An LC/MS/MS method for simultaneous quantitation of two homoisoflavones: Protosappanin B and brazilin with hypoglycemic activity in rat plasma and its application to a comparative pharmacokinetic study in normal and streptozotocin-treated rats

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A B S T R A C T
Ethnopharmacological relevance: The heartwood of Caesalpinia sappan L. (Leguminosae), a widely used Chinese medicine in folk, has been used for the treatment of traumatic injury, stasis pain, amenorrhea, dysmenorrhea, as well as stabbing pain in the chest, abdomen and so on. Protosappanin B and brazilin, as the major bioactive homoisoflavones of Sappan Lignum, are used as the marker components for the quality control of the herb in China Pharmacopoeia.

Aim of the study: To establish a sensitive LC/MS/MS method for investigating the pharmacokinetic properties of protosappanin B and brazilin in rats after oral administration of Sappan Lignum extract, and compare their pharmacokinetics difference between normal and streptozotocin-treated rats.

Material and methods: A rapid, selective and sensitive LC/MS/MS method was developed and validated for the simultaneous quantification of protosappanin B and brazilin in rat plasma. Normal and streptozotocin-treated rats were orally administered with the Sappan Lignum extract at the same dose of 2.83 g extract/kg body weight (equivalent to 35.56 mg/kg of protosappanin B and 52.25 mg/kg of brazilin), respectively.

Results: After oral administration of Sappan Lignum extract, a remarkable increase (p < 0.05) in the value of AUC 0–24 h, C max and T 1/2 associated with protosappanin B and brazilin was observed in the streptozotocin-treated group. Compared with the normal rats, elimination of both compounds in the streptozotocin-treated rats was slower.

Conclusion: The established method was successfully applied to compare the pharmacokinetic behaviors of protosappanin B and brazilin in rat plasma after oral administration of Sappan Lignum extract between normal and streptozotocin-treated groups; the results might suggest the accumulation of both compounds in diabetic pathologic states and the adverse reaction should be considered when it was used.

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1. Introduction

Sappan Lignum has a long history of use in Chinese herbal folk medicine (Editorial Committee of Zhonghua Bencao National Traditional Chinese Herb Administration, 1999; Zhonghua Bencao). It is prepared from the dried heartwood of Caesalpinia sappan L. (Leguminosae), and has been firstly recorded in the China Pharmacopoeia since 1963 (Editorial Committee of Chinese Pharmacopoeia, 1963). This medicine has been used for the treatment of traumatic injury, stasis pain, amenorrhea, dysmenorrhea, as well as stabbing pain in the chest, abdomen and so on (Jiangsu New Medical College, 1986). Modern pharmacological studies showed that the herb has a wide range of pharmacological activities, including antibacterial (Xu and Lee, 2004; Batubara et al., 2010), antimicrobial (Kim et al., 2004; Srinivasan et al., 2012), antioxidant (Wetwitayaklung et al., 2005; Saenjum et al., 2010), anticarcinogenic (Benabdajji et al., 2004; Ueda et al., 2002), and anti-inflammatory activities (Jeong et al., 2008; Wu et al., 2011). Recent studies on endocrine disorders have shown that the extract of Sappan Lignum can significantly reduce the blood glucose level of rats with diabetic renal disease, alleviate renal damage, and...
improve renal function (Hu and Li, 2011; Zhang et al., 2011). Moreover, brazilin, as one bioactive marker compound in the herb, also exhibited potent hypoglycemic action in streptozotocin-induced diabetic rats by improving glucose metabolism and stimulating glucose transport in 3T3-L1 cells (Kim et al., 1995). Therefore it is significant to measure the concentration of main effective ingredients in Sappan Lignum in

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\text{protosappanin B} \quad m/z = 303
\]

\[
\text{brazilin} \quad m/z = 285
\]

\[
\text{isovitexin} \quad m/z = 431
\]

Fig. 1. Proposed MS/MS fragmentation pathways of protosappanin B (a), brazilin (b) and isovitexin (internal standard, c).
biological matrix for their pharmacokinetic study. Protosappannin B and brazilin (Fig. 1) are two major bioactive homoisoavones isolated from Sappan Lignum, which are used as marker components for the quality control of the herb in the current version of China Pharmacopoeia (Editorial Committee of Chinese Pharmacopoeia, 2010).

Few high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection methods for the simultaneous determination of protosappannin B and brazilin in herbal medicine had been reported in the literature (Zhao et al., 2010; Chen et al., 2010, 2012). However, because of the poor selectivity and long run time, the HPLC methods were not suitable for the simultaneous determination of the two compounds in vivo. In regard to the quantitative study of protosappannin B and brazilin in biological samples, a paper based on liquid chromatography tandem mass spectrometry (LC/MS/MS) method has been recently published online (Deng et al., 2013), but the method is only applicable for assaying braziliin in rat plasma samples, and is not suitable for the simultaneous determination of the both compounds in vivo. Thus far, there is no report of the simultaneous determination of both compounds in biological samples using LC/MS. In the present study, a fully validated LC/MS/MS method was established for the simultaneous quantification of both isoavones (protosappannin B and brazilin) in rat plasma following oral administration of Sappan Lignum extract, and the possible pharmacokinetic differences in normal and streptozotocin-treated rats were investigated.

2. Materials and methods

2.1. Chemicals and reagents

Protosappannin B, brazilin and isovitexin (internal standard, IS) were purchased from Tauto Biotech Co., Ltd. (Shanghai, China). Water used for the LC/MS/MS analysis was prepared using a Milli-Q purification system procured from Millipore (Bedford, MA, USA). HPLC grade methanol was purchased from Honeywell Burdick & Jackson (Ulsan, Korea). Ammonium acetate and acetic acid of HPLC grade were supplied by Tedia (Fairfield, OH, USA). Streptozotocin was purchased from Sigma-Aldrich Corporation (St Louis, MO).

2.2. Animals

Twelve male Wistar rats weighing 200–220 g were purchased from the Experimental Animal Center of Liaoning Medical University (Jinzhou, China). The rats were housed under controlled environmental conditions (temperature, 22 ± 2 °C; humidity, 50 ± 10%) and allowed free access to food and water. The animal study was approved by the Institutional Animal Ethics Committee and performed according to the Regulations of Experimental Animal Administration issued by the State Commission of Science Technology of the People's Republic of China.

Twelve male rats were divided randomly into two groups with six rats in each. Diabetes in rats was induced by the chemical reagents method as described previously (Jaiswal et al., 2009; Talari et al., 2010). Briefly, the rats in the diabetic group were given intraperitoneal injection of streptozotocin solution (16 mg/mL, dissolved in a 10 mM citrate buffer at pH 4.5) at a single dose of 50 mg/kg body weight; an equal volume of citrate buffer was injected into rats of the control group. 72 h after the single injection of streptozotocin solution, the rats showing fasted glucose levels (≥300 mg/dL) were considered to be diabetic and those with consecutive seven-day hyperglycemia (≥300 mg/dL) were included in the study (Jaiswal et al., 2009).

2.3. Instruments and conditions

The chromatographic separation was achieved on an Agilent ZORBAX Eclipse XDB C18 column (150 × 4.6 mm², particle size, 5 μm) at 30 °C. Gradient elution was performed with solvents consisting of 10 mM ammonium acetate in water containing 0.1% acetic acid (v/v) (solvent A) and methanol (solvent B). The elution gradient was 30–100% B (0–3 min), and 100–30% B (3–6.2 min) at a flow rate of 0.6 mL/min. The gradient solutions were filtered through a 0.45 μm membrane filter before use.

LC/MS/MS detection was conducted on an Agilent Technologies 1200 series system and an Agilent 6460 triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. Negative ionization mode was used, and the ions were monitored in the multiple reaction monitoring (MRM) mode. ESI source parameters were as follows: drying-gas flow 10 L/min; drying-gas temperature 350 °C; nebulizer pressure 45 psi; and capillary voltage +3.5 kV. Compound-dependent parameters such as fragmentation voltage and collision energy were optimized at 140 V and 12 eV for braziliin, 140 V and 10 eV for protosappannin B, as well as 150 V and 20 eV for the IS, respectively.

2.4. Preparation of Sappan Lignum extract

Sappan Lignum was purchased from Liaoning Traditional Chinese Medicine Co., Ltd. (Shenyang, China). The herb Sappan Lignum (400 g) was extracted with 5 L of 95% ethanol under reflux two times each lasting for 2 h (Hu et al., 2008; Wu et al., 2011). The filtrate was concentrated under reduced pressure to obtain about 200 mL of residual liquid, and then the liquid was extracted with 800 mL of ethyl acetate. Afterward, the extract was evaporated to dryness, yielding 11.3 g of Sappan Lignum extract.

2.5. Preparation of calibration standards and quality control samples

Stock standard solutions (1.00 mg/mL) of protosappannin B, brazilin and the internal standard (IS) isovitexin were prepared in methanol and used to prepare working standard solutions. Calibration curves for protosappannin B and brazilin were prepared in blank rat plasma at concentrations of 3.00, 10.0, 30.0, 100, 300, 1000 and 3000 ng/mL by the serial dilution method. Quality control (QC) samples representing the low, medium and high concentration levels (10.0, 100 and 2700 ng/mL, respectively) were separately prepared for each analyte. A working concentration of the IS (1000 ng/mL of isovitexin) solution was also prepared by the dilution method.

2.6. Sample preparation

A simple protein precipitate method was employed for extraction of braziliin, protosappannin B and IS from rat plasma. A 100 μL volume of the plasma sample was transferred to a 1.5 mL plastic test tube, and then 100 μL of the working concentration of the IS (1000 ng/mL) was added to the test tube. After another 300 μL of methanol was added for adequately precipitating protein, the sample mixture was vortexed for 1 min and then centrifuged at 11,000 rpm for 5 min to remove the protein precipitate. An aliquot of this supernatant was transferred to a clean glass tube and was evaporated to dryness under gentle nitrogen stream at 40 °C. The dry residue was reconstituted in 100 μL of the mobile phase, and a 10 μL aliquot of supernatant was analyzed by LC/MS/MS.

2.7. Method validation

The validation of the method was performed according to the guidelines for bioanalytical methods (US Food and Drug
Validation parameters such as specificity, sensitivity, accuracy, precision, recovery, matrix effect, and stability were investigated.

### 2.7.1. Specificity

Specificity was evaluated by comparing the chromatograms of blank rat plasma processed by protein precipitation with the chromatogram spiked with respective standards to detect any peaks interfering the analytes. Six different specimens of blank rat plasma were evaluated for the specificity.

### 2.7.2. Linearity and lower limit of quantification

A line was fitted through the standard curve whose range was based on a weighted linear regression (weight = 1/x$^2$) of the peak

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**Fig. 2.** Representative spectra of precursor ion and product ion of protosappanin B (a, b), brazilin (c, d) and isovitexin (e, f).
Fig. 3. Representative MRM chromatograms of a blank plasma sample (a), a blank plasma sample spiked analytes and IS (LLOQ) (b), a plasma sample at 10 min after oral administration of Sappan Lignum extract to normal rats (c), and a plasma sample at 10 min after oral administration of Sappan Lignum extract to streptozotocin-treated rats (d).
area ratio of protosappanin B and brazilin to IS (y) versus the actual concentration of the analyte (x). LLOQ was defined as the lower limit of quantification in the calibration curve that can be accurately measured, for which the relative standard deviation (RSD) was better than 20%.

2.7.3. Accuracy and precision

To evaluate the intra-day precision (specified as RSD) and accuracy (calculated by the ratio of determined concentration to nominal concentration), six samples at three QC concentration levels (low, 10.0 ng/mL; middle, 100 ng/mL; and high 2700 ng/mL for protosappanin B and brazilin, respectively) were prepared for analysis in 1 day. The inter-day precision and accuracy was determined with the same QC samples on three consecutive days. The variation of under 15% for the precision and accuracy was acceptable.

2.7.4. Recovery and matrix effect

Recovery of protosappanin B and brazilin was evaluated by comparing peak area ratios of extracted QC samples with those of reference QC solutions reconstituted in blank plasma extracts. The matrix effect of the assay was evaluated by comparing the peak area ratios of analytes resolved in the reconstituted solution of the blank plasma (six different specimens) extracts with those resolved in the mobile phase.

2.7.5. Stability

Stability of processing including three repeated freeze-thaw cycles (from −20 °C to 25 °C) and post-preparative stability (ambient, 4 h), room temperature stability (25 °C for 2 h), and long-term storage (−20 °C for 22 days) was assessed by analyzing triplicates of QC samples.

2.8. Application to pharmacokinetic study

The animals were fasted overnight with free access to water. The Sappan Lignum extract (11.3 g) was suspended with 0.5% carboxymethyl cellulose sodium (CMC-Na) aqueous solution to yield a concentration of 0.283 g/mL. Following intragastric administration to rats at a dose of 2.83 g extract/kg body weight

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/mL)</th>
<th>Precision (RSD, %)</th>
<th>Accuracy (RE, %)</th>
<th>Extract recovery (%)</th>
<th>Matrix effect (%)</th>
</tr>
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<tbody>
<tr>
<td>Protosappanin B</td>
<td>10.0</td>
<td>10.4</td>
<td>5.9</td>
<td>-1.8</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8.7</td>
<td>10.2</td>
<td>1.5</td>
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<td></td>
<td>2700</td>
<td>6.1</td>
<td>7.5</td>
<td>0.0</td>
<td>-2.4</td>
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<tr>
<td>Brazilin</td>
<td>10.0</td>
<td>6.3</td>
<td>11.8</td>
<td>-4.1</td>
<td>-9.1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.2</td>
<td>12.0</td>
<td>-6.0</td>
<td>-0.6</td>
</tr>
<tr>
<td></td>
<td>2700</td>
<td>4.4</td>
<td>4.1</td>
<td>4.5</td>
<td>5.0</td>
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</table>

<table>
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<tr>
<th>Compound</th>
<th>Nominal concentration (ng/mL)</th>
<th>Found concentration (Mean ± SD; ng/mL)</th>
<th>Precision (% RSD)</th>
<th>Accuracy (%)</th>
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<tr>
<td>Protosappanin B</td>
<td>0 h</td>
<td>10.0</td>
<td>9.59 ± 0.83</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
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<td>2700</td>
<td>2526 ± 79.9</td>
<td>3.2</td>
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<td></td>
<td>Room temperature stability</td>
<td>10.0</td>
<td>10.2 ± 10</td>
<td>9.6</td>
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<tr>
<td></td>
<td></td>
<td>2700</td>
<td>2546 ± 79.9</td>
<td>1.9</td>
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<td>Freeze-thaw stability</td>
<td>10.0</td>
<td>9.66 ± 0.72</td>
<td>7.5</td>
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<td></td>
<td>2700</td>
<td>2749 ± 115</td>
<td>4.2</td>
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<tr>
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<td>10.4 ± 0.9</td>
<td>8.7</td>
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<tr>
<td></td>
<td></td>
<td>2700</td>
<td>2756 ± 208</td>
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<td>Long-term stability</td>
<td>10.0</td>
<td>10.4 ± 0.6</td>
<td>5.6</td>
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<tr>
<td></td>
<td></td>
<td>2700</td>
<td>2621 ± 154</td>
<td>5.9</td>
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<tr>
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<td>9.77 ± 0.16</td>
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<td>9.83 ± 1.00</td>
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<td>2621 ± 671</td>
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<td>Freeze-thaw stability</td>
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<td>10.3 ± 0.9</td>
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<td></td>
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<td>2797 ± 213</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>Post-preparative stability</td>
<td>10.0</td>
<td>10.1 ± 0.8</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2700</td>
<td>2645 ± 63.1</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Long-term stability</td>
<td>10.0</td>
<td>9.52 ± 0.36</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2700</td>
<td>2836 ± 209</td>
<td>7.4</td>
</tr>
</tbody>
</table>
(equivalent to 35.56 mg/kg of protosappanin B and 52.25 mg/kg of brazilin), approximately 200 μL of blood samples were collected into heparinized plastic tubes via oculi chorioideae vein from each rat at pre-dose (0), 2 min, 5 min, 10 min, 25 min, 40 min, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h. Plasma was harvested by centrifugation (3000 rpm for 10 min) and then frozen at −20 °C until LC/MS/MS analysis.

Pharmacokinetic analysis was performed by non-compartmental model using DAS 2.0 software program (Mathematical Pharmacology Professional Committee of China, Shanghai, China) to calculate AUC, C_{max}, \text{T}_{1/2} and CL/F. The statistical analysis between two groups was performed using the unpaired Student’s t-test. P≤0.05 was considered statistically significant for all the tests. All data were presented as the mean±SD.

3. Results and discussion

3.1. Method development and optimization

To develop the LC/MS/MS method, the MS responses of precursor ions and product ions of protosappanin B, brazilin and the IS were tuned in both positive and negative ionization modes with a syringe pump infusion. However, a better response with good sensitivity, reproducibility and fragmentation was found in negative ionization mode than positive mode for each analyte. The deprotonated form [M−H]− of each analyte was the precursor ion in the Q1 spectrum and used as the precursor ion to obtain the corresponding product ions in Q3 scan spectra. The mass spectrum of the deprotonated molecular ions [M−H]− of protosappanin B (m/z 303.0) showed the formation of product ions at m/z 184.8, 213.2, 230.8 and 242.9 (Fig. 2b). The most sensitive mass transition was monitored from m/z 303.0 to 230.8. The mass spectrum of the product ion scan of brazilin (m/z 285.1) showed the formation of characteristic product ions at m/z 121.0, 134.9, 162.9, 229.0 and 266.8 (Fig. 2d), and the most sensitive mass transition was observed from m/z 285.1 to 162.9. Similarly, the product ion mass spectrum of the IS (isovitexin, m/z 431.1) showed the formation of characteristic product ions at m/z 282.8, 311.1 and 340.8 (Fig. 2f). The most sensitive mass transition was from m/z 431.1 to 311.1. Fig. 1 shows the details of the fragmentation patterns of protosappanin B, brazilin and the IS.

The presence of a small amount of acetic acid buffer in the mobile phase improved MS detection with good responses in the negative ionization mode. A gradient elution program was finally adopted with an acceptable run time of 6.2 min.

3.2. Method validation

3.2.1. Specificity

Fig. 3 shows the typical MRM chromatographic profiles of blank rat plasma samples, plasma samples spiked with protosappanin B, brazilin, and the IS, as well as plasma samples at 10 min obtained after oral administration of Sappan Lignum extract. The retention times of protosappanin B, brazilin and the IS were 4.75, 4.66 and 5.63 min, respectively. No interference was observed at the respective time of the analytes and IS under the specified LC/MS/MS conditions.

3.2.2. Precision and accuracy

Table 1 summarizes the intra- and inter-day precision and accuracy values for protosappanin B and brazilin. The intra- and inter-day precisions (RSD) were less than 12.0% for each QC level of both analytes. The accuracies (RE) were less than 1.5% for protosappanin B and within 5.0% for brazilin. The above obtained values met the acceptable criteria, and the method was thus judged to be accurate and precise.

3.2.3. Recovery and matrix effect

The recoveries in rat plasma were 102.4±3.8%, 104.8±5.7 and 99.5±4.7% for protosappanin B at concentrations of 10.0, 100 and 2700 ng/mL, as well as 96.2±13.0%, 101.3±5.5%, and 99.5±4.7% for brazilin at concentrations of 10.0, 100 and 2700 ng/mL, respectively. For the IS, the mean recovery was 101.8±9.0%.

In terms of matrix effects, the ratios of the peak responses were 101.2±7.2%, 91.5±7.9%, and 89.2±11.0% at 10.0, 100, and 2700 ng/mL for protosappanin B, as well as 90.2±6.4%, 94.5±4.1%, and 99.0±3.2% at 10.0, 100, and 2700 ng/mL for brazilin, respectively. The value for the IS was 95.4±10.0%. The results indicated that no significant ion suppression or enhancement from the plasma matrix was observed under the present conditions.

3.2.4. Stability

The stability result shows good stability for both analytes under four different conditions with <5.0% concentration variation compared with the initial values (Table 2). Thus, the data conformed to the acceptance criteria.
streptozotocin-treated groups are presented in Fig. 4, and the main intraperitoneally administered can cause liver damage and induce homoiso
everal studies indicated that a significant difference was observed in pharmacokinetic parameters, including AUC0–24 h, AUC0–∞, Cmax, T1/2, and Ce max for protosappanin B and brazilin; however, no significant difference was observed in Tmax between two groups. After oral administration of Sappan Lignum extract, the streptozotocin-treated group exhibited a remarkable increase (p < 0.05) in the values of AUC0–24 h, AUC0–∞, Cmax, and T1/2 associated with protosappanin B and brazilin. This finding indicated that the systemic exposure (AUC) of both compounds significantly increased after oral administration of Sappan Lignum extract in the diabetic state. Protosappanin B and brazilin are homoisoavonoids with similar structures, and may have similar absorption and metabolic pathways. On the other hand, diabetes might have induced a decrease in gastric emptying, resulting in a significant increase in the absorption of protosappanin B and brazilin in plasma (Ishiguchi et al., 2002).

Elimination of both compounds was slower in streptozotocin-treated rats than in normal rats. T1/2 in streptozotocin-treated rats was about twofold compared with normal rats, whereas Ce max/F in streptozotocin-treated rats decreased fourfold compared with normal rats. Hyperglycemia induced when streptozotocin was intraperitoneally administered can cause liver damage and induce changes in liver metabolic enzymes, which may prolong the residence time and reduce the elimination of drug (Baig et al., 2001; El-serag et al., 2004). Moreover, the damage to kidney induced by streptozotocin, which was also coincident with diabetic nephropathy, may be the key reason why the elimination of both drugs in streptozotocin-treated rats slowed down (Qin et al., 2009; Cruzado et al., 2004).

### 3.3. Pharmacokinetic study

The validated method was successfully applied to the pharmacokinetics of protosappanin B and brazilin in rat plasma after oral administration of Sappan Lignum extract at a dose of 2.83 g/kg. The plasma drug–time curves of both analytes in normal and streptozotocin-treated groups are presented in Fig. 4, and the main pharmacokinetic parameters are summarized in Table 3. As shown in Table 3, statistically significant differences (p < 0.05) were observed in pharmacokinetic parameters, including AUC0–24 h, AUC0–∞, Cmax, T1/2, and Ce max/F for protosappanin B and brazilin; however, no significant difference was observed in Tmax between two groups. After oral administration of Sappan Lignum extract, the streptozotocin-treated group exhibited a remarkable increase (p < 0.05) in the values of AUC0–24 h, AUC0–∞, Cmax, and T1/2 associated with protosappanin B and brazilin. This finding indicated that the systemic exposure (AUC) of both compounds significantly increased after oral administration of Sappan Lignum extract in the diabetic state. Protosappanin B and brazilin are homoisoavonoids with similar structures, and may have similar absorption and metabolic pathways. On the other hand, diabetes might have induced a decrease in gastric emptying, resulting in a significant increase in the absorption of protosappanin B and brazilin in plasma (Ishiguchi et al., 2002).

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### 4. Conclusion

A rapid, selective, and sensitive LC/MS/MS method for the simultaneous determination of protosappanin B and brazilin in rat plasma was developed and validated for the first time. A simple protein precipitation procedure was used to prepare the samples using 100 μL of plasma with high recovery and minimal matrix effect. The established method was successfully used to compare the pharmacokinetic behaviors of protosappanin B and brazilin in rat plasma after oral administration of Sappan Lignum extract between normal and streptozotocin-treated groups. The results indicated that a significant difference in pharmacokinetic parameters for protosappanin B and brazipent in the two groups. Both compounds had larger absorption and slower elimination in streptozotocin-treated rats than in normal rats. The study provided a preliminary discussion on the pharmacokinetic difference of both drugs in normal and streptozotocin-treated rats, and suggested that the accumulation of drugs in pathologic states, as well as the resulting adverse reactions, should be considered when the drugs are used.

### Table 3

Non-compartmental pharmacokinetic parameters for protosappanin B and brazilin in rat plasma after oral administration of Sappan Lignum extract between normal and streptozotocin-treated groups (mean ± SD, n = 6).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Normal group</th>
<th>Streptozotocin-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protosappanin B</td>
<td>Brazilin</td>
</tr>
<tr>
<td>AUC0–10 (ng h/mL)</td>
<td>503.3 ± 99.9</td>
<td>1784 ± 386</td>
</tr>
<tr>
<td>AUC0–∞ (ng h/mL)</td>
<td>518.8 ± 98.2</td>
<td>1796 ± 385</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>2.90 ± 0.54</td>
<td>2.31 ± 0.84</td>
</tr>
<tr>
<td>Ce max (ng/mL)</td>
<td>712 ± 17.1</td>
<td>30.5 ± 8.09</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.306 ± 0.234</td>
<td>0.333 ± 0.130</td>
</tr>
<tr>
<td></td>
<td>239.2 ± 57.9</td>
<td>1820 ± 257</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with normal group.
the pharmacokinetic of metformin hydrochloride between normal and diabetic
Antioxidant activity and protective effects on DNA damage of Caesalpinia
activity of Caesalpinia sappan L. Asian Pacific Journal of Tropical Biomedicine 2,
S136–S139.
prepared by two methods of in situ micronization: pharmacokinetic studies in
diabetic and normal rats. AAPS PharmSciTech 11, 786–792.
Ueda, J.Y., Tezuka, Y., Banskota, A.H., Tran, Q.L., Tran, Q.K., Harimaya, Y., Saiki, I.,
Biological and Pharmaceutical Bulletin 25, 753–760.
of Caesalpina sappan L. heartwood in various ages. Naresuan University Journal
13, 43–45.
Wu, S.Q., Otero, M., Unger, F.M., Goldring, M.B., Phrutivorapongkul, A., Chiari, C.,
ethanolic Caesalpinia sappan extract in human chondrocytes and macrophages.
Phytotherapy Research 18, 647–651.
protopsappanin B in Caesalpinia sappan L. by HPLC. West China Journal of