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Plasma pharmacokinetics and tissue distribution study of cajaninstilbene acid in rats by liquid chromatography with tandem mass spectrometry

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ABSTRACT

Cajaninstilbene acid (CSA; 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid) is a major active constituent of pigeonpea leaves, has been proven to be effective in clinical treatment of diabetes, hepatitis, measles and dysentery. A rapid and sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) method was developed and validated for the determination of CSA in rat plasma and various tissues (brain, heart, lung, liver, spleen, small intestine and kidney) of rat for the first time. Rat plasma and tissue distribution pre-treated by protein precipitation with acetoacetate was analyzed using LC–MS/MS with an electrospray ionization (ESI) interface, and isoliquiritigenin was used as an internal standard. Chromatographic separation was achieved on a HIQ Sil C_{18} column with the mobile phase of water and methanol (9:91, v/v) containing 0.1% formic acid and resulted in a total run time of 10 min. The isocratic elution mode pumped at a flow rate of 1.0 mL/min. The lower limit of quantification (LLOQ) which was 10 ng/mL. The calibration curve was linear from 10 to 6000 ng/mL ($R = 0.9967$) for plasma samples and 10 to 6000 ng/mL ($R \geq 0.9974$) for tissue homogenates. The intra- and inter-day assay of precision in plasma and tissues ranged from 0.6% to 6.1% and 1.5% to 6.6%, respectively, and the intra- and inter-day assay accuracy was 93.5–104.6% and 93.3–107.5%, respectively. Recoveries in plasma and tissues ranged from 95.0% to 106.8%. The method was successfully applied in pharmacokinetic and tissue distribution studies of CSA after oral administration to rats. The pharmacokinetics of CSA showed rapid absorption and elimination (T_{max}, 10.7 ± 0.31 min; $t_{1/2}$, 51.40 ± 6.54 min). After oral administration in rats, CSA was rapidly and widely distributed in tissues. High concentrations were found in liver and kidney indicating that CSA was possibly absorbed by liver and eliminated by kidney.

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1. Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the most precious hardy grain legume crops grown in many developing countries in semiarid tropics and subtropics. Nowadays, pigeonpea is used for the treatment of many diseases, such as diabetes, hepatitis, measles and dysentery, in India, Africa and South America [1,2]. In the folk medicine of China, pigeonpea leaves are used for treating certain symptoms, including bedsores, pain, infections, etc. [3,4]. In addition, pigeonpea is used in traditional Chinese medicine (TCM) for the therapy of ischemic necrosis of femoral head [5–7]. Pigeonpea leaves exhibit notable vulnerary, anti-inflammatory, anti-biotic, abirritative effects and inhibit capillar permeability and proliferation of *Leishmania* parasites [8–12]. The main chemical classes of bioactive compounds in pigeonpea leaves are stilbenes and flavonoids [13].

Stilbenes exist in many plant species [14–20]. These compounds have attracted much attention for their biological effects [21–25]. Initial investigations of active constituents of pigeonpea leaves showed that cajaninstilbene acid (CSA, chemical name: 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid) is present in low amounts in plants [26]. This stilbene exerts some remarkable pharmacological activities. For example, it acts as an anti-hypoglycemic agent and reduces blood triglyceride levels [27,28]. It has also attracted much attention for its significant anti-inflammatory, impermeability and analgesic effects [29]. Furthermore, it is also considered to be highly effective in treating postmenopausal osteoporosis [8]. In our previous studies, CSA had shown significant high

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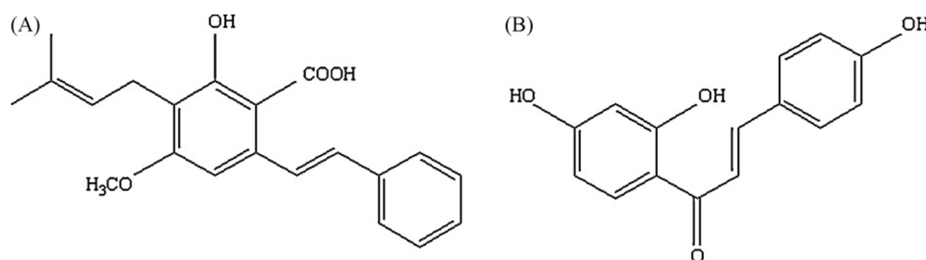

Fig. 1. Chemical structures of (A) cajaninstilbene acid (CSA) and (B) isoliquiritigenin (ISL).

anti-oxidant activity [30]. In general, CSA has a great potential as novel food supplement or phytotherapeutic drug for human health in the future. The chemical structures of CSA and the chemically related compound isoliquiritigenin, which served as internal standard, are shown in Fig. 1.

To our knowledge, CSA identification and quantification methods have sparsely been reported. In an earlier study, we had used high performance liquid chromatography (HPLC) was used to determine CSA content in pigeonpea extracts [31]. However, this method cannot be used to study pharmacokinetics of CSA, due to the fact that the blood sample cannot exceed 10% of the total blood volume of an animal, if multiple time points need to be measured. On the other hand, the CSA levels that can be reached in blood plasma are very low. Therefore, a sensitive and precise method to determine CSA levels in small sample volumes is required.

In the present study, a simple and highly sensitive LC–MS/MS method was developed for the first time to determine CSA levels in rat plasma and tissue samples. The conditions for sample preparation and analysis were optimized, and the method was validated in terms of selectivity, sensitivity, accuracy, precision and recovery. The newly developed method was applied in pharmacokinetic and tissue distribution studies following intragastric administration of CSA (2 mg/kg) in rats.

2. Experimental

2.1. Chemicals and reagents

Cajaninstilbene acid (CSA, purity 98%, HPLC grade) was separated and purified in laboratory. The structure was confirmed by comparing the IR, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and MS data with reported data [31]. Isoliquiritigenin (ISL, purity ≥98%) was purchased from Sigma–Aldrich, Inc. (Taufkirchen, Germany). Other chemicals used in the present investigation were nitrogen gas (purity 99.99%, Liming Gas Corp., China), methanol, ethyl acetate (J&K Chemical Ltd., China), and formic acid (96%, DIMA Technology Inc., USA). Deionized water used for all experiments was of Milli-Q quality (Millipore Corp., Bedford, MA, USA).

2.2. Animals

Female Kunming rats, weighing 220–250 g, were obtained from Harbin Veterinary Research Institute Animal Co., Ltd. (Harbin, Heilongjiang, China), After a single dose of CSA (2 mg/kg) to healthy rats (n = 6), blood samples were collected using heparinized tubes at 0, 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, and 8 h post-dose, then immediately centrifuged at 3000 × g for 15 min, and stored at −70 °C until analysis. The protocol of the study was approved by the Ethics Committee of the Chengdu University of Traditional Chinese Medicine. The investigation was conducted in accordance with the ethical principles of animal use and care.

To study the tissue distribution of CSA, four groups of overnight fasted rats (n = 5 per group) were orally administered 2 mg/kg CSA per animal by gastric intubation. The rats were sacrificed by decapitation at 0, 10, 30, and 60 min post-dosing, respectively. The tissues or organs, including brain, heart, lung, liver, spleen, small intestine and kidney, were excised, trimmed of extraneous fat, residual muscle and connective tissue, thoroughly rinsed with physiological saline solution, and then blotted dry.

2.3. Instrumentations and chromatographic conditions

An Agilent 1100 series HPLC system (Agilent Technologies, San Jose, CA, USA) for chromatographic analysis consisted of a G1312A binary pump, a 7725i manual injector, and a G1379A degasser. The column effluent was monitored by an API3000 triple-stage quadrupole mass spectrometer (Applied Biosystems, Concord, Canada) equipped with an electrospray ionization (ESI) source. Chromatographic separations were performed on a HIQ Sil C_{18} column (4.6 mm × 250 mm, KYA TEACH, made in Japan) maintained at 20 °C. The mobile phase consisted of water and methanol (9:91, v/v) containing 0.1% formic acid. The flow rate was 1.0 mL/min, and the sample injection volume was 10 μL. The ion spray voltage was set at −4500 V. Compound parameters viz., declustering potential (DP), collision energy (CE), entrance potential (EP) and collision exit potential (CXP) were −60, −25, −10, −5 V for CSA and −80, −35, −10, −5 V for ISL, respectively. The mass spectrometer was operated in ESI negative ion mode, and the detection of the ions was performed in the multiple reaction monitoring (MRM) mode, monitoring the transition of m/z 337.1 precursor ion $[M-H]^-$ to the m/z 293.0 product ion for CSA, and m/z 255.1 precursor ion $[M-H]^-$ to the m/z 119.3 product ion for ISL.

2.4. Standard and quality control sample preparation

A stock solution of CSA was prepared in methanol at a final concentration of 100 μg/mL. All solutions were stored at 4 °C. Calibration standards were prepared by spiking working standard solutions and the ISL (50 μL, 400 ng/mL in methanol) into 100 μL of blank mouse plasma and different tissue homogenates of untreated rat. The final concentrations of standard curve samples were 10, 20, 50, 100, 200 500, 1000, 3000 and 6000 ng/mL. Quality control (QC) samples at four concentrations of 10, 100, 1000 and 4000 ng/mL for plasma and three concentrations of 10, 100 and 1000 ng/mL for tissue analyte samples (the three concentrations of small intestine were 10, 500 and 5000 ng/mL) were prepared separately. Standard calibration samples and QC samples were stored at −20 °C until analysis.

2.5. Sample preparation

The ISL solution (20 μL, 400 ng/mL in methanol) was added to 100 μL of rat plasma sample. The mixture was then precipitated with 500 μL ethyl acetate. After vortexing for 2 min, the samples were centrifuged at 13,200 × g for 10 min. The supernatant fluid was transferred to a glass insert and evaporated to dryness under

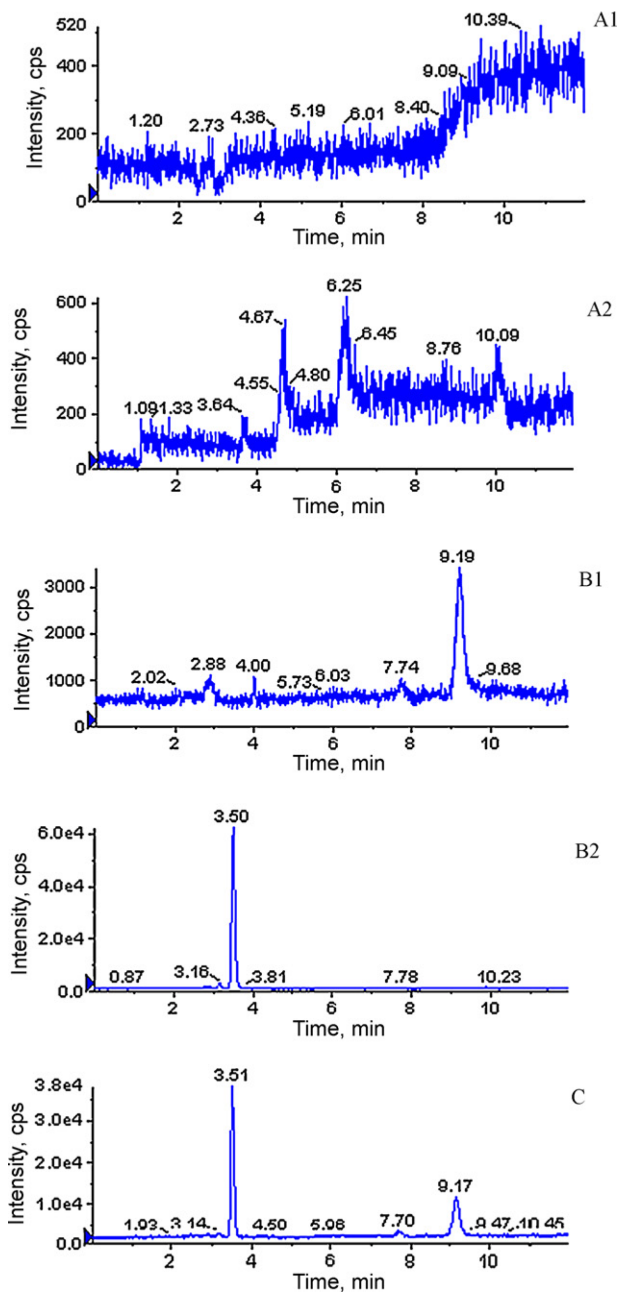

Fig. 2. Representative chromatograms of (A1) CSA and (A2) ISL obtained by extraction of (A) blank rat plasma, (B) plasma spiked with CSA (10 ng/mL, LLOQ, B1) and ISL (400 ng/mL, B2), (C) plasma obtained 15 min after intragastric administration of CSA (2 mg/kg) to rats.

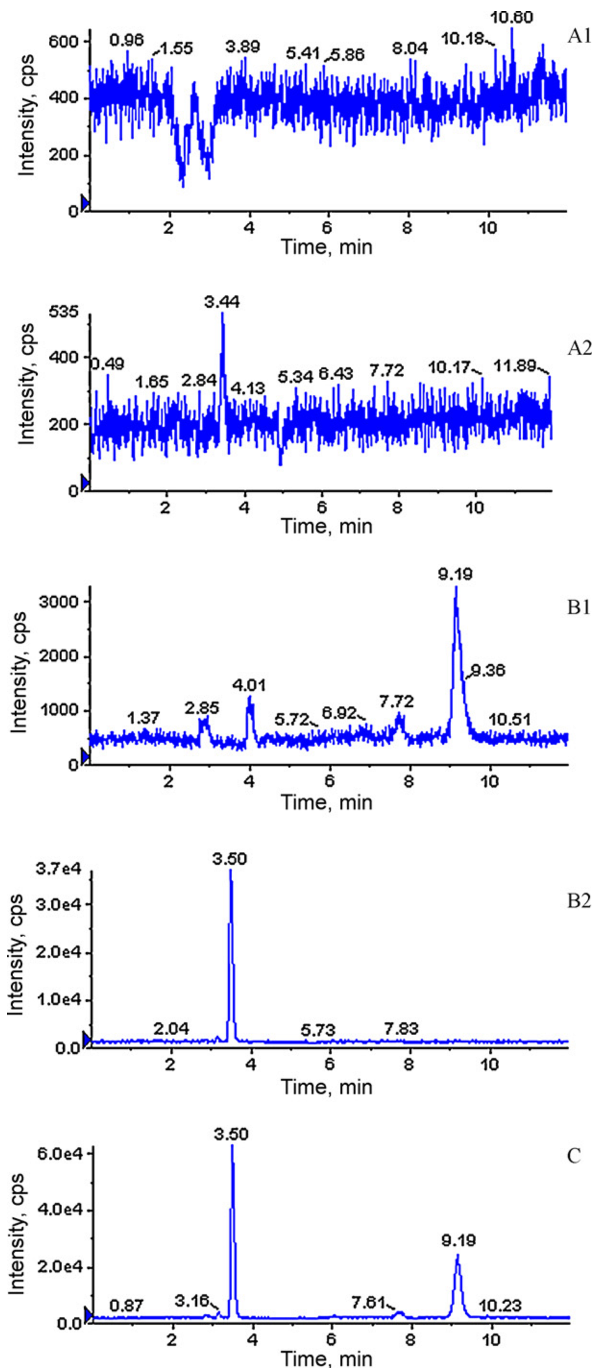

Fig. 3. Representative chromatograms of (A1) CSA and (A2) ISL obtained by extraction of (A) blank rat liver homogenates, (B) Liver homogenates spiked with CSA (10 ng/mL, LLOQ, B1) and ISL (400 ng/mL, B2), (C) Liver homogenates at 15 min after intragastric administration of CSA (2 mg/kg) to rats.

vacuum using a speedVac concentrator for a quick speed. The residue was reconstituted in 200 μL of the mobile phase, and 10 μL was injected on the column.

To study tissue distribution, small slices of tissues (300 mg) were individually homogenized with ethyl acetate (4.0 mL), vortexed and centrifuged at 13,200 × *g* for 15 min at 4 °C. The supernatant was separated and evaporated under N_2 gas, and the residue was reconstituted in 200 μL methanol before HPLC analysis. Protein concentrations in tissue samples were higher. Therefore, methanol was used as an alternative, in order to thoroughly precipitate proteins for subsequent analyses with high accuracy and precision. The internal standard (20 μL, 400 ng/mL in methanol) was added in tissue analyte samples before homogenization.

2.6. Method validation

Method validation assays were carried out according to the currently accepted U.S. Food and Drug Administration (FDA) bioanalytical method validation guidance [32].

2.6.1. Specificity

Blank plasma control samples and CSA-containing plasma samples after single oral administration from six different rats were analyzed. For tissue distribution, blank liver homogenates control samples and CSA-containing liver homogenates sam-

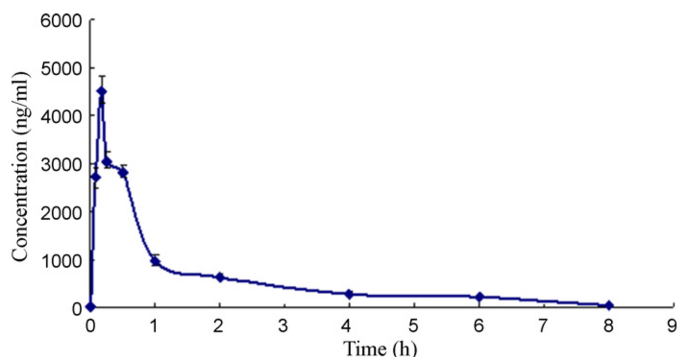

Fig. 4. Mean plasma concentration–time curve of CSA in rats ($n = 5$) obtained after intragastric administration of CSA (2 mg/kg).

ples after single oral administration from six different rats were analyzed. Their chromatograms were examined for endogenous compounds, which might possibly interfere with and disturb CSA quantification. The apparent responses at the retention times of CSA and ISL were compared with the response at the limit of quantification.

2.6.2. Calibration curve

Calibration curves were calculated based on the relationship between the ratio of the peak area of CSA to that of the internal standard and the theoretical concentration of analyte. Calibration standards obtained for plotting the calibration curve were 10, 20, 50, 100, 200 500, 1000, 3000 and 6000 ng/mL. The results were fitted to linear regression analysis using $1/x^2$ as weighting factor. Quality control and stability samples were calculated from the resulting area ratios of CSA to internal standard and the regression equation of the calibration curve.

2.6.3. Accuracy and precision

Intra-day accuracy and precision were evaluated by analysis of the four QC samples with five determinations per concentration at the same day. The inter-day accuracy and precision were measured over six days. Different concentrations were analyzed to cover the entire range of the calibration curve. The criteria for acceptability of the data included accuracy of ±15% standard deviation (S.D.) from the nominal values and a precision of ±15% relative standard deviation (R.S.D.), except for LLOQ, where it should not exceed ±20% of accuracy as well as precision.

2.6.4. Recovery

The extraction recoveries of CSA were determined at low, medium and high concentrations. Recoveries were calculated by comparing the analyte/internal standard peak area ratios ($R1$) obtained from extracted plasma samples and tissues homogenates with those ($R2$) from the standard solutions at the same concentration.

2.6.5. Stability

Freeze and thaw stability. The QC samples at three different concentrations were stored at −20 °C for 24 h and thawed at room temperature. When completely thawed, the samples were refrozen for 12–24 h under the same conditions. After three cycles, the percent loss of the analyte was determined by comparing the concentrations with those obtained before freezing.

Short-term temperature stability. The QC samples at different concentrations were thawed at room temperature, kept at this temperature from 4 to 24 h, and analyzed.

Long-term stability. QC plasma and tissue distribution samples at different concentration levels kept at low temperature (−20 °C) were studied for a period of 2 weeks.

Post-preparative stability. The autosampler stability was conducted by reanalyzing extracted QC samples kept under autosampler conditions (4 °C) for 12 h.

2.7. Application to pharmacokinetic study

Pharmacokinetic parameters were calculated using the Bioavailability Program Package (BAPP, Version 2.0, Center of

Table 1
Precision, accuracy and recovery of CSA in plasma samples and tissue homogenates of rats ($n = 5$).

Biosamples	Concentration (ng/mL)	Intra-day ($n = 5$)		Inter-day ($n = 5$)		Recovery	
		Accuracy (mean %)	Precision (R.S.D., %)	Accuracy (mean %)	Precision (R.S.D., %)	Mean ± S.D.%	R.S.D. (%)
Plasma	10	103.4	3.7	107.5	6.6	103.4 ± 3.6	4.3
	100	102.3	3.8	105.4	3.8	102.7 ± 3.0	3.6
	1000	99.2	1.8	102.0	2.2	99.1 ± 1.9	2.4
	4000	96.5	3.5	96.3	2.8	104.6 ± 5.6	4.7
Heart	10	94.6	5.3	95.4	4.3	96.2 ± 4.0	4.8
	100	95.7	3.6	95.6	4.4	97.0 ± 3.5	4.0
	1000	98.6	2.1	94.3	5.0	96.8 ± 3.6	3.9
Liver	10	104.6	3.5	106.4	5.3	103.6 ± 3.9	4.1
	100	98.6	1.2	96.8	3.6	102.3 ± 2.0	1.5
	1000	97.6	2.3	96.0	3.5	106.8 ± 7.1	7.9
Spleen	10	93.5	6.1	93.3	5.8	102.6 ± 3.0	2.8
	100	94.3	5.9	95.8	4.6	98.6 ± 1.8	2.0
	1000	97.3	3.7	95.7	4.5	97.6 ± 2.5	2.7
Lung	10	103.6	3.6	103.5	4.3	104.8 ± 5.3	5.1
	100	102.9	2.5	104.6	4.1	103.2 ± 3.8	3.8
	1000	97.8	3.1	93.8	5.4	100.9 ± 1.0	1.2
Kidney	10	99.5	0.6	97.5	3.0	96.3 ± 4.1	4.5
	100	98.6	1.1	96.9	2.9	102.6 ± 3.0	3.1
	1000	98.0	1.9	95.8	4.7	97.6 ± 2.3	3.3
Intestine	10	94.6	5.3	95.2	4.3	95.6 ± 3.8	4.0
	500	95.6	3.2	97.5	1.9	96.8 ± 3.0	2.8
	5000	94.8	5.0	98.3	1.5	95.0 ± 4.8	5.0
ISL	400	99.1	0.6	98.5	1.3	95.6 ± 5.2	5.9

Table 2
Stability of CSA in plasma samples and tissue homogenates of rats ($n = 5$).

Biosamples	Concentration (ng/mL)	Freeze and thaw stability Accuracy (mean ± S.D., %)	Short-term stability Accuracy (mean ± S.D., %)	Long-term stability Accuracy (mean ± S.D., %)	Post-preparative stability Accuracy (mean ± S.D., %)
Plasma	10	106.2 ± 6.7	102.7 ± 3.0	101.4 ± 1.2	98.4 ± 2.7
	100	100.3 ± 0.7	104.2 ± 4.3	99.1 ± 1.4	102.4 ± 2.7
	1000	105.4 ± 4.7	103.2 ± 3.3	100.7 ± 1.1	102.4 ± 2.9
	4000	103.3 ± 2.1	98.6 ± 2.3	104.9 ± 4.6	97.3 ± 1.6
Heart	10	95.6 ± 5.9	97.1 ± 2.0	93.7 ± 5.5	95.3 ± 3.6
	100	90.1 ± 3.6	98.3 ± 2.8	97.5 ± 5.2	98.6 ± 2.0
	1000	92.0 ± 6.6	98.9 ± 3.3	103.5 ± 5.6	105.3 ± 6.2
Liver	10	95.5 ± 6.2	93.6 ± 6.3	95.3 ± 6.7	98.3 ± 3.9
	100	103.6 ± 5.4	93.1 ± 2.6	95.1 ± 5.2	96.5 ± 4.3
	1000	94.1 ± 6.8	94.4 ± 4.7	94.7 ± 4.9	94.3 ± 3.2
Spleen	10	85.19 ± 6.2	90.79 ± 4.8	86.48 ± 5.9	88.17 ± 3.2
	100	88.89 ± 5.6	86.85 ± 3.4	86.24 ± 6.2	85.91 ± 4.3
	1000	91.17 ± 5.3	86.84 ± 6.1	85.75 ± 4.8	86.44 ± 5.5
Lung	10	87.04 ± 3.7	88.6 ± 6.3	91.3 ± 5.2	86.3 ± 5.3
	100	89.82 ± 3.1	85.45 ± 4.0	89.6 ± 3.2	94.2 ± 6.3
	1000	86.56 ± 4.8	85.96 ± 3.1	93.7 ± 4.5	88.6 ± 5.2
Kidney	10	100.8 ± 2.8	94.5 ± 5.7	93.4 ± 4.3	97.3 ± 3.4
	100	100.6 ± 3.9	97.7 ± 5.4	97.8 ± 6.6	101.0 ± 3.8
	1000	105.7 ± 4.5	107.9 ± 5.3	95.5 ± 6.5	96.2 ± 3.2
Intestine	10	96.9 ± 0.3	90.9 ± 5.1	93.5 ± 4.6	95.2 ± 5.0
	500	101 ± 2.4	97.0 ± 3.8	97.2 ± 3.4	96.7 ± 7.5
	5000	106 ± 4.2	93.2 ± 1.5	92.71 ± 4.2	97.27 ± 0.5

Drug Metabolism and Pharmacokinetics, China Pharmaceutical University). C_{max} was the observed maximum concentration, and the T_{max} was the time taken to reach the maximum drug concentration. The area under the plasma CSA concentration *versus* time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the linear trapezoidal rule. Mean residence time (MRT) was calculated as AUMC/AUC. Terminal half-life ($t_{1/2}$) was calculated as 0.693× MRT.

3. Results and discussion

3.1. Chromatographic method

The negative mass spectra of CSA and ISL are shown in Fig. 2. In the full scan mass spectrum, the most abundant ions for CSA and ISL were m/z 337.1 precursor ion $[M-H]^-$ to the m/z 293.0 product ion and m/z 255.1 precursor ion $[M-H]^-$ to the m/z 119.3 product ion, respectively. The two compounds were subjected to separation by reverse-phase HPLC on an HIQ Sil C_{18} column using water and methanol (9:91, v/v) containing 0.1% formic acid as mobile phase at 20 °C. The ratio of peak area between CSA and ISL were optimized, in order to achieve a sufficient accuracy. The addition of 0.1% formic acid to the mobile phase increased the accuracy of the analytes. Ion chromatograms of CSA and ISL obtained by protein precipitation from rat plasma and liver homogenates are shown in Figs. 3 and 4, respectively. Both CSA and ISL were eluted within 10 min with retention times of approximately 9.19 and 3.50 min, respectively. The retention times were short and suitable for high throughput sample determination in pharmacokinetic studies.

3.2. Method validation

3.2.1. Specificity

Specificity was assessed by analyzing blank plasma and CSA in rat plasma after single oral administration from six different rats. For tissue distribution, blank liver homogenates control samples and CSA-containing liver homogenates samples after single oral administration from six different rats were analyzed. CSA and ISL eluted at approximately 9.19 and 3.50 min, respectively. There was no endogenous interference from plasma and tissue distribution at any time point in the chromatogram.

3.2.2. Calibration curve

The standard curves of the peak area (Y) to the concentration (C) were constructed using $1/x^2$ weighted linear least-squares regression model. The results are listed in Table 1. The calibration curves were prepared daily and showed good linearity in the range 10–6000 ng/mL for CSA. The calibration curves for all matrices showed good linearity ($R > 0.9967$) over the concentration ranges tested. The lowest concentration of 10 ng/mL with R.S.D. < 20% was taken as LLOQ.

3.2.3. Accuracy and precision

The results of accuracy and precision measurements assessed by analyzing quality control samples at the three concentrations are presented in Table 2. For plasma, the intra-day accuracy ranged from 99.2% to 103.4%, and the inter-day accuracy ranged from 102.0% to 107.5%. The mean intra- and inter-day precision was between 1.8% and 3.8% and 2.2–6.6%, respectively. For tissue homogenates, the intra-day accuracy ranged from 93.5% to 104.6%,

Table 3
Concentration of CSA found in plasma samples at different time points.

Time (h)	0	0.083	0.167	0.25	0.5	1	2	4	6	8
Concentration (ng/mL)	0	2719.22	4510.10	3042.10	2810.24	956.86	629.38	271.78	213.60	34.05

Table 4
Relevant pharmacokinetic parameters of CSA in rats ($n=5$) obtained after intragastric administration of CSA (2 mg/kg).

Parameter	Unit	Administration
$AUC_{0\rightarrow t}$	ng min/mL	343,372.89 ± 2546.66
MRT	min	74.17 ± 16.36
C_{max}	ng/mL	4500 ± 345.83
T_{max}	min	10.7 ± 0.31
$t_{1/2}$	min	51.40 ± 6.54

and the inter-day accuracy ranged from 93.3% to 106.4%. The mean intra- and inter-day precision was in the ranges of 0.6–6.1% and 1.5–5.8%, respectively.

3.2.4. Recovery

Recoveries of CSA were measured by comparing the analyte/internal standard peak area ratios ($R1$) obtained from extracted plasma and tissue distribution samples with those ($R2$) from the standard solutions at the same concentrations. The mean recoveries ($n=5$) for CSA (10, 100 and 1000 ng/mL) were 103.4 ± 3.6%, 102.7 ± 3.0% and 99.1 ± 1.9%, the mean recoveries in all tissue samples were above 95.0 ± 4.8%, while the recovery of the internal standard determined at 400 ng/mL was 95.6 ± 5.2% (Table 2).

3.2.5. Stability

The results of short-term stability, freeze/thaw stability, autosampler stability, and long-term stability are shown in Table 3. Maximum deviations of 6.7% in plasma, and 7.5% in tissue homogenates were observed. The results were found to be within the assay variability limits during the entire process.

3.3. Application to pharmacokinetic studies

Each rat was administered an oral dose of 2 mg/kg CSA. Blood samples were collected at 0, 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, and 8 h. The samples were centrifuged and the separated plasma samples were frozen at −20 °C. A plot of the plasma concentration–time profile of CSA in rats is shown in Fig. 4. The pharmacokinetic parameters of CSA are listed in Table 4. As for intragastric administration, the terminal half-life ($t_{1/2}$) was 51.40 ± 6.54 min. The area under the plasma concentration curve ($AUC_{0\rightarrow\infty}$) of CSA after intragastric administration was 343,372.89 ± 2546.66 ng min/mL. Other pharmacokinetic parameters of this study are shown in Table 4.

3.4. Tissue distribution studies

Tissue distribution of CSA was investigated following a single oral dose of 2 mg/kg CSA to rats. The results (Table 5) indicated that the CSA underwent a rapid and wide distribution in tissues and organs throughout the whole body within the time course examined. Following 10 min of CSA administration, most of the analyzed tissues contained a significant amount of CSA. Except brain, CSA showed substantial disposition in heart, lungs, liver, spleen, small intestine and kidneys. The highest levels were detected in small intestine, followed by liver and kidneys. In terms of compound clearance, the liver initially absorbed much more CSA than the kidneys did. The results from the current study indicate that after oral administration CSA rapidly distributed to various organs. The highest concentrations were found in small intestine may be attributable mainly attributed to the residual drug content. The high liver concentrations indicate that CSA was possibly absorpted in liver. On the contrary, increasing concentrations of CSA in kidneys over time indicate that renal excretion might be a major elimination route for CSA. Meanwhile, CSA was not found in brain, suggesting that CSA did not efficiently cross the blood–brain barrier.

Table 5
Concentration of CSA (ng/mg) in rats ($n=5$) 10, 30 and 60 min after intragastric administration of CSA (2 mg/kg).

Tissue	10 min	30 min	60 min
Heart	0.29	0.41	0.16
Liver	0.84	3.64	1.64
Spleen	0.20	0.85	0.38
Lung	0.16	0.66	0.46
Kidney	0.73	0.77	0.83
Brain	n.d.	n.d.	n.d.
Intestine	18.88	8.75	5.86

n.d., not detectable.

4. Conclusion

For the first time, a highly sensitive and specific method for the determination of CSA in rats was developed using high-performance liquid chromatographic separation with tandem mass spectrometric detection. The LC–MS/MS method was characterized by rapid separation and specific identification of CSA from rat plasma and different tissues (brain, heart, lung, liver, spleen, small intestine and kidney). In the present study, the T_{max} of CSA in plasma was <15 min indicated that CSA was rapidly absorpted. Half-life of CSA in plasma was <1 h indicated that CSA was rapidly eliminated. In tissue distribution studies, we found that CSA was probably absorpted in liver and eliminated through kidneys. Furthermore, CSA did not efficiently cross the blood–brain barrier.

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