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# Simultaneous determination of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid in rat plasma after oral administration of Danggui Buxue Tang extract for their pharmacokinetic studies by liquid chromatography–mass spectrometry

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**Abstract**

A sensitive and reliable high-performance liquid chromatography–mass spectrometry (HPLC–MS) was developed and validated for simultaneous quantification of five main bioactive components, i.e., calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid in rat plasma after oral administration of Danggui Buxue Tang (DBT) extract. Plasma samples were extracted with solid-phase extraction (SPE) separated on an Inertsil ZORBAX $C_{18}$ column and detected by MS with electrospray ionization (ESI) interface in negative selective ion monitoring (SIM) mode. Calibration curves offered linear ranges of two orders of magnitude with $r^2 > 0.99$. The method had the lower limit quantification of 0.55, 0.46, 1.07, 1.12 and 4.6 ng/mL for calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid, respectively, with precision less than 10%. The RSD of intra- and inter-day variations ranged from 2.10% to 6.19% and 2.37% to 6.72%. This developed method was subsequently applied to pharmacokinetic studies of the five compounds in rats successfully.


*Keywords:* HPLC–MS; Calycosin-7-*O*-β-D-glucoside; Ononin; Astragaloside IV; Astragaloside I; Ferulic acid; Danggui Buxue Tang extract; Pharmacokinetic

## 1. Introduction

Danggui Buxue Tang (DBT) is a most classical formulae of traditional Chinese medicines (TCMs), comprising Radix Astragli and Radix Angelica Sinensis (5:1, w/w). This formula has been widely used since 1247 AD to nourish the "Blood" (body circulation) and raise the "Qi" (vital energy). Modern pharmacological researches demonstrated that DBT has the abilities to promote hematopoietic functions, to stimulate cardiovascular circulation, to prevent osteoporosis, to increase anti-oxidation activity and to stimulate the immune system [1,2]. Nowadays, it is commonly applied not only as an efficacious medicinal prescription but also as a healthy food supplement in Asia.

Calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid are five main bioactive components of DBT [3–5]. Simultaneous determination of these five main bioactive components in biological fluids for pharmacokinetic investigations is then required, not only to evaluate the clinical applications of DBT, but also to achieve a better understanding of the pharmacological action mechanism, explaining a variety of events related to the efficacy and toxicity of TCMs.

Several methods have been previously described to determine each of these analytes in plasma separately [6,7]. However, there are few reports available regarding physiological disposition and pharmacokinetic studies of DBT. Up to our knowledge, there is no method established for the simultaneous

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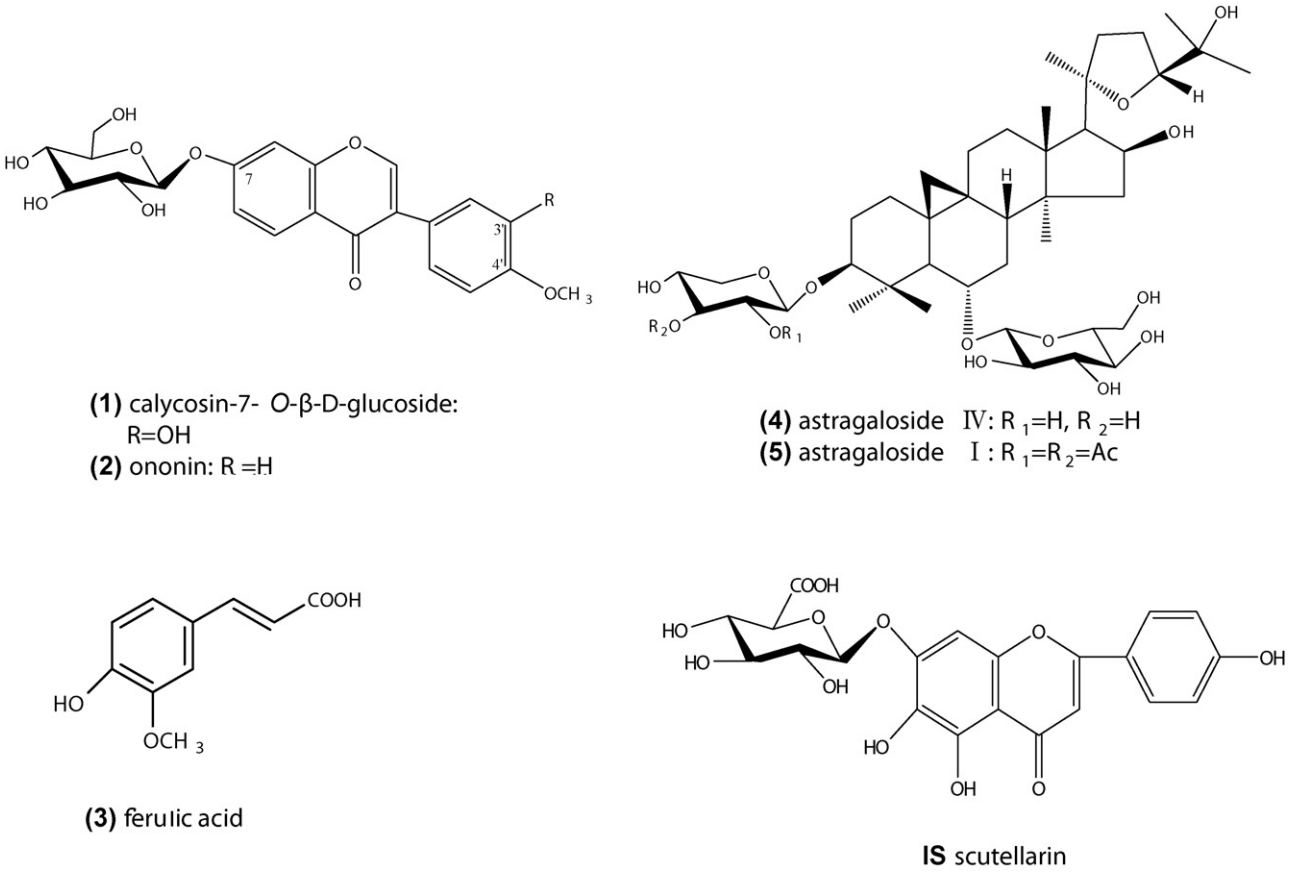


Fig. 1. Chemical structures of the calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid and scutellarin (IS).

estimation of these five active constituents for pharmacokinetic investigations. Being a common analytical tool for various compounds, LC–MS has several advantages over HPLC in terms of sensitivity, selectivity, etc. Therefore, this study was aimed at developing a sensitive and validated high-performance liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) method for simultaneous determination of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid in rat plasma after oral administration of DBT, and for their pharmacokinetic studies, so as to take a limited view of their pharmacokinetic profiles.

## 2. Experimental

### 2.1. Herbal materials and chemicals

Radix Angelica sinensis and Radix Astragali were collected from Min County of Gansu Province and Hunyuan County of Shanxi Province of China, respectively, and authenticated by one of us, Prof. Ping Li. Ferulic acid and scutellarin (internal standard, IS) were bought from the Chinese National Institute for Control of Pharmaceutical and Biological Products (Beijing, China); other reference compounds, i.e., calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, and astragaloside I were isolated from Radix Astragali in the authors' laboratory. And their structures were elucidated in terms of their spectral data (IR, MS, $^{1}$H NMR and $^{13}$C NMR). The purity of each compound was determined to be higher than 98% by normalization of the peak area detected by HPLC. The chemical structures of the reference compounds are shown in Fig. 1.

Acetonitrile was of HPLC grade from Merck (Darmstadt, Germany); distilled water was further purified by Milli-Q system (Millipore, Milford, MA, USA); formic acid was purchased from the first chemical company of Nanjing (Jiangsu, China); other chemicals were of analytical grade. All solvents and samples were filtered through 0.22 μm filter membrane before injecting into HPLC.

### 2.2. HPLC–MS analysis

Analyses were performed on an Agilent Series 1100 series liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a dual pump, an auto-sampler and an Agilent SL G1946D single quadrupole mass spectrometer (USA) with an ESI source. All the operations, the acquiring and analysis of data were controlled by Chemstation software (Agilent Technologies, USA).

The HPLC analysis was performed on a ZORBAX $C_{18}$ column (250 mm × 4.6 mm I.D., 5 μm) and a ZORBAX $C_{18}$ guard column (12.5 mm × 4.6 mm I.D., 5 μm). The mobile phases consisted of 0.1% formic acid water (A) and acetonitrile (B) using a gradient elution of 20–20% (v/v) B at 0–12 min; 20–32% B at 12–20 min; 32–34% B at 20–30 min; 34–45% B at 30–39 min; 45–45% B at 39–42 min; 45–60% B at 42–50 min. The flow-rate

was 0.8 mL/min, the column temperature was kept at 25 °C, and the injection volume was 10 μL.

The ESI-MS data were acquired in negative mode and conditions of MS analysis were as follows: drying gas ($N_2$) flow-rate, 8 L/min; drying gas temperature, 300 °C; nebulizing gas ($N_2$) pressure, 16 psi; capillary voltage, 3500 V; quad temperature, 100 °C; fragmentor, 100 V.

The standard solution and extracted samples were analyzed in selective ion monitoring (SIM) mode using the ESI source by monitoring the HCOO-adducts ions. SIM for each compound were restricted to specific retention time windows 0–15 min, *m/z* 491.10; 15–22 min, *m/z* 475.20; 22–50 min, *m/z* 301.10 by channel 1, and 0–15 min, *m/z* 193.10; 15–30 min, *m/z* 301.10; 30–40 min, *m/z* 829.10; 40–50 min, *m/z* 913.10 by channel 2.

### 2.3. Animals

Sprague–Dawley rats (220–250 g) were obtained from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China) and housed with unlimited access to food and water except for fasting 12 h before the experiment. The animals were maintained on a 12-h light/12-h dark cycle (light on at 8:00) at ambient temperature (22–25 °C) and at 60% relative humidity. Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of China Pharmaceutical University (Nanjing, China) and protocol was approved by the Animal Ethics Committee of this institution.

### 2.4. Determination of five main compounds in DBT

To calculate the administered dose of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid, their contents in DBT were quantitatively determined. Thirty grams of mixed powder of Radix Astragali and Radix Angelica Sinensis (5:1, w/w) was immersed in 500 mL 75% ethanol for 1 h, and then refluxed for 2 h at 85 °C. The solvent was removed with rotary evaporation under vacuum at 45 °C, and the residue was dissolved in 30 mL phosphate buffer and filtered through a 0.22 μm membrane, and then 10 μL of this solution was injected into the HPLC system for analysis. The HPLC analysis of the five constituents in DBT was previously method established by the authors [8]. The contents of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid were 0.408, 0.116, 0.120, 0.681 and 0.037 mg/mL in extract, respectively.

### 2.5. Sample preparation

Supelclean™ LC-18 solid phase extraction (SPE) columns (1 mL/100 mg volume, Supelco, USA) were conditioned and equilibrated with methanol, followed by deionized water before use. Samples of plasma (500 μL), mixed with 10 μL of IS solution (2 μg/mL in water) and water (500 μL), were loaded onto SPE columns. After washed off with deionized water, the SPE columns were eluted using 3 mL 90% methanol. The eluant was evaporated to dryness in a water bath at 45 °C under a nitrogen stream. The residues were reconstituted in 200 μL aliquots of HPLC mobile phase, and centrifuged at 15,000 × *g*, 4 °C for 10 min. Supernatants (10 μL) were injected into the HPLC system.

### 2.6. Calibration curve

The mixture of stock standard solution containing calycosin-7-*O*-β-D-glucoside (1.1 mg/mL), ononin (0.93 mg/mL), astragaloside IV, (1.07 mg/mL), astragaloside I (1.12 mg/mL) and ferulic acid (0.99 mg/mL) was prepared in methanol. The IS stock solution of 1 mg/mL was also prepared in methanol, and kept at 20 μg/mL in each working solution and samples. Calibration samples in plasma were prepared by spiking aliquots of the stock solutions into drug-free plasma samples to obtain final concentrations in the range of 5.50–1100 ng/mL for calycosin-7-*O*-β-D-glucoside, 4.65–1023 ng/mL for ononin, 5.35–1177 ng/mL for astragaloside IV, 5.60–1232 ng/mL for astragaloside I, and 4.95–990 ng/mL for ferulic acid. Quality control (QC) samples were also prepared in the same way (10, 100, 1000 ng/mL for calycosin-7-*O*-β-D-glucoside; 9.3, 93, 930 ng/mL for ononin; 10.7, 107, 1070 ng/mL for astragaloside IV; 11.2, 112, 1120 ng/mL for astragaloside I; 9.1, 91, 910 ng/mL for ferulic acid). All solutions were stored at 4 °C before use. For a standard curve, the ratio of the chromatographic peaks area (analytes/IS) as ordinate variables were plotted versus the concentration of these drugs as abscissa. The limit of detection (LOD) was considered as the final concentration producing a signal-to-noise (S/N) ratio of 3 and the limit of quantification (LOQ) concentration producing a S/N ratio of 10.

### 2.7. Precision and accuracy

The precision and accuracy of method were assessed by performing replicate analyses of QC samples against calibration standards. The precision was determined from inter-day and intra-day using five determinations of low, middle, and high concentration and expressed as relative standard deviation (RSD).

### 2.8. Extraction recovery and stability

The extraction recovery was determined by calculating the ratio of the amounts of QC samples finally obtained against those originally spiked in the drug-free plasma. The freeze/thaw stability was evaluated by analyzing QC samples at three concentrations after undergoing for freeze (−80 °C) and thaw (room temperature) cycles in 1 month.

### 2.9. Application of the method and pharmacokinetic study

Blood samples were collected from rats as part of a tissue distribution study. Groups of six rats were sacrificed by decapitation at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 h after oral administration of DBT (13.5 mL/kg). Within 30 min after blood withdrawal, the samples were centrifuged and the

Table 1
Extraction recovery of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid in rat plasma ($n = 5$)

| Compounds | Spiked concentration (ng/mL) | Recovery (%) (mean ± SD) | RSD (%) |
|---|---|---|---|
| Calycosin-7-*O*-β-D-glucoside | 10 | 62.03 ± 5.34 | 8.61 |
| | 100 | 70.12 ± 2.68 | 3.82 |
| | 1000 | 76.32 ± 1.21 | 1.59 |
| Ononin | 9.3 | 66.72 ± 5.51 | 8.26 |
| | 93 | 72.38 ± 3.16 | 4.37 |
| | 930 | 75.24 ± 1.37 | 1.82 |
| Ferulic acid | 9.1 | 60.95 ± 4.28 | 7.02 |
| | 91 | 63.58 ± 3.26 | 5.13 |
| | 910 | 69.80 ± 1.09 | 1.56 |
| Astragaloside IV | 10.7 | 62.27 ± 4.36 | 7.00 |
| | 107 | 69.72 ± 2.22 | 3.18 |
| | 1070 | 75.65 ± 1.68 | 2.22 |
| Astragaloside I | 11.2 | 61.32 ± 3.61 | 5.89 |
| | 112 | 68.15 ± 2.28 | 3.35 |
| | 1120 | 72.18 ± 1.26 | 1.75 |

separated plasma samples were frozen in polypropylene tubes at −20 °C prior to analysis.

PK parameters including area under concentration–time curve (AUC), maximum plasma concentration ($C_{max}$), time to reach the maximum concentrations ($T_{max}$), oral clearance (CL/F), and mean residence time (MRT) were estimated using a non-compartmental analysis using Drug And Statistics 2.0 (DAS 2.0) (Mathematical Pharmacology Professional Committee of China, Shanghai, China).

## 3. Result and discussion

### 3.1. SPE method establishment

The aim of sample preparation was to remove interferences from plasma samples with a suitable recovery rate involving a minimum number of working steps. Several extraction procedures were tested, including SPE and the liquid–liquid extraction method. It was estimated that SPE could remove proteins and other interfering components in rat plasma with satisfactory drug recovery. Proteins and interfering compounds can be removed by deionized water, and five analytes retained on the Supelclean™ LC-18 SPE were completely eluted with 90% methanol.

### 3.2. Extraction recovery

The extraction recoveries of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid from rat plasma are shown in Table 1.

The extraction recoveries were determined for five replicates of rat plasma spiked with low, medium and high concentrations of the five analytes. The mean recoveries of the samples were more than 60% and the average extraction recovery of internal standard was 76.27%. The data indicated that the extraction recoveries of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I, ferulic acid and I.S. from the plasma were concentration-independent in the concentration range evaluated and were acceptable.

### 3.3. Selectivity

Fig. 2 shows the chromatographic profiles of blank plasma, blank plasma spiked with five analytes and IS, and plasma obtained 15 min after oral administration of DBT. The retention time of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I, ferulic acid and IS were 6.84, 20.67, 32.52, 45.22, 10.75 and 8.16 min, respectively. A baseline separation of these compounds was obtained under the specified chromatographic conditions. No interfering peaks were detected and therefore a high and well-acceptable selectivity was obtained by this method.

### 3.4. Linearity and sensitivity

All the linear regression of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid in rat plasma displayed good linear relationships over the range of 5.50–1100 ng/mL, 4.65–1023 ng/mL, 5.35–1177 ng/mL, 5.60–1232 ng/mL and 4.95–990 ng/mL, respectively. The mean values of regression equation of the analytes in rat plasma were: $y = 1.8752x - 0.0054$ ($r^2 = 0.9986$, calycosin-7-*O*-β-D-glucoside), $y = 2.2552x - 0.0083$ ($r^2 = 0.9979$, ononin), $y = 3.0916x - 0.0052$ ($r^2 = 0.9956$, astragaloside IV), $y = 2.49x + 0.0034$ ($r^2 = 0.9934$, astragaloside I) and $y = 2.7891x - 0.0148$ ($r^2 = 0.9972$, ferulic acid).

Sensitivity was evaluated by the LOD and LOQ determinations, which are defined as the lowest concentration that can be reliably and reproducibly measured at least five replicates. To determine the LOD, pooled plasma samples were spiked to contain 0.28 ng/mL calycosin-7-*O*-β-D-glucoside, 0.23 ng/mL ononin, 0.27 ng/mL astragaloside IV, 0.28 ng/mL astragaloside I and 2.03 ng/mL ferulic acid, respectively, and analyzed on five different days. The peak area in chromatograms for the spiked plasma samples containing the above lowest concentrations was compared with the noise signal. The LOD had to have precision of ≤10% and S/N ratio ≥ 3. As to the LOQ, the same procedures were performed with contents of analytes in

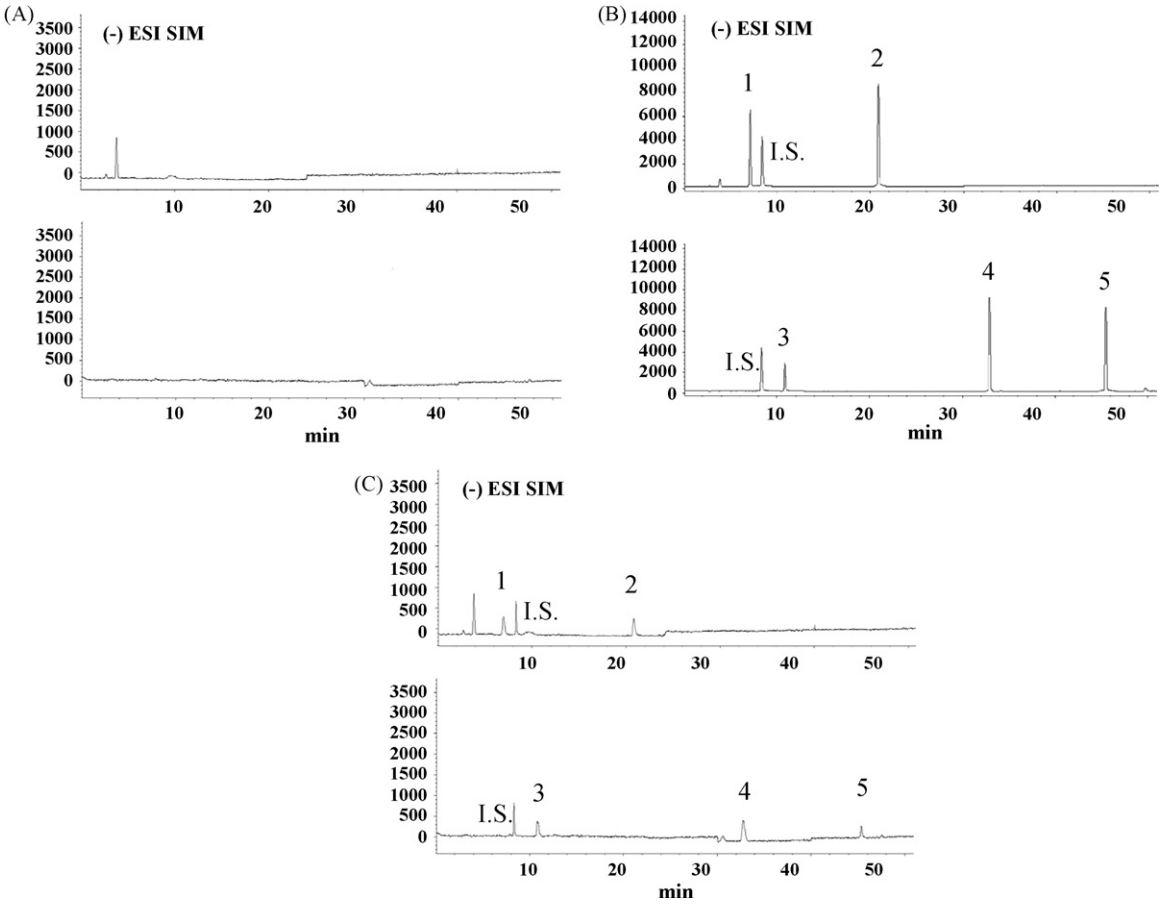


Fig. 2. Selected ion monitoring chromatograms from (A) blank plasma, (B) blank plasma spiked with calycosin-7-*O*-β-D-glucoside (1, 0.3 μg/mL), ononin (2, 0.46 μg/mL), ferulic acid (3, 0.36 μg/mL), astragaloside IV (4, 1.07 μg/mL), astragaloside I (5, 0.42 μg/mL), (C) a rat plasma sample containing 1 (0.018 μg/mL), 2 (0.017 μg/mL), 3 (0.038 μg/mL), 4 (0.08 μg/mL), 5 (0.015 μg/mL), respectively 0.25 h after oral administration of DBT.

pooled plasma samples spiked to 0.55 ng/mL calycosin-7-*O*-β-D-glucoside, 0.46 ng/mL ononin, 1.07 ng/mL astragaloside IV, 1.12 ng/mL astragaloside I, and 4.6 ng/mL ferulic acid, respectively. The LOQ had to have precision of ≤10% and a S/N ratio ≥ 10.

### 3.5. Precision and accuracy

The precision and accuracy of the method were assessed in plasma by performing replicate analyses of spiked samples against calibration standards. The procedures were repeated on the same day and between five different days on the same spiked standard series. The intra-day and inter-day precision and accuracy of the method are shown in Tables 2 and 3. The precisions (RSD) were all less than 10%. The data indicated that the precision and accuracy of this method were acceptable.

### 3.6. Stability

Stability data are summarized in Table 4 and indicated that the five analytes were stable in plasma for at least five freeze/thaw cycles. Moreover, the results of the stability showed that all the investigated compounds were stable for at least 4 weeks when kept frozen at −80 °C.

### 3.7. Application of the analytical method in pharmacokinetics study

TCMs combination is used to cure diseases as a whole. Each herb that is comprised in the formula is necessary to the integral effect. As the chemical constituents of the formula are complex, the pharmacokinetic study is usually focused on the main active constituents. In particular, calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid were usually used in quality control studies of DBT [8,9]. Therefore, the current investigation was designed to administer DBT to animal subjects, and then to determine the plasma profiles and pharmacokinetic parameters of the five analytes in DBT. Ligustilide from Radix Angelicae Sinensis is another major marker component in DBT. However, in our preliminary experiments, the ligustilide could not be observed in rat serum after oral administration of DBT. The plasma concentration–time profiles for the five main active components of DBT are shown in Fig. 3 and the main pharmacokinetic parameters in rats are presented in Table 5. Following the administration of the DBT the concentrations of calycosin-7-*O*-β-D-glucoside, ononin and astragaloside I, ferulic acid were fitted to a one-compartment model, except for that stragaloside IV was, on the other hand, fitted to a two-compartment model.

Table 2
Intra-day precision and accuracy values for calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid from the assay QC standards ($n = 5$)

| Compounds | Spiked concentration (ng/mL) | Measured concentration (ng/mL) (mean ± SD) | Precision (RSD%) | Accuracy percent error (%) |
|---|---|---|---|---|
| Calycosin-7-*O*-β-D-glucoside | 10 | 10.18 ± 0.32 | 3.14 | 1.80 |
| | 100 | 103.32 ± 2.65 | 2.56 | 3.32 |
| | 1000 | 980.52 ± 16.32 | 1.66 | −1.95 |
| Ononin | 9.3 | 9.10 ± 0.42 | 4.62 | −2.15 |
| | 93 | 90.89 ± 2.89 | 3.18 | −2.27 |
| | 930 | 918.59 ± 15.96 | 1.74 | −1.27 |
| Ferulic acid | 9.1 | 9.39 ± 0.38 | 4.05 | 3.19 |
| | 91 | 89.26 ± 3.58 | 5.99 | −1.91 |
| | 910 | 889.35 ± 25.38 | 2.86 | −2.27 |
| Astragaloside IV | 10.7 | 10.08 ± 0.56 | 5.56 | −5.79 |
| | 107 | 103.52 ± 4.20 | 4.18 | −3.25 |
| | 1070 | 1032.56 ± 25.68 | 4.06 | −3.49 |
| Astragaloside I | 11.2 | 11.65 ± 0.63 | 5.41 | 4.02 |
| | 112 | 109.52 ± 2.66 | 2.43 | −2.21 |
| | 1120 | 1152.61 ± 26.95 | 2.39 | 2.91 |

Table 3
Inter-day precision and accuracy values for calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid from the assay QC standards ($n = 5$)

| Compounds | Spiked concentration (ng/mL) | Measured concentration (ng/mL) (mean ± SD) | Precision (RSD%) | Accuracy percent error (%) |
|---|---|---|---|---|
| Calycosin-7-*O*-β-D-glucoside | 10 | 10.31 ± 0. 46 | 4.46 | 3.10 |
| | 100 | 106.26 ± 3.18 | 2.99 | 6.26 |
| | 1000 | 960.46 ± 18.28 | 1.90 | −3.95 |
| Ononin | 9.3 | 9.04 ± 0.62 | 6.78 | −2.80 |
| | 93 | 90.26 ± 3.63 | 4.02 | −2.95 |
| | 930 | 916.12 ± 30.96 | 3.38 | −1.50 |
| Ferulic acid | 9.1 | 9.69 ± 0.59 | 6.09 | 6.48 |
| | 91 | 88.36 ± 5.29 | 5.99 | −2.90 |
| | 910 | 882.26 ± 39.68 | 4.49 | −3.05 |
| Astragaloside IV | 10.7 | 9.98 ± 0.60 | 6.01 | −6.72 |
| | 107 | 100.56 ± 4.20 | 4.18 | −6.02 |
| | 1070 | 1012.56 ± 30.29 | 3.04 | −5.36 |
| Astragaloside I | 11.2 | 11.82 ± 0.61 | 5.16 | 5.54 |
| | 112 | 108.68 ± 3.76 | 3.46 | −2.96 |
| | 1120 | 1160.61 ± 30.94 | 2.66 | 3.63 |

As shown in Table 5 and Fig. 3, owing to the similar structures, calycosin-7-*O*-β-D-glucoside and ononin have parallel pharmacokinetic parameters *in vivo*, being absorbed rapidly and eliminated quickly with the similar rate. However, the contents of calycosin-7-*O*-β-D-glucoside and ononin in DBT were 0.408 and 0.116 mg/mL, respectively, whereas the content of the ononin in plasma was higher. This might result from the relatively weak polarity of ononin.

With $t_{max}$ at 0.43 h and $t_{1/2}$ at 0.75 h, ferulic acid was absorbed and eliminated the most rapidly among five tested compounds, which was in conformity with the previous references [10,11].

As seen from Fig. 1, astragaloside I is the derivative of astragaloside IV, differing only in the two acyl groups in β-D-xylose at ring C-3 position, but their pharmacokinetic parameters are significant different. Astragaloside IV was fitted to a two-compartment model in coincidence with published reports [6], while astragaloside I was fitted to a one-compartment mode. The contents of astragaloside I and astragaloside IV were 0.681 and 0.120 mg/mL in DBT, respectively, whereas the values of $C_{max}$ and AUC of astragaloside I were much lower than those of astragaloside IV. This phenomenon might be attributed to the biotransformation of astragaloside I to astragaloside IV by

Table 4
Freeze and thaw stability study ($n = 5$)

| Compounds | Amount added (ng/mL) | Mean amount found (±SD ng/mL) |
|---|---|---|
| Calycosin-7-*O*-β-D-glucoside | 10 | 10.03 ± 0.115 |
| | 1000 | 990.90 ± 0.889 |
| Ononin | 9.3 | 10.01 ± 0.043 |
| | 930 | 920.38 ± 1.57 |
| Ferulic acid | 9.1 | 11.03 ± 0.435 |
| | 910 | 900.83 ± 21.89 |
| Astragaloside IV | 10.7 | 10.13 ± 0.023 |
| | 1070 | 1050.12 ± 3.554 |
| Astragaloside I | 11.2 | 11.05 ± 0.041 |
| | 1120 | 1009.83 ± 3.302 |

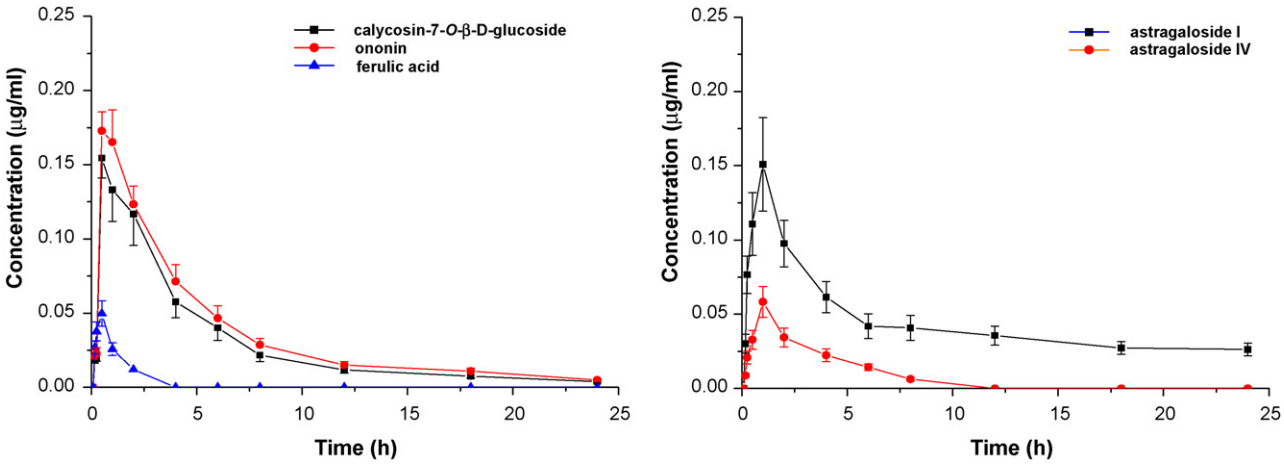


Fig. 3. Mean plasma concentration–time profile for calycosin-7-*O*-β-D-glucoside, ononin, ferulic acid (A), astragaloside IV and astragaloside I (B) in rat plasma after oral administration of DBT.

Table 5
Mean pharmacokinetic parameters of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid in rat serum ($n = 5$) after oral administration DBT

| Compounds | Parameter | | | | | | |
|---|---|---|---|---|---|---|---|
| | $t_{max}$ (h) | $C_{max}$ (μg mL$^{-1}$) | $T_{1/2\alpha}$ (h) | $T_{1/2\beta}$ (h) | CL (mL kg$^{-1}$ h$^{-1}$) | $AUC_{0\rightarrow\infty}$(μg h$^{-1}$ mL$^{-1}$) | MRT (h) |
| Calycosin-7-*O*-β-D-glucoside | 0.48 | 0.15 | – | 2.18 | 141.0 | 0.72 | 3.26 |
| Ononin | 0.53 | 0.17 | – | 2.42 | 117.6 | 0.87 | 3.65 |
| Ferulic acid | 0.43 | 0.049 | – | 0.75 | 546.4 | 0.065 | 1.26 |
| Astragaloside IV | 1.16 | 0.15 | 0.87 | 8.36 | 289.6 | 2.09 | 13.68 |
| Astragaloside I | 1.08 | 0.032 | – | 2.27 | 389.6 | 0.24 | 3.52 |

intestinal bacteria and enzymes *in vivo*, giving rise to great increase of astragaloside IV in plasma. Besides, it is likely that constituents in herbal preparations may be substrates, inhibitors, or inducers of CYPs, and thus have an impact on the pharmacokinetics of each other [12,13].

## 4. Conclusion

This paper described a simple, sensitive and validated LC–MS method for simultaneous determination of calycosin-7-*O*-β-D-glucoside, ononin, astragaloside IV, astragaloside I and ferulic acid in rat plasma after oral administration of DBT, and investigated on their pharmacokinetic studies as well. The assay provided adequate recovery with good precision and accuracy. The pharmacokinetic results contribute to a better understanding of the pharmacological action mechanism, and provide a firm basis for evaluating the clinical efficacy of DBT. In addition, the established method has now been extended to determine the concentrations of these bioactive components in other bio-samples, such as in tissues, in urine and in bile, etc., and the results would be published later.

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