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A rapid reversed phase high-performance liquid chromatographic method for determination of sophoridine in rat plasma and its application to pharmacokinetics studies

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Abstract

A high-performance liquid chromatography (HPLC) method for determining sophoridine in rat plasma was developed for application in the pharmacokinetic studies. The plasma was deproteinized with acetonitrile that contained an internal standard (ephedrine) and was separated from the aqueous layer by adding sodium chloride and sodium carbonate. The HPLC assay was carried out using a YMC-ODS column. The mobile phase was methanol–ethanol–0.01 mol l^{-1} ammonium acetate buffer–triethylamine (10:0.5:89.5:0.03, v/v/v/v) (pH 6.80). The flow rate was 0.8 ml min^{-1}. The detection wavelength was set at 210 nm. The method was used to determine the concentration–time profiles of sophoridine in the plasma following oral administration or injection of sophoridine aqueous solution. The fractions of sophoridine reaching the systemic circulation were estimated for the first time by a deconvolution method.

Keywords: Sophoridine; HPLC–DAD; Rat plasma

1. Introduction

Sophora alopecuroides L. is a bush that grows throughout northwest China and is an important source of Chinese medicine [1]. Ku-dou-zi, the aired root of *Sophora alopecuroides* L., is a common traditional Chinese herb used to treat acute or chronic gastroenteritis [2]. The herb is known to contain quinolizidine alkaloids as bioactive constituents and a previous study has shown that sophoridine (SRI, structure shown in Fig. 1) is one of the main alkaloids in this herb [3]. The studies of pharmacological effect have shown that SRI has wide pharmacological effects including anti-arrhythmic [4], anti-tumor [5], immunological enhancement [6], immunosuppression [7], antiseptic and central nervous system excitation effects [8].

SRI's various pharmacological effects and its fate in body have been well described [9,10]. However, these pharmacokinetic studies have focused only on the processes after intravenous injection. In spite of Ku-dou-zi is usually administered orally, there is a lack of information concerning the extent to which SRI is absorbed following oral administration. Because most Chinese medicines are administered orally in the form of a crude extract, the absorption rates of the active components from the gastrointestinal tract could be used as a suitable reference in clinical applications.

Several methods have been reported for the determination of SRI in plasma, including gas chromatography [9], capillary gas chromatography [10], HPLC–UV method [11] and HPLC–MS method [12]. However, these methods all have disadvantages. GC methods have complicated sample pretreatments and they seem to be unsuitable for quantitative determination of SRI in rat plasma because only a small amount of blood is normally used in pharmacokinetic studies. The HPLC–UV method is unsuitable for microanalysis of SRI in plasma because of poor quantitation limits (350 ng ml^{-1}). The HPLC–MS method is not common because of the high analysis cost and the expensive apparatus required.

The aim of the present work is to develop a simple, sensitive and specific HPLC assay for the quantitation of SRI in rat plasma and application of the HPLC method developed in this investigation for pharmacokinetic studies.

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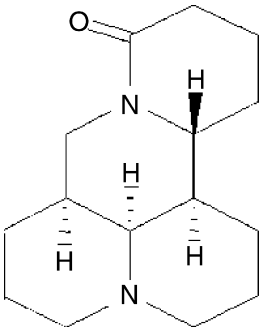

Fig. 1. Structure of SRI.

2. Experiment

2.1. Chemicals and reagents

The SRI used in analysis was analytical grade and that used in animal experiment was drug grade, purchased from the Chinese Medicine Control Institute (Beijing, China) and the Yanchi Pharmaceutical Factory (Ningxia, China), respectively. Acetonitrile, ethanol and methanol were HPLC grade, obtained from Shandong Yuwang Co. Ltd. (Shandong, China). HPLC grade water was used.

2.2. Chromatographic system

The HPLC system was carried out using a Shimadzu LC-20A HPLC system (Kyoto, Japan) which consisted of a binary gradient pump (model LC-20A), a SPD-20MA diode-array detector, a SiL-20A auto sampler and a DGU-20A3 degasser. The apparatus was interfaced to a DELL PC compatible computer using LC solution software.

The separation was performed on a YMC-pack ODS-A column, 4.6 mm × 150 mm, 5 μm (YMC Co. Ltd., Kyoto, Japan). The mobile phase consisted of methanol, ethanol, 0.01 mol l^{-1} ammonium acetate buffer and triethylamine in a volume ratio of 10:0.5:89.5:0.03. The pH of the mobile phase was adjusted to 6.80 with glacial acetic acid. Prior to use, the mobile phase was filtered through a 0.45 μm hydrophilic membrane filter. The mobile phase was delivered at a flow rate of 0.8 ml min^{-1}. Detection was performed at a wavelength of 210 nm at room temperature. The sample injection volume was 20 μl.

2.3. Animals and blood sampling

Male wistar-strain rats (190–220 g) were obtained from the Laboratory Animal Center of Lanzhou University and fasted for 12 h with free access to water, prior to the experiments. A polyethylene tube (0.28 mm, I.D., 0.61 mm, O.D.) was inserted into the right femoral artery of the rat while the animal was under anesthesia with ether. SRI aqueous solution was then orally administered to the rat at a dose of 40 mg kg^{-1} or was injected into the blood at a single dose of 40 or 4 mg kg^{-1}. Blood samples (0.25 ml) were collected at 0, 10, 20, 30, 45, 60, 75, 90, 120, 180, 240, 360 and 480 min after oral administration or 0, 5, 10, 20, 30, 45, 60, 75, 90, 120, 180 and 240 min after injection. After each sampling, loss of blood volume was supplemented with an equal volume of saline containing 100 IU ml^{-1} heparin.

2.4. Preparation of plasma samples

Centrifugations were done in a 1.5 ml microcentrifuge at 10,800 rpm. Each collected blood sample was immediately transferred to a heparinized microcentrifuge tube and centrifuged for 5 min. The plasma (0.1 ml) was then vortex-mixed with 0.2 ml of acetonitrile containing ephedrine (10 μg ml^{-1}) as internal standard (IS) for 30 s. After 10 min the mixture was centrifuged for 5 min to separate precipitated proteins. The supernatant was transferred into 1.5 ml tubes containing 40–50 mg sodium chloride and 10–20 mg sodium carbonate. The suspension was vortex-mixed and placed for 10 min. After vortex-mixing and centrifuging once again for 5 min, 20 μl solution of acetonitrile layer was directly injected into the chromatography. The same sample processing was applied to the recovery and to the precision in plasma.

2.5. Calibration curve

Calibration curves in the concentration range of 0.5–70 μg ml^{-1} for SRI were constructed by plotting the peak-area ratio of each analyte/IS versus SRI concentration in rat plasma. In order to avoid undue bias, the calibration curve was split into two ranges: 0.5–5.0 and 5.0–70.0 μg ml^{-1}. Least squares linear regression analysis was used to determine the slope, intercept and correlation coefficient.

2.6. Recovery

For the recovery study, four concentrations of SRI solution were prepared in acetonitrile and plasma. Plasma samples were spiked with SRI at concentrations of 1.0, 5.0, 10.0, 35.0 and 70.0 μg ml^{-1}. The recovery was determined as the ratio of peak-area (analyte/IS) of plasma to that of acetonitrile.

2.7. Precision and limit of quantitation

Plasma samples were spiked with SRI at concentrations of 0.5, 1.0, 5.0, 10.0, 20.0, 35.0 and 70.0 μg ml^{-1}. Samples were processed in replicates ($n = 6$) and subjected to HPLC analysis. The precision was calculated as the relative standard deviation of measurements.

To determine the limit of quantification (LOQ) dilutions of 0.1, 0.15, 0.2, 0.3, 0.4 μg ml^{-1} SRI in plasma were prepared using a solution of 1.0 μg ml^{-1} SRI in plasma for calibration.

2.8. Selectivity

The selectivity of the method was determined by examining the separation of SRI from endogenous plasma constituents in blank plasma. The effect of concomitant elution of IS was also investigated. Solutions of SRI in acetonitrile and in plasma were injected under the same conditions, and the retention times of SRI and IS were determined.

2.9. Assay application

The present method was used to determine the plasma concentration of SRI in rat plasma after oral administration or injection of SRI aqueous solution from which the fractions of SRI reaching the systemic circulation were estimated by a deconvolution method.

3. Results and discussion

3.1. HPLC chromatograms

Under the conditions described above, the HPLC chromatograms of blank plasma, plasma spiked with SRI at a concentration of 35.0 μg ml^{-1} and the plasma obtained 1 h after oral administration of SRI aqueous solution at a dose 40 mg kg^{-1}, are shown in Fig. 2. It can be seen that plasma proteins do not interfere with the elution of SRI and IS. The retention times for IS and SRI were approximately 5.6 and 7.9 min, respectively. The peaks were sharp and symmetrical with good baseline resolution and minimal tailings.

The separation of SRI, IS and endogenous plasma by reverse phase HPLC, was investigated by choice of mobile phase, the pH of the mobile phase, mobile phase flow rate, and the amounts of modifier. The initial mobile phase system consisted of methanol–water (13:87, v/v). To improve the peak shapes and separation of SRI and IS, ethanol was added in the mobile phase and its optimum volume ratio was 0.5/100 of the mobile mixture. Water in the mobile phase was replaced by 0.01 mol l^{-1} ammonium acetate buffer to obtain better baseline stability. Triethylamine was added as a modifier to improve peak tailing and the best volume ratio was 0.03/100 of the mobile phase. The optimum mobile phase system was therefore methanol–ethanol–0.01 mol l^{-1} ammonium acetate buffer–triethylamine (10:0.5:89.5:0.03, v/v/v/v). The retention time of SRI in the mobile phase was pH-dependent and the optimum pH was identified as 6.8. The optimum mobile phase flow rate was found to be 0.8 ml min^{-1}.

The common pretreatment methods of SRI in plasma that have been described [9,10,11,12] are complicated. In our study, there was only one process from alkalization to extraction. And the HPLC method we developed was simple in terms of sample pretreatment, convenience and low analysis cost.

3.2. Calibration curves

SRI was dissolved in acetonitrile and diluted to give a series of standard solutions (0.5, 1.0, 5.0, 10.0, 20.0, 35.0 and 70.0 μg ml^{-1}) for the calibration curves of the drug in rat plasma. The linear regression analysis of the standard calibration plot for rat plasma were $Y = 13.1X + 0.158$ for the low concentration range (0.5–5.0 μg ml^{-1}) and $Y = 10.2X + 1.953$ for the high concentration range (5.0–70.0 μg ml^{-1}) with the same r^2 values of 0.9995, where Y and X represented the peak-area ratio and SRI concentration, respectively.

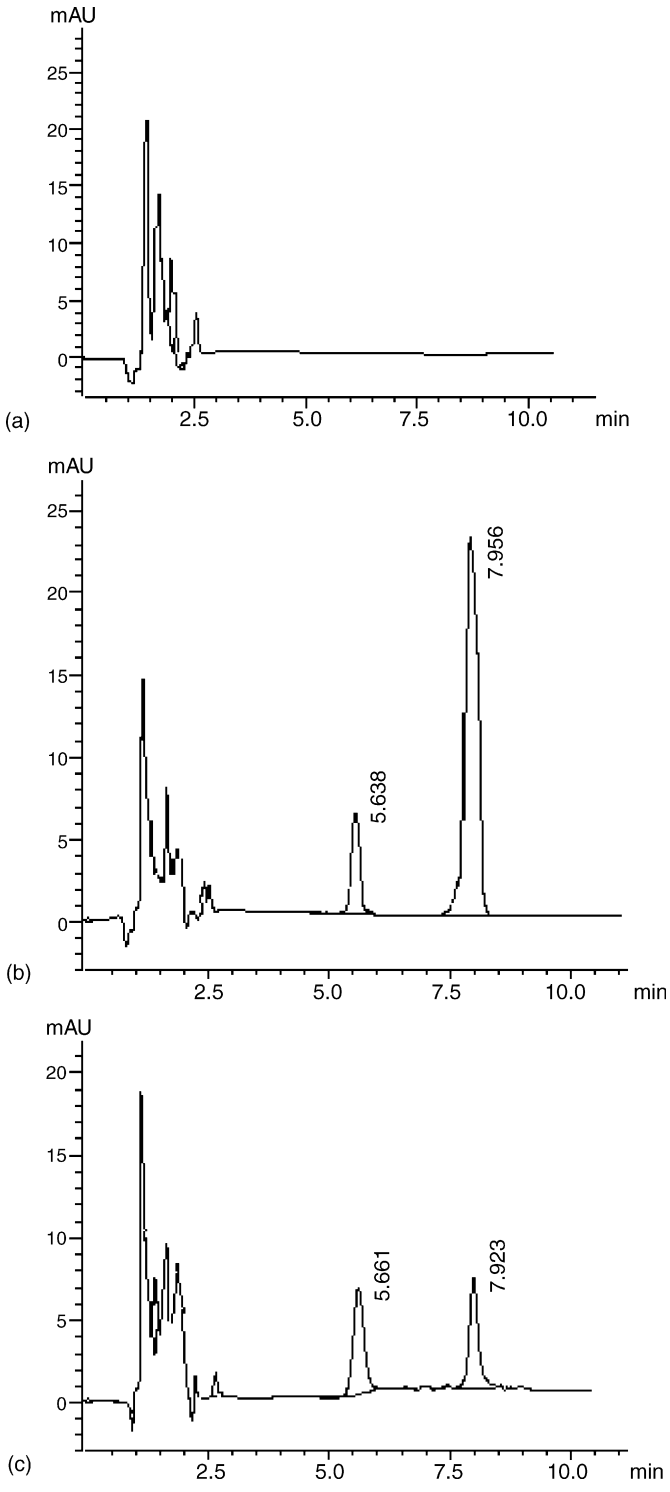

Fig. 2. Chromatograms of SRI in rat plasma: (a) blank plasma; (b) plasma spiked with sophoridine at a concentration of 35.0 μg ml^{-1} and ephedrine (IS); (c) plasma sample obtained 1 h after oral administration of SRI aqueous solution at a dose 40 mg kg^{-1}. Retention times of IS and SRI were approximately 5.6 and 7.9 min, respectively.

3.3. Recovery

The recovery of the assay was assessed by comparing the peak-area ratios (analyte/IS) obtained from spiked plasma sam-

Table 1
Recovery of the sophoridine assay

Skied concentration ($\mu g\,ml^{-1}$)	Peak-area ratio		Recovery (%)	CV (%)
	Untreated	Treated		
1.0	0.06 ± 0.002	0.057 ± 0.003	95.00 ± 2.95	3.11
5.0	0.41 ± 0.004	0.38 ± 0.021	92.68 ± 2.50	2.27
10.0	0.90 ± 0.017	0.83 ± 0.03	92.22 ± 1.79	1.94
35.0	3.36 ± 0.148	3.04 ± 0.093	90.50 ± 2.33	2.58
70.0	7.02 ± 0.16	6.15 ± 0.12	87.61 ± 1.58	1.81

Each value represents the mean ± SD ($n = 5$).

Table 2
Validation of the intra-day assay

Spiked concentration ($\mu g\,ml^{-1}$)	Measured concentration ($\mu g\,ml^{-1}$)	Accuracy (%)	CV (%)
0.5	0.51 ± 0.035	102.00	6.86
1.0	0.96 ± 0.029	96.00	3.02
5.0	5.07 ± 0.058	101.40	1.14
10.0	10.59 ± 0.24	105.90	2.26
20.0	20.77 ± 0.69	103.85	3.32
35.0	35.80 ± 0.75	102.28	2.09
70.0	68.95 ± 2.40	98.50	3.48

Each value represents the mean ± SD ($n = 5$).

ples of different SRI concentrations with the peak-area ratios for the samples containing the equivalent amounts of analyte and IS directly dissolved in acetonitrile. The recovery of SRI from rat plasma is shown in Table 1 and shows that the recovery by this method exceeded 87%. The average recovery of SRI was approximately 65% when it was extracted into the acetonitrile layer from the aqueous layer by adding sodium chloride [11]. The average recovery was improved to 87% by adding sodium chloride and sodium carbonate to alkalize the supernatant. This allowed the alkalization and extraction to take place in the same process.

3.4. Accuracy, reproducibility and limit of quantitation

Accuracy, reproducibility and precision data are summarized in Tables 2 and 3, where the values are expressed as mean detected concentration and coefficient of variation (CV). The reproducibility of the method was assessed by examining both

Table 3
Validation of the inter-day assay

Spiked concentration ($\mu g\,ml^{-1}$)	Measured concentration ($\mu g\,ml^{-1}$)	Accuracy (%)	CV (%)
0.5	0.52 ± 0.05	104.00	9.61
1.0	0.94 ± 0.03	94.00	3.19
5.0	5.03 ± 0.32	100.60	6.36
10.0	10.36 ± 0.36	103.60	3.47
20.0	20.02 ± 0.64	100.10	3.20
35.0	34.38 ± 1.83	98.22	5.32
70.0	70.65 ± 3.22	100.93	4.56

Each value represents the mean ± SD ($n = 5$).

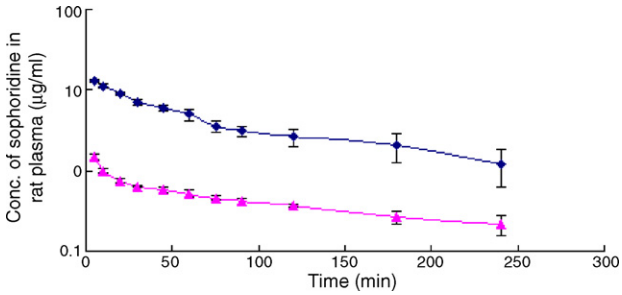

Fig. 3. Plasma concentration profiles of SRI after injection at a dose of 40 mg kg^{-1} (♦) and 4 mg kg^{-1} (▲). Data are means ± SD of three experiments.

intra- and inter-day variance. The data shows that the CV of SRI low to high concentrations was better than 7 and 10% for intra-day and inter-day assays, respectively. Assay accuracy was better than 94% (Tables 2 and 3). These validations demonstrate the reliability of our method [13]. The limit of quantitation was deemed to be 150 ng ml^{-1}.

3.5. Application

In order to estimate the absorption rate of SRI in the gastrointestinal tract, the plasma concentration of SRI was analyzed in rat plasma, following oral administration or injection of SRI aqueous solution. The plasma concentration of SRI was determined at 0, 10, 20, 30, 45, 60, 75, 90, 120, 180, 240, 360 and 480 min after oral dosing or 0, 5, 10, 20, 30, 45, 60, 75, 90, 120, 180 and 240 min after injection. Fig. 3 shows the mean ± SD plasma concentration–time profile of SRI after being injected with doses of 40 and 4 mg kg^{-1}. When the injection dose of SRI was increased from 4 to 40 mg kg^{-1}, there were no significant differences in the apparent total body clearance, the elimination half-life, or the volume of distribution. The area under the curve of SRI appeared to increase proportionally from 4 to 40 mg kg^{-1} (data not shown). These results suggested that the pharmacokinetics of SRI are a linear process. Fig. 4 shows the mean ± standard error of the mean (SEM) plasma concentration–time profile of SRI after oral administration of 40 mg kg^{-1}. The mean maximum concentration of SRI in plasma was 2.36 μg ml^{-1} at 60 min after oral dosing. The fractions of SRI absorbed were estimated by a deconvolution

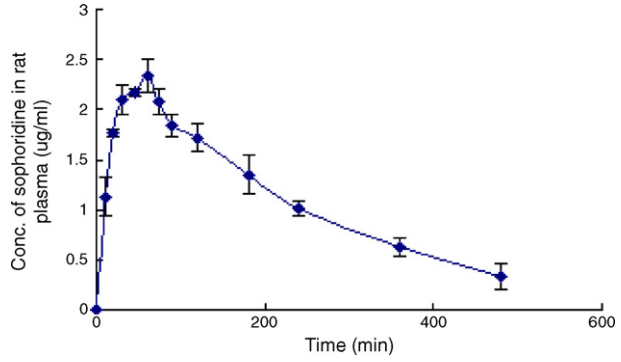

Fig. 4. Plasma concentration–time curve of sophoridine in rat after oral administration of SRI aqueous solution at a dose of 40 mg kg^{-1}. Data are means ± SEM of five experiments.

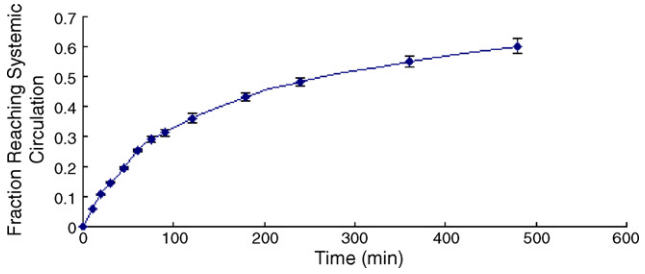

Fig. 5. Time-courses of fractions of SRI reaching systemic circulation after oral administration of SRI aqueous solution. Data are means ± SEM of five eperiments.

method (Fig. 5). The fractions of SRI reaching the systemic circulation rose rapidly to 0.43 within the first 2 h and then slowed to 0.60 by 8 h.

In conclusion, the HPLC method used, was simple, sensitive and specific, and could be used for the analysis of large numbers of plasma samples. The assay was validated to meet the requirements of pharmacokinetic studies. The fractions of SRI reaching the systemic circulation following oral dosing could be used as a suitable reference in clinical application.

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