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Short communication

Validation of an HPLC method for the determination of scutellarin in rat plasma and its pharmacokinetics

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Abstract

A validated HPLC method was developed for the quantification of scutellarin in rat plasma using a liquid–liquid extraction and an ultraviolet detection. Chromatographic separation of scutellarin in plasma was performed on a C_{18} column, with a mobile phase of acetonitrile–water (23:77, v/v), adjusted to pH 2.5 with 1 M phosphoric acid, and rutin was used as an internal standard. The calibration curve was linear over the range 0.1–100 μg/ml in rat plasma. The average extraction recoveries were 85.9 ± 8.9, 71.0 ± 4.6, 72.7 ± 1.2% ($n = 3$) at concentrations of 0.1, 2, 100 μg/ml, respectively, and the within-day and between-day precisions were less than 15%. After intravenous administration to rats over the doses range of 10–40 mg/kg, the plasma concentration–time curve of scutellarin was best conformed to three-compartment open model. The AUC of scutellarin was proportional to dose, and the systemic clearance (Cl), elimination half-life ($t_{1/2}\beta$) and apparent volume of distribution (V_c) were not significantly different among the three doses, suggestive of the linear pharmacokinetics characteristic of scutellarin after intravenous administration.

Keywords: Scutellarin; HPLC; Pharmacokinetics

1. Introduction

Flavonoids are the most prevalent classes of polyphenolic compounds widely distributed in edible plants and thus are important constituents of human diet [1]. Epidemiological observations strongly suggest flavonoids to be preventive in coronary heart disease [2,3], stoke [4] and certain cancers [5–7]. Thus, currently much attention is paid to flavonoids because of the beneficial pharmalogical activities.

Scutellarin, a flavone glucuronide (Fig. 1), extracted from a Chinese herb *Erigero breviscapus* (vant.) hand.-Mazz., is drawing particular interest because it significantly dilates blood vessel, improves microcirculation, increases cerebral blood flow, and inhibits platelet aggregation activity. To date, it has been widely used in the treatment of cerebral infarction and sequelae, cerebral thrombus, coronary heart disease and angina pectoris [8].

In our studies, scutellarin in rats was eliminated rapidly after intravenous administration, and the plasma concentration became low quickly. Therefore a sensitive HPLC assay is required to determine the plasma levels. Thus far, a number of HPLC methods have been published [8–11]. However, the limit of quantitation reported in some studies was insufficient to determine the low plasma concentration achieved [9,10]. Thereby, the objective of this study was to develop a sensitive and precise analytical method for determining scutellarin in biological samples, and further to understand its pharmacokinetic characteristics in rats after intravenous administration.

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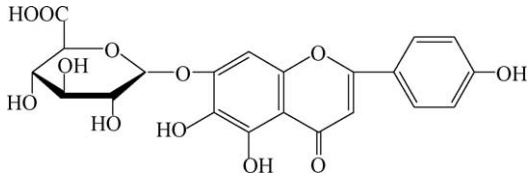

Fig. 1. The chemical structure of scutellarin.

2. Materials and methods

2.1. Chemicals and reagents

Scutellarin was provided by Yunnan Yuxi Wanfang Natural Medicine Co., Ltd. (95.5%, Kunming, China). The reference standard of scutellarin was purchased from Delta information center for natural organic compounds (98.5%, Xuancheng, China). The reference standard of scutellarein was provided by Beijing Tianbo Fangzhou Pharmaceutical and Chemical Technology Co., Ltd. (99.1%, Beijing, China). The internal standard, rutin, was purchased from National Institute for the Control of Pharmaceutical and Biological Products (99.8%, Beijing, China). Acetonitrile was of HPLC grade from Shandong Yuwang Co., Ltd. All other reagents were of analytical grade.

2.2. Apparatus and chromatographic conditions

The Shimadzu HPLC system (Kyoto, Japan) consisted of a LC-10AT pump, a SPD-10A UV detector, and a 7725i sample injector. The analytical column used was Hypersil C_{18} (4.6 mm × 200 mm, 5 μm) from Dalian Elite Analytical Instruments Co., Ltd. (Dalian, China). The chromatographic analysis was performed at room temperature with a flow rate of 0.8 ml/min and the eluate was monitored at 335 nm. The mobile phase consisted of acetonitrile–water (23:77, v/v), adjusted to pH 2.5 with 1 M phosphoric acid.

2.3. Sample preparation

To aliquots of 100 μl plasma sample, 10 μl internal standard solution (100 μg/ml, rutin in methanol) and 50 μl 0.5 M phosphoric acid were added and mixed for a few seconds. A volume of 2 ml of ethyl acetate was added to the solution and shaken on horizontal shaker for 5 min, followed by centrifugation at 1000 × *g* for 10 min. The organic layer was transferred to another tube and the solvent was evaporated to dryness in a water bath at 40 °C under a flow of nitrogen. The residue was dissolved in 50 μl mobile phase and 20 μl was injected into the HPLC.

2.4. Stability study of plasma sample

To evaluate the stability of sample during analysis time and also upon storage for a limited time, normal rat plasma samples spiked with 20 μg/ml of scutellarin were analyzed at different times after preparation and at three different temperatures.

2.5. Pharmacokinetics study

All animal studies were performed according to the Guidelines for the Care and Use of Laboratory Animals that was approved by the Committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical University.

Wistar rats (male and female, 240–300 g) were provided by the Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Animals were housed in a room with controlled temperature and humidity, and allowed to freely access to food and water. They were fasted overnight before the experiments. Three groups (6 rats/group) were randomly assigned to receive scutellarin solution via the tail vein injection at a single dose of 10, 20, 40 mg/kg, respectively. Right external jugular vein was cannulated for sample collection. Blood samples were collected from the jugular vein catheter at 0.05, 0.17, 0.33, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, and 4.0 h after administration. Samples were centrifuged at 1000 × *g* for 10 min. Plasma was transferred into microcentrifuge tubes and stored at −20 °C until analysis.

2.6. Data analysis

The pharmacokinetic parameters were calculated by the three-compartment method using 3p97 program (a practical pharmacokinetic program, developed by the Chinese Society of Mathematical Pharmacology), and the compartment model was evaluated based on AIC rule.

3. Results and discussion

3.1. Chromatographic separation

The composition of the mobile phase is a critical factor for separating scutellarin from internal standard (rutin) and endogenous substance. The resolution between scutellarin and rutin became poor by the addition of methanol in the mobile phase, and thus acetonitrile was employed as an organic modifier. When pH value of the mobile phase decreased from 6.0 to 2.5, the HETP (height equivalent to one theoretical plate) of peaks became lower, but the resolution higher. The underlying reason was that the ionization of the weak acidic phenolic group and carboxyl group, as well as the interactions of these groups with residual traces of metals in the stationary phase, was suppressed [12]. The mutual separation of scutellarin, rutin and endogenous substances was optimized by using the mobile phase with acetonitrile–water (23:77, v/v), adjusted to pH 2.5 with the use of an acid modifier, phosphoric acid, as shown in Fig. 2.

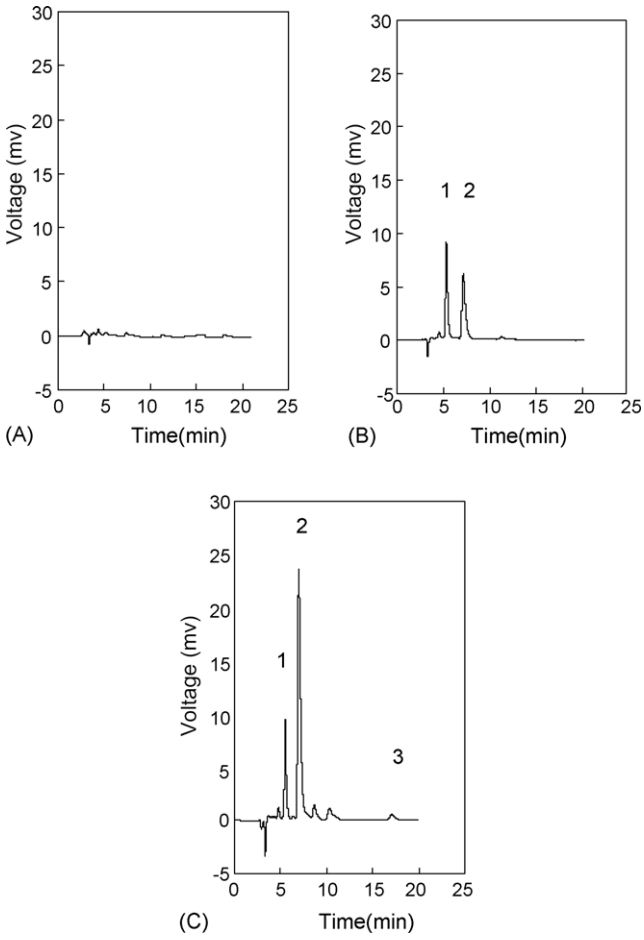

Fig. 2. Chromatograms of blank plasma (A); blank plasma spiked with internal standard (10 μg/ml) and scutellarin (5 μg/ml) (B); rat plasma sample at 20 min after intravenous administration of scutellarin at a dose of 40 mg/kg (27.5 μg/ml) (C). 1, Rutin; 2, scrutellarin; 3, scutellarein.

In our study, we found a metabolite of scutellarin in the plasma samples identified as scutellarein, with the same retention time by HPLC method compared with the reference standard of scutellarein, but the concentration of scutellarein was very low.

Furthermore, the chromatographic conditions established in our studies not only were adapt to quantitate the plasma level of scutellarin but also were adapt to quantitate scutellarein (a metabolite of scutellarein) simultaneously. However, the scutellarein was not detected in the chromatographic conditions of the references above mentioned.

3.2. Method validation tests

3.2.1. Linearity and limit of quantification

Calibration curves for the plasma assay developed with peak–area ratio (y) of scutellarin to internal standard versus drug concentration (C) were found to be linear over the concentration range of 0.1–100 μg/ml using weighted least square method, and the weight was $1/C^2$. The linear regression equation of the calibration curve was $Y = 0.1707 + 0.2445 \times C$, and r was 0.998. The limit of quantification (LOQ) was 100 ng/ml.

3.2.2. Precision and accuracy

The precision and accuracy of the method were examined by adding known amounts of scutellarin to rat blank plasma. For intra-day precision and accuracy, three replicate quality control samples at each concentration were assayed on the same day. The inter-day precision and accuracy were evaluated on three different days. The results are summarized in Table 1. The intra-day and inter-day precisions were within 15% for scutellarin, which indicated the method was reproducible. The extraction recoveries under the liquid–liquid extraction conditions were 85.9 ± 8.9, 71.0 ± 4.6, $72.7 \pm 1.2\%$ ($n = 3$) at concentrations of 0.1, 2, 100 μg/ml, respectively. Therefore, the criteria of precision, accuracy and recovery for analyzing biological samples were fulfilled in the developed analytical method.

3.3. Stability study of scutellarin in plasma samples

The stabilities of scutellarin in plasma (20 μg/ml) at different temperatures are shown in Table 2. About 5% decrease in plasma samples after 24 h at room temperature was observed, and after 24 h at 37 °C, about 95% of scutellarin degraded, but the frozen samples were still stable, which were stored at −20 °C for 1 month or treated after three freeze and thaw cycles. Scutellarin in plasma sample was unstable at 37 °C because of the existence of polyphenolic hydroxyl group which was apt to be oxidized.

3.4. Pharmacokinetics study

The mean plasma concentration–time profiles after intravenous administration of scutellarin 10, 20, and 40 mg/kg to rats are shown in Fig. 3.

Table 1
Precision and accuracy of scutellarin determination in rat plasma ($n = 3$)

Added (μg/ml)	Intra-day			Inter-day		
	Measured (μg/ml)	R.S.D. (%)	Accuracy (%)	Measured (μg/ml)	R.S.D. (%)	Accuracy (%)
0.100	0.098	8.5	98.0	0.102	12.5	102
2	2.05	6.2	102.4	1.98	7.8	99.4
100	98.4	3.6	98.4	97.6	4.6	97.6
Mean		6.1	99.6		8.3	99.7

Table 2
Stability of scutellarin in rat plasma (20 μg/ml) at three different temperatures (mean ± S.D., $n = 3$)

Temperature	Content (%)				
	4 h	8 h	12 h	24 h	48 h
Frozen (−20 °C)	102.1 ± 1.7	100.8 ± 2.3	101.5 ± 1.1	101.1 ± 1.2	102.0 ± 0.7
Room temperature	99.7 ± 1.4	98.4 ± 0.9	96.7 ± 1.3	94.3 ± 2.7	59.9 ± 1.8
37 °C	96.7 ± 1.6	91.7 ± 1.1	84.5 ± 2.1	5.1 ± 3.2	0
Thawing[a]	99.8 ± 1.4	98.1 ± 1.5	95.7 ± 1.3	93.8 ± 2.5	54.2 ± 3.4

[a] The stability of scutellarin in the frozen plasma sample after thawing at room temperature for different time.

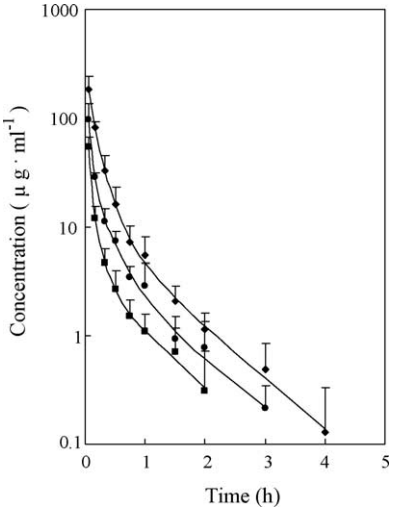

Fig. 3. Mean plasma concentration–time curves of scutellarin in rats (mean ± S.D., $n = 6$) after a single intravenous dose of scutellarin at 10 mg/kg (■), 20 mg/kg (●) and 40 mg/kg (♦).

Table 3
Pharmacokinetic parameters for scutellarin in rats (mean ± S.D., $n = 6$) after a single intravenous dose of scutellarin at 10, 20, 40 mg/kg dose levels

Parameter	10 mg/kg	20 mg/kg	40 mg/kg
α(1/h)	4.68 ± 1.90	4.78 ± 2.01	3.06 ± 1.06
β(1/h)	0.96 ± 0.35	0.96 ± 0.36	0.99 ± 0.57
γ (1/h))	17.57 ± 4.70	13.77 ± 4.50	9.82 ± 6.07
V_c (l/kg)	0.09 ± 0.03	0.14 ± 0.08	0.16 ± 0.08
$t_{1/2\ (\gamma)}$ (h)	0.04 ± 0.01	0.06 ± 0.04	0.09 ± 0.04
$t_{1/2\ (\alpha)}$ (h)	0.16 ± 0.08	0.17 ± 0.06	0.25 ± 0.08
$t_{1/2\ (\beta)}$ (h)	0.84 ± 0.41	0.91 ± 0.64	1.02 ± 0.72
K_{12} (1/h)	2.10 ± 1.79	1.87 ± 2.27	1.58 ± 1.96
K_{21} (1/h)	5.50 ± 2.23	5.91 ± 1.48	3.94 ± 2.06
K_{13} (1/h)	3.41 ± 2.29	2.03 ± 2.11	0.71 ± 0.40
K_{31} (1/h)	1.25 ± 0.40	1.23 ± 0.60	1.16 ± 0.64
K_{10} (1/h)	11.32 ± 3.17	8.47 ± 2.72	6.48 ± 2.98
AUC (h mg/l)	11.05 ± 2.68	21.02 ± 5.33	45.71 ± 9.53
CL (s) (h l/kg)	0.95 ± 0.23	1.01 ± 0.27	0.91 ± 0.20

Scutellarin was eliminated rapidly from the plasma. Accordingly, plasma concentration of scutellarin was detectable only up to 4 h in rats. The scutellarin concentration–time data were best fitted to a three-compartment open model after intravenous administration at three doses and the pharmacokinetic parameters were summarized in Table 3. The AUC values were 11.05 ± 2.68, 21.02 ± 5.33, 45.71 ± 9.53 h mg/l for 10, 20, 40 mg/kg, respectively. In addition, the AUC values versus doses were linear over the administered doses range ($r = 0.998$). The results suggested that the AUC of scutellarin in rat plasma was proportional to doses and the systemic clearance (Cl), elimination half-life ($t_{1/2}\beta$) and apparent volume of distribution (V_c) were not significantly different at doses range of 10–40 mg/kg, suggestive of the linear pharmacokinetic characteristic of scutellarin in rats after intravenous administration.

4. Conclusions

In conclusion, a simple, specific and sensitive HPLC method for the determination of scutellarin in rat plasma was developed, which was applied to pharmacokinetic investigation in rats after intravenous administration of scutellarin at doses of 10, 20, 40 mg/kg, and scutellarin exhibited linear pharmacokinetic characteristics over the dose range studied.

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