0.10	0.08 ± 0.01	0.01 ± 0.01	0.07 ± 0.01	0.01 ± 0.01
$t_{1/2\alpha}$ (h)	0.16 ± 0.02	0.17 ± 0.03	0.07 ± 0.03	0.14 ± 0.05	1.09 ± 0.15	0.13 ± 0.07	0.66 ± 0.05
$t_{1/2\beta}$ (h)	2.01 ± 0.66	2.46 ± 1.24	2.82 ± 0.88	8.83 ± 0.85	72.40 ± 11.31	10.52 ± 1.31	68.04 ± 4.11
k_{12} (1/h)	2.07 ± 0.28	0.87 ± 0.53	6.35 ± 0.55	3.71 ± 0.12	0.48 ± 0.06	2.44 ± 0.61	0.67 ± 0.11
k_{10} (1/h)	1.43 ± 0.39	3.27 ± 0.73	0.84 ± 0.29	0.92 ± 0.07	0.05 ± 0.02	0.12 ± 0.05	0.04 ± 0.03
k_{21} (1/h)	1.26 ± 0.55	0.44 ± 0.17	2.88 ± 0.54	0.43 ± 0.21	0.13 ± 0.03	2.94 ± 0.51	0.35 ± 0.16
Vl (l)	0.10 ± 0.03	0.20 ± 0.09	0.11 ± 0.01	0.31 ± 0.13	0.12 ± 0.03	0.65 ± 0.32	0.20 ± 0.05
CL (l/h)	0.13 ± 0.04	0.55 ± 0.22	0.10 ± 0.03	0.05 ± 0.01	0.05 ± 0.01	0.15 ± 0.05	0.05 ± 0.01
AUC (h μg/ml)	7.91 ± 1.82	23.29 ± 4.13	4.50 ± 1.53	13.80 ± 2.46	379.5 ± 61.32	5.15 ± 0.89	47.54 ± 14.98

[a] A: Concentration intercept at time zero of the distribution phase; B: concentration intercept at time zero of the elimination phase; α: distribution rate constant; β: elimination rate constant; $t_{1/2\alpha}$: distribution half life; $t_{1/2\beta}$: elimination terminal half life; k_{12}: central to peripheral compartment rate constant; k_{10}: central compartment elimination rate constant; k_{21}: peripheral to central compartment the rate constant; Vl: volume of central compartment; Cl: total clearance; AUC: Area under plasma concentration time curve.

HPLC-MS method provides a useful alternative for analysis of the pharmacokinetics of multi-bioactive components in plasma samples.

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