—Regular Article—

HPLC Determination of Strychnine and Brucine in Rat Tissues and the Distribution Study of Processed Semen Strychni

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A simple and low-cost HPLC method with UV absorbance detection was developed and validated to simultaneously determine strychnine and brucine, the most abundant alkaloids in the processed Semen Strychni, in rat tissues (kidney, liver, spleen, lung, heart, stomach, small intestine, brain and plasma). The tissue samples were treated with a simple liquid-liquid extraction prior to HPLC. The LOQs were in the range of 0.039～0.050 μg/ml for different tissue or plasma samples. The extraction recoveries varied from 71.63 to 98.79%. The linear range was 0.05–2 μg/ml with correlation coefficient of over 0.991. The intra- and inter-day precision was less than 15%. Then the method was used to measure the tissue distribution of strychnine and brucine after intravenous administration of 1 mg/kg crude alkaloids fraction (CAF) extracted from the processed Semen Strychni. The results revealed that strychnine and brucine possessed similar tissue distribution characterization. The highest level was observed in kidney, while the lowest level was found in brain. It was indicated that kidney might be the primary excretion organ of prototype strychnine and brucine. It was also deduced that strychnine and brucine had difficulty in crossing the blood-brain barrier. Furthermore, no long-term accumulation of strychnine and brucine was found in rat tissues.

Key words——Semen Strychni; HPLC; tissue distribution; strychnine; brucine

INTRODUCTION

Strychnos nux-vomica L. (Loganiaceae) is grown extensively in southern Asian countries. The dried seed of this plant, Semen Strychni, officially listed in the Chinese Pharmacopoeia, has been used clinically for improving blood circulation, relieving rheumatic pain and treating cancer for a long history. As a major ingredient, it has been included in many prescriptions of traditional Chinese medicine such as ''Jiu-fen-san'', ''Feng-shi-ma-qian-pian'', *etc*. Because of violent toxicity, the unprocessed Semen Strychni is strictly controlled by government and required to be processed before use.[1] Traditionally, raw Semen Strychni is processed in hot sand (above 220°C) for 3–4 minutes, which is intended to reduce toxicity.[2,3] The results of acute toxicity revealed that the LD50 of the crude alkaloid fractions (CAF) from the processed seeds was 2.35 mg/kg, while that of the unprocessed seeds was 1.21 mg/kg.[3]

The major bioactive constituents of the processed Semen Strychni had been identified to be alkaloids. Among CAF of the processed Semen Strychni, strychnine and brucine (Fig. 1) take up more than 70%.[4] Furthermore, strychnine and brucine have been proved to show analgesic, anti-inflammatory and anti-tumor effects,[5,6] despite their toxicity in nature. In addition, strychnine nitrate injection has been in clinical application in China. Therefore, there is of great importance to establish a simple, low-cost and sensitive screening method for the determination of strychnine and brucine.

A sensitive and specific thin-layer chromatography (TLC) densitometric method has been developed for the simultaneous quantitation of strychnine and brucine from Strychnous formulations.[7] This method is simple and low-cost, but it is not suitable for the analysis of bio-samples.

Much attention has been paid to simultaneous determination of strychnine and brucine in body fluids. Capillary electrophoresis (CE) with field-amplified sample stacking was reported to determine strychnine

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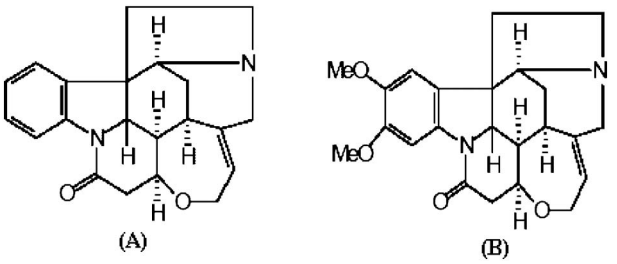

Fig. 1. Chemical Structures of Strychnine (A) and Brucine (B)

and brucine quantitatively in human urine.[8] The limits of quantification (LOQs) were 8 and 10 ng/ml for strychnine and brucine, respectively. However, 5 ml urine sample was needed for the extraction of strychnine and brucine from the sample. The volume was too large to apply the method for the quantification of strychnine and brucine in other biosamples. In addition, a high performance liquid chromatography-electrospray mass spectrometric (HPLC-ESI-MS) method was recently developed for the simultaneous analysis of strychnine and brucine in plasma.[9] Although excellent sensitivity can be obtained with MS analysis, it has a higher operation cost and operators need to be well trained. Solid-phase extraction (SPE) procedure has been widely used in the preparation of body fluids samples for the simultaneous determination of strychnine, brucine and 3–11 kind of other alkaloids.[10–12] However, for the determination of strychnine and brucine without the interferences from other alkaloids, SPE might not be necessary.

Compared with methods to determine strychnine and brucine in biological fluids, little data is available concerning the simultaneous determination in tissues. The published method was described for strychnine determination in tissue samples by gas chromatography-mass spectrometry (GC-MS).[13] The strychnine was isolated from tissue samples using a liquid-liquid extraction procedure and the LOQ was 0.1 μg/ml.

In the present paper, a reliable, simple and low-cost HPLC with UV detection method is described for the simultaneous determination of strychnine and brucine in rat tissues. The validated method has been applied to a tissue distribution study after intravenous administration of CAF of the processed Semen Strychni. Then the tissue distribution characteristics of strychnine and brucine are compared. To our knowledge, there is little information available about the tissue distribution of strychnine and brucine.

MATERIALS AND METHODS

Plant Material The processed Semen Strychni was supplied by Nanjing Company of Chinese Herbal Drugs (Nanjing, China). Voucher specimens were maintained at Nanjing University of Chinese Medicine, China.

Chemicals and Reagents Reference substances of strychnine, brucine and the internal standard (I.S.) huperzine A were supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade acetonitrile was purchased from Tedia (USA). Distilled water, prepared from demineralized water, was used throughout the experiment. All the other chemicals were purchased from Nanjing Chemical Reagent Corporation (Nanjing, China) and of analytical grade.

Animals Male Spargue-Dawley rats, weighting 200–250 g, were purchased from Shanghai Slac Laboratory Animal Co. Ltd (Shanghai, China) with the license number SCXK (Shanghai) 2007–0005. The rats were acclimatized for at least 1 week in a 12 h light/dark cycle with free access to standard chow and water. They were fasted overnight before drug administration. Animal experiments were carried out in accordance to the Guidelines for Animal Experiments of Nanjing University of Chinese Medicine, and the protocols of animal experiments were approved by the Animals Ethics Committee of Nanjing University of Chinese Medicine.

Chromatographic Conditions All analytical procedures were performed using a Shimadzu HPLC-UV system (Shimadzu Company, Japan) consisting of a LC-20AT pump and a SPD-20A UV detector. Data was collected by a HPLC chromatography workstation (N2000, Intelligence and information institute of Zhejiang University). Chromatographic separation was achieved on a Kromasil C_{18} analytical column (4.6 mm$\times$250 mm, 5 μm, Kromasil, Sweden) coupled with a C_{18} guard cartridge (4.6 mm$\times$10 mm, 5 μm, Hanbang, China), maintained at 35°C. The mobile phase consisted of 24% acetonitrile and 76% buffer (isometric mixture of 0.01 mol/l sodium heptane sulfonate and 0.02 mol/l potassium dihydrogen phosphate, the pH value was adjusted to 2.8 with 10 % phosphoric acid), which was selected according to the Pharmacopoeia of the People's Republic of China with minor modification. The prepared mobile phase

was filtered using a vacuum filter system equipped with 0.45 μm filter and was delivered at a flow rate of 1.0 ml/min. The detection wavelength was set at 264 nm.

Preparation of Standard and Quality Control Samples Stock solution of strychnine and brucine were prepared in methanol at concentration of 1 mg/ml. Both stock solutions were diluted with methanol to get a combined standard working solution of 100 μg/ml for strychnine and 100 μg/ml for brucine. Then the combined standard working solution was further diluted with methanol to provide a series of standard solutions with desired concentrations. The I.S. was prepared in methanol at a concentration of 40 μg/ml. All the solutions were stored at -20℃ and were brought to room temperature before use. The calibration standards were prepared by spiking 0.5 ml blank tissue homogenates or plasma with appropriate amounts of working solutions to yield final concentrations in the range of 0.05–2 μg/ml for both strychnine and brucine. Combined quality control (QC) samples were prepared at low, medium and high concentration levels of 0.05, 0.3 and 2 μg/ml for both strychnine and brucine. The calibration standards and QC samples were performed using the procedure described below.

Sample Extraction Procedure The tissues were dissected and homogenized by the addition of physiological saline solution at the ratio of 1 : 2 (v/v). To avoid over-heating, tissues were homogenized in ice-water bath. The 500 μl tissue homogenate or plasma was spiked with 20 μl I.S. Then 50 μl aqueous ammonia was added and the samples were vortex-mixed for 1 min. Three ml chloroform was then added. The mixture was vortex-mixed for 3 min and subsequently centrifuged for 10 min at 2200$\times g$ at ambient temperature. The organic layer was transferred into a glass tube and the residue was re-extracted with 3 ml chloroform in a similar manner. The combined chloroform was evaporated at 50℃ under a gentle stream of nitrogen gas, until a completely dried residue was left over. The residue was reconstituted in 100 μl methanol and centrifuged for 5 min at 12000$\times g$. Finally, 20 μl of the supernatant were injected into HPLC system for analysis.

Assay Validation

Calibration Curve The calibration curves were constructed from the concentration of calibration standard *vs* the peak area ratio of calibration standards and the I.S. Each calibration curve consisted of at least six concentration levels. A weighting factor ($1/x^2$) was used to correct for bias in the calibration line. The calibration curve had to have a correlation coefficient (r^2) of 0.99 or better.

Accuracy and Precision The intra-day precision and accuracy were calculated by analyzing QC samples in six replicates in one day. The inter-day precision and accuracy were performed by replicate analysis of QC samples on four different days. Accuracy was defined as a percentage (RE%). It was calculated using the formula RE% = (E–T)/T*100%, in which E is the computed value and T is the true concentration. The criteria for acceptability of the data included accuracy within $\pm$15% deviation from the nominal values and precision within $\pm$15% relative standard deviation (R.S.D.).

Limit of Quantification The limit of quantification (LOQ) was investigated in a series of different diluted tissue standard samples and defined as the lowest concentration that produced a signal-to-noise (S/N) ratio of 10.

Extraction Recovery The extraction recoveries of strychnine and brucine were tested at three QC levels by comparing the peak areas from extracted tissues samples with those found by direct injection of standard solutions at the same concentration.

Stability The stabilities of strychnine and brucine in tissue samples were tested with QC samples. These QC samples were stored at room temperature for 12 h and then analyzed. The long-term storage stabilities in the treated samples at -20℃ were studied. The concentration after storage for a period of time was compared with the initial concentration as determined for freshly prepared samples. The freeze-thaw stability was determined after three freeze and thaw cycles. In each cycle, the QC samples were stored at -20℃ for 24 h and thawed unassisted at room temperature. The cycle was repeated twice and then the samples were analyzed after the third cycle.

Preparation of CAF CAF was prepared according to the previous paper with minor modification.[14] Slices of the processed Semen Strychni were ground into a fine powder and passed through a sieve (20 mesh). Each powder sample (100 g) was moisten with 10 ml 15% aqueous ammonia and kept for 2 h. The moistened powder was soaked in 300 ml chloroform and stirred for 24 h. The solution was filtered and the residue was re-extracted with chloroform in a

similar manner for three times. The combined solution was evaporated and re-soluted with a solution of 1 mol/l HCl. The resulted acidic solution was adjusted to pH 12.0 with 40% NaOH and extracted six times with chloroform (50 ml each). The combined chloroform solutions were gathered and evaporated to give a CAF. The obtained CAF was dissolved in pH 7.4 PBS. The percentages of strychnine and brucine in the resulted 0.5 mg/ml CAF solution were determined to be 42.70±2.55% and 28.13±1.05% (n =3) respectively, based on the HPLC method.[15]

Tissue Distribution Study After intravenous administration of 1 mg/kg CAF solution to rats by tail vein injection, the animals were sacrificed at predetermined time point. The tissues (brain, liver, kidney, lung, spleen, heart, stomach, small intestine) and blood samples were obtained at 2, 5, 15, 30, 60,

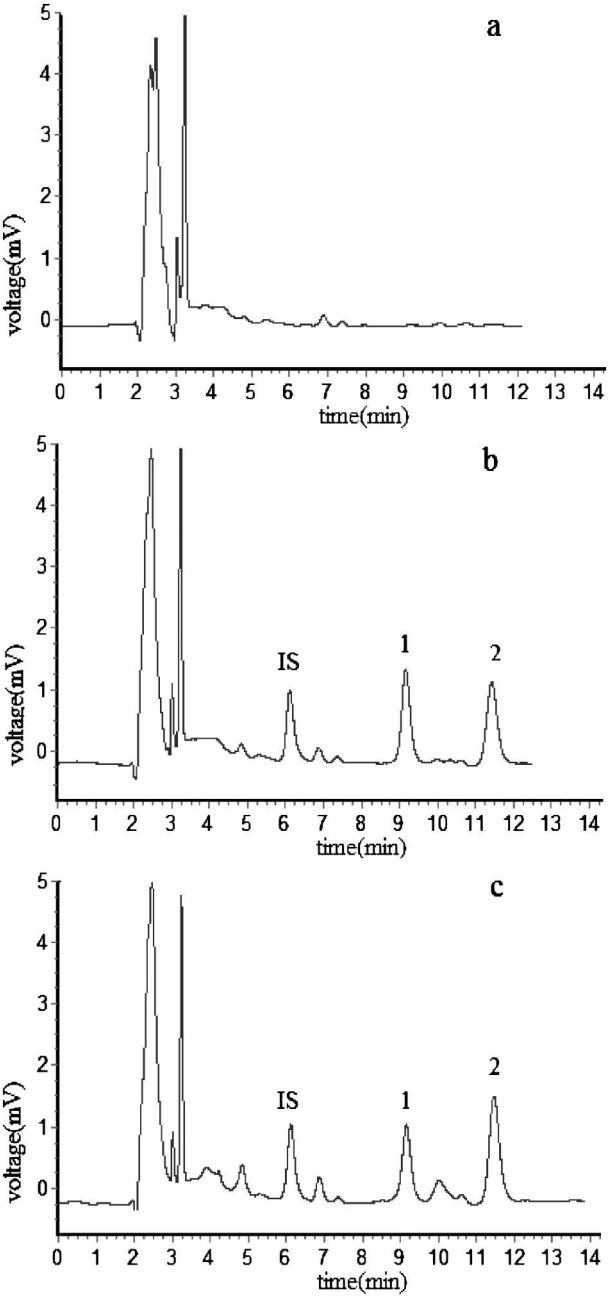

Fig. 2. HPLC Chromatograms of Plasma Samples

a: blank rat plasma, b: rat plasma spiked with 0.3 μg/ml brucine (1), strychnine (2) and huperzine A (I.S.), c: rat plasma 5 min after intravenous administration of 1 mg/kg CAF solution.

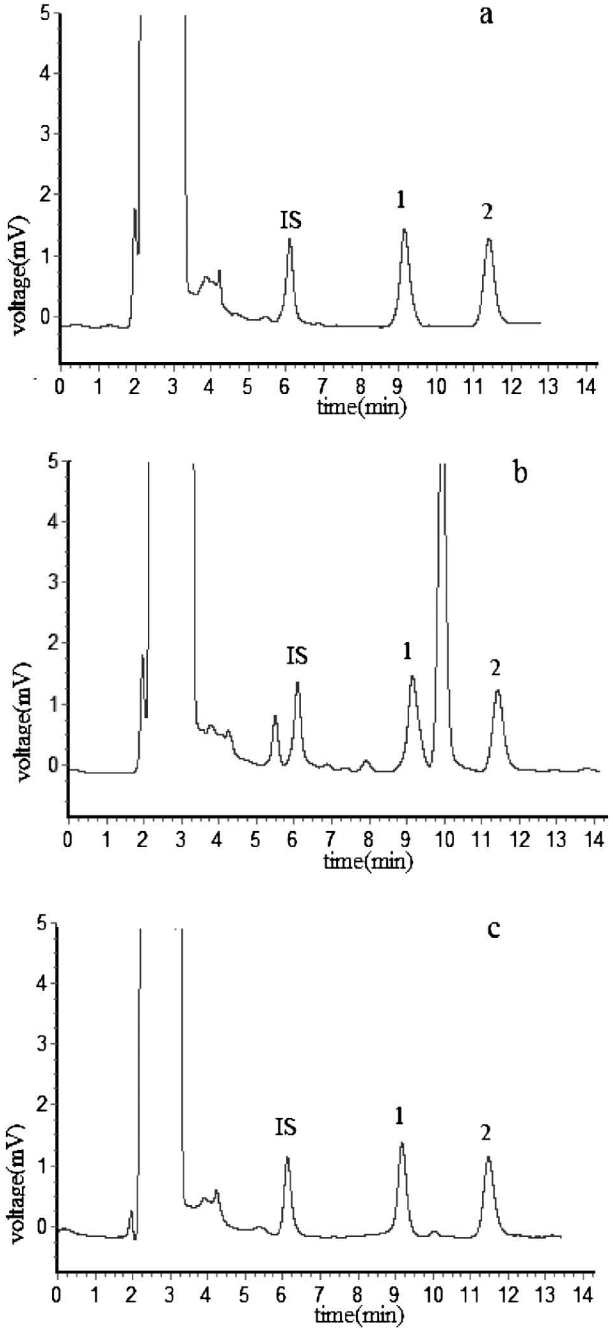

Fig. 3. HPLC Chromatograms of Spleen Samples

a: blank rat spleen, b: rat spleen spiked with 0.3 μg/ml brucine (1), strychnine (2) and huperzine A (I.S.), c: rat spleen 5 min after intravenous administration of 1 mg/kg CAF solution.

100 min, respectively. Tissue samples were weighed rapidly and put into physiological saline solution to remove the blood or content, blotted on filter paper, and then weighed for wet weight. Plasma samples were prepared from blood by centrifugation at 4000 $\times g$ for 5 min. The obtained tissue or plasma samples were stored at -20 ℃ until analysis.

RESULTS

Specificity Typical chromatograms obtained from plasma are shown in Fig. 2. Figure 2a was the chromatogram of organic extract of the blank plasma. Figure 2b represents the blank plasma sample spiked with strychnine and brucine (0.3 μg/ml). Figure 2c shows a plasma sample obtained at 5 min after a single i.v. dose of 1 mg/kg CAF. There are no interference peaks co-eluting with the compounds of interest. Separation of strychnine, brucine and I.S. in the rat tissue samples was achieved successfully and the retention times were approximately 9.2 min, 11.5 min and 6.1 min shown in Figs 2b and 2c, respectively. Chromatograms obtained from spleen are shown in Fig. 3.

Calibration Curves The calibration curves were constructed from the concentration of calibration standards (C) *vs* the peak area ratio of calibration standards and the I.S. (Y). The spiked standard samples were analyzed in three separate analytical runs. A weighting factor ($1/x^2$) was used to correct for bias in the calibration line. The calibration curve of assay of strychnine and brucine showed an acceptable linearity with a correlation coefficient of over 0.9910 using every control tissue homogenate in the concentration range from 0.05 to 2.0 μg/ml. The LOQs were

Table 1. Accuracy and Precision for Determination of Strychnine in Tissue Samples

Tissues	Nominal concentration (μg/ml)	Intra-day precision		Inter-day precision	
		Accuracy (RE%)	Precision (RSD%)	Accuracy (RE%)	Precision (RSD%)
Brain	0.05	1.3	2.8	0.5	8.8
	0.3	−1.8	0.8	−7.3	2.2
	2	−0.7	0.4	−9.4	4.9
Liver	0.05	−1.2	7.8	−2.0	8.5
	0.3	−6.8	0.5	6.2	3.3
	2	1.9	1.5	4.5	1.8
Kidney	0.05	4.4	4.0	9.0	3.0
	0.3	0.4	0.6	9.9	0.9
	2	8.6	0.7	7.4	2.2
Spleen	0.05	−3.8	9.7	−13.9	8.0
	0.3	−0.8	6.6	0.3	5.6
	2	3.3	2.1	9.0	3.6
Lung	0.05	−2.8	8.1	−3.0	10.5
	0.3	−2.2	1.6	−4.4	6.0
	2	1.6	0.4	−1.5	8.2
Heart	0.05	5.0	3.0	8.1	5.9
	0.3	4.7	3.3	13.9	4.4
	2	7.1	2.0	3.6	3.4
Stomach	0.05	−0.9	5.4	3.8	8.3
	0.3	5.0	2.8	8.6	3.0
	2	−2.4	0.8	8.8	0.5
Small intestine	0.05	2.9	2.5	11.0	5.6
	0.3	−3.0	3.0	−1.3	6.1
	2	0.0	1.4	6.7	4.7
Plasma	0.05	1.5	2.3	4.0	3.6
	0.3	0.4	0.7	6.8	3.8
	2	−2.8	1.1	9.3	1.2

Intra-day: $n=6$; Inter-day: $n=4$ days with 5 replicates per day.

determined in five replicates as a signal-to-noise ratio (S/N) of 10 and in the range of 0.039～0.050 μg/ml for different tissue or plasma samples.

Recovery and Precision Six replicates of blank homogenate supernatants of tissue, 500 μl spiked with low, medium and high concentration combined standard solutions, were treated and analyzed by HPLC as the described above. The method showed acceptable precision and accuracy. Tables 1 and 2 summarize the intra- and inter- precision and accuracy for strychnine and brucine in QC samples, respectively.

Extraction Recovery The extraction recoveries of strychnine and brucine were tested at three QC levels by comparing the peak areas from extracted tissues samples with those found by direct injection of standard solutions at the same concentration. Extraction recovery experiments were carried out to investigate extraction efficiency. The extraction recoveries varied from 71.6 to 98.8% (Table 3).

Stability The stabilities of strychnine and brucine were investigated under a variety of storage and process conditions. The analytes were found to be stable (RE within ±15%) after three cycles of freeze (−20℃) and thaw (room temperature) in rat tissues. The analyte was also shown to be stable in the reconstituted solution for 12 h at room temperature (RE within ±15%). No signs of degradation were found under the freeze condition (−20℃) for 7 days.

Tissue Distribution Study Tissue distribution of strychnine and brucine was determined in liver, kidney, lung, spleen, heart, stomach, small intestine, brain and plasma. The concentration-time profiles of strychnine and brucine in various tissues after in-

Table 2. Accuracy and Precision for Determination of Brucine in Tissue Samples

Tissues	Nominal concentration (μg/ml)	Intra-day precision		Inter-day precision	
		Accuracy (RE%)	Precision (RSD%)	Accuracy (RE%)	Precision (RSD%)
Brain	0.05	3.4	2.0	3.1	9.9
	0.3	−5.1	0.6	−7.9	1.4
	2	0.0	0.4	−9.1	1.6
Liver	0.05	−0.8	6.0	3.8	8.0
	0.3	−9.4	0.7	−4.7	1.4
	2	4.8	1.2	−1.8	1.7
Kidney	0.05	1.4	2.4	10.2	3.3
	0.3	2.4	1.7	1.7	1.3
	2	7.3	0.8	5.6	7.2
Spleen	0.05	−3.4	5.5	3.7	8.0
	0.3	0.2	5.8	4.4	5.6
	2	5.2	1.1	4.0	3.6
Lung	0.05	−4.7	8.4	−9.2	4.4
	0.3	−2.5	1.1	−7.4	6.0
	2	−0.8	0.4	−2.8	8.9
Heart	0.05	−9.6	5.4	3.4	8.1
	0.3	2.2	2.5	11.5	2.5
	2	4.7	1.8	2.8	4.4
Stomach	0.05	−0.6	1.5	−4.7	10.4
	0.3	3.9	0.9	3.1	5.1
	2	−0.2	0.4	1.3	1.4
Small intestine	0.05	1.3	1.8	3.9	5.6
	0.3	−1.2	2.2	1.9	6.1
	2	−0.4	1.6	2.6	4.7
Plasma	0.05	2.5	4.7	10.9	7.1
	0.3	4.0	1.1	−0.5	4.6
	2	3.1	1.2	1.7	2.9

Intra-day: $n=6$; Inter-day: $n=4$ days with 5 replicates per day.

Table 3. Extraction Recoveries (%) for Determination of Strychnine and Brucine in Tissue Samples (n=6)

Tissues	Analytes	Added concentration (μg/ml)		
		0.05	0.3	2
Brain	strychnine	84.4±4.3	79.5±10.2	79.4±8.0
	brucine	71.6±3.9	78.1±4.7	75.3±7.4
Liver	strychnine	82.0±5.3	83.2±1.2	87.0±3.1
	brucine	90.8±3.8	89.1±2.4	83.3±3.8
Kidney	strychnine	86.5±10.6	89.5±3.6	83.0±6.4
	brucine	88.1±3.8	80.8±2.7	82.2±7.0
Spleen	strychnine	91.8±6.4	91.8±3.1	76.7±2.3
	brucine	88.0±9.6	85.8±4.0	74.3±1.6
Lung	strychnine	95.5±5.6	84.9±7.4	89.5±2.2
	brucine	84.7±9.9	81.8±9.0	82.8±3.3
Heart	strychnine	82.6±2.1	86.8±4.9	92.1±4.8
	brucine	82.1±3.9	85.1±4.8	86.8±5.0
Stomach	strychnine	85.7±6.8	95.4±7.6	81.9±10.9
	brucine	86.3±3.8	86.6±8.7	78.8±7.9
Small intestine	strychnine	89.8±2.9	98.8±5.4	89.7±5.9
	brucine	88.8±7.8	94.3±3.2	87.5±6.2
Plasma	strychnine	86.0±8.6	90.6±1.4	90.9±2.3
	brucine	91.0±4.3	88.3±4.9	95.4±4.1

travenous administration of 1 mg/kg CAF to rats are shown in Fig. 4. In brain, the concentration of brucine is lower than LOD and cannot be quantified. The area under the concentration *versus* time curve up to the last quantifiable time point, AUC_{0-t} was obtained by the linear trapezoidal summation. The AUC_{0-t} of different tissues is shown in Fig. 5.

DISCUSSION

Seletction of I.S. To select a suitable internal standard, several components including huperzine A, ephedrine, aconitine, matrine and caffeine having structural or chemical similarity to the analytes, were considered. However, the resolutions between of endogenous substances in blank tissues and matrine or caffeine were unsatisfied under several chromatographic conditions. Aconitine and ephedrine were both found to be unstable in the process of sample preparation. Therefore, huperzine A, being chemical-

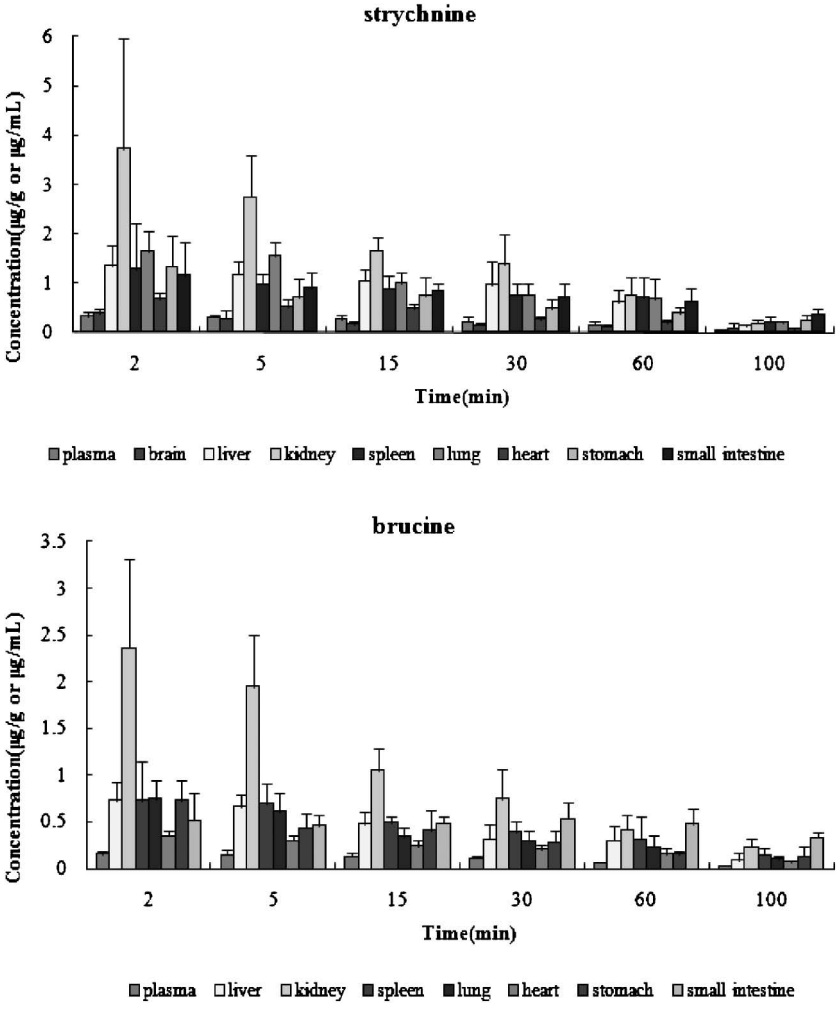

Fig. 4. Distribution of Strychnine and Brucine in Various Tissues after Intravenous Administration of 1 mg/kg CAF to Rats (n=6)

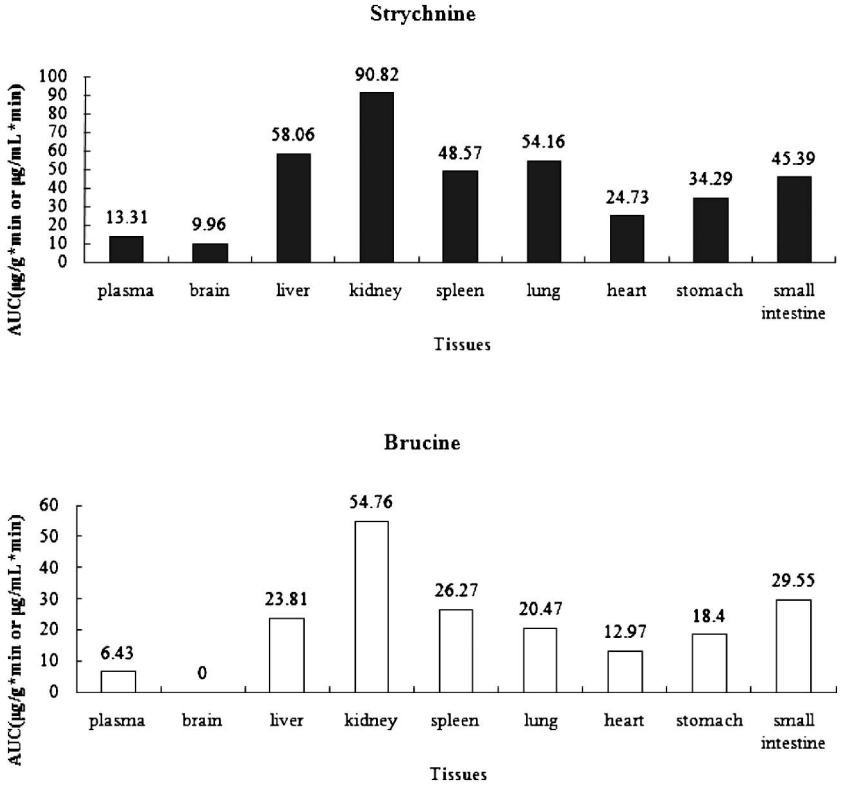

Fig. 5. AUC_{0-t} of Strychnine and Brucine in Various Tissues after Intravenous Administration of 1 mg/kg CAF to Rats ($n=6$)

ly similar to the analyte and no interference around in the whole process, finally, was chosen as the I.S. for the assay.

Sample Preparation As to liquid-liquid extraction step, ethyl acetate and ethyl ether were also tested as extraction solvents. At last, chloroform was used for the liquid-liquid extraction by virtue of its satisfactory recoveries. Due to the alkaline nitrogen atoms in the structures of strychnine and brucine, alkalization with aqueous ammonia before extraction could help improve extraction efficiency.

Tissue Distribution Study The main bioactive components of Semen Strychni are alkaloids. The most abundant alkaloids existing in the processed Semen Strychni are strychnine and brucine, which have been reported to possess analgesic, anti-inflammatroy and anti-tumor effects. Recently, strychnine and brucine were reported to possess anti-proliferative and cytotoxic activity against RPMI8226, the human multiple myeloma (MM) cell line.[16] Since MM is an incurable hematological malignancy, the investigation of strychnine and brucine has been gaining attention. However, the tissue distribution characterization of strychnine and brucine remains largely unknown.

After intravenous administration of CAF to rats, strychnine and brucine were widely distributed into tissues. Since strychnine and brucine in most tissues could not be detected at more than 100 min, there was no long-term accumulation in rat tissues. AUC of these two alkaloids in all tissues except brain was significantly higher than that of plasma. The high values were found in most abundant blood-supply tissues such as kidney, small intestine, liver, spleen and lung, which implied that the distribution of strychnine and brucine was depended on the blood flow or perfusion rate of the organ. The highest level was observed in kidney, which demonstrated that kidney might be the primary excretion organ of prototype strychnine and brucine. Moreover, high drug levels of strychnine and brucine have been reported to be found in urine samples. It was reported that the urine samples of a healthy female volunteer treated with a dose equivalent to 160 μg of strychnine and 150 μg brucine were analyzed.[8] The urine levels of strychnine and brucine could be detected even at 24 h after administration. In another report, biological fluids of a clinical strychnine poisoning case were collected and analyzed.[13] The concentrations of blood and urine were 0.35 and 15.3 μg/ml at the same time point, respectively. Furthermore, it was deduced that glucuronide conjugate

was the major metabolite of strychnine in human urine based on chromatographic and MS data.[17]

Inversely, the lowest AUC level was found in brain. Strychnine could be detected and quantified in brain, while brucine was even under the LOQ. The possible reason was related to the structure of brucine with methoxyl groups, which resulted in its higher polarity compared with strychnine. Hence it was more difficult for brucine to cross the blood-brain barrier and thus could not be quantified in brain.

As seen in Fig. 5., it could be deduced that the tissue distribution characteristics of strychnine and brucine were similar. The AUC percentages of certain tissue were not significantly different between strychnine and brucine. The content ratio of strychnine to brucine in administered CAF solution was about 1.5. However, the AUC ratios of strychnine to brucine were 1.63 in kidney, 2.23 in liver, 1.89 in spleen and 2.64 in lung, respectively. These values were all more than 1.5. Strychnine and brucine had been reported to possess high permeability and the dominant mechanism of the absorption was passive diffusion. The apparent permeability coefficients (P_{app}) for strychnine and brucine were $(3.11\pm0.17)\times10^{-5}$ and $(1.67\pm0.65)\times10^{-5}$ cm/s, respectively.[18] The higher permeability across biomembrane of strychnine might be related with higher tissue acceptability.

For tissue distribution study of a drug, the tissues are often selected as heart, liver, spleen, kidney and lung.[19] Brain is selected because the processed Semen Strychini possesses severe central nervous toxicity. According to the fact that Semen Strychini is applied for the treatment of gastric disease, the concentrations of strychnine and brucine are also determined in stomach and small intestine.

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