Original Article

LC–MS/MS determination and pharmacokinetic study of bergenin, the main bioactive component of Bergenia purpurascens after oral administration in rats

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Abstract Bergenin, a C-glucoside of 4-O-methyl gallic acid from Bergenia purpurascens, is a naturally antitussive and expectorant agent. A rapid and sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) method has been developed and validated for the determination of the active component—bergenin, in rat plasma after oral administration of aqueous B. purpurascens extract. The plasma samples were pretreated by protein precipitation with acetonitrile and chromatographic separation was achieved on a Diamonsil® C18 column (150 mm × 4.6 mm, 5 μm) with isocratic elution using a mobile phase consisting of water–methanol (30:70, v/v) at a flow rate of 0.6 mL/min. The detection was accomplished by a triple-quadrupole tandem mass spectrometer in multiple-reaction monitoring (MRM) scanning via an electrospray ionization (ESI) source operating in the negative mode. The optimized mass transition ion-pairs (m/z) for quantitation were 327.3/192.0 for bergenin, and 431.1/311.1 for IS. The time for each analysis run was only 3.5 min between injections. The calibration curve exhibited good linearity (r² > 0.99) over a range of 1.00–2000 ng/mL for bergenin. The lower limit of quantitation (LLOQ) was 1.00 ng/mL. The intra- and inter-day precisions were no more than 11.8%, and relative errors (RE) were within the range of 0.0–4.4%. The validated method was successfully applied to investigate the pharmacokinetics of bergenin after oral administration of B. purpurascens extract in rats.

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1. Introduction

Yanbaicai, the rhizome of Bergenia purpurascens, is a well-known and widely used traditional Chinese medicine (TCM) for relief against cough and asthma in Chinese folk [1,2]. Biological and
pharmacological studies had shown that B. purpurascens extract showed antioxidant, anti-inflammatory and immunologic enhancement activities [3–5]. Phytochemical investigation indicated that more than 20 compounds had been identified from the plant B. purpurascens, in which bergenin was the principal compound contributing to the biological activity [6–9]. Bergenin, a C-glucoside of 4-O-methyl gallic acid isolated from B. purpurascens has a variety of pharmacological activities, including antiarrhythmic, antipatotoxic, antulcerogenic, anti-inflammatory, anti-HIV, antimicrobial, immunomodulatory and neuroprotective properties [10–13]. To date, there is no report about the pharmacokinetics of the B. purpurascens extract in vivo, even though several reports for pharmacokinetics have estimated the concentration of bergenin after oral administration of bergenin tablets by LC–MS/MS [14,15]. Compared with the published papers [14,15], in the present study, we have developed a much more sensitive (LLOQ, 1.00 ng/mL) and rapid (running time, 3.5 min) LC–MS/MS method to measure bergenin in rat plasma for the first time after oral administration of aqueous B. purpurascens extract.

2. Materials and methods

2.1. Chemicals and reagents

Bergenin (purity > 99.0%, determined by HPLC) was isolated from B. purpurascens in our laboratory. The structure of bergenin (Fig. 1A) was fully characterized by 1H, 13C NMR and MS data (see the supporting information, Table S1). Isovitexin (purity > 98%, determined by HPLC, Fig. 1B) was used as an internal standard (IS), which was isolated from the leaves of Santalum album L. and characterized by NMR technique (see the supporting information, Table S1). HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Deionized water was prepared by a Milli-Q system (Millipore, MA, USA).

2.2. Instrumentation

An Agilent 1200 LC system consisting of a degasser, a binary pump, an SL autosampler and a thermostatic column compartment was coupled with a 6410 triple-quadrupole mass spectrometer (Agilent Technologies, USA), which was equipped with an electrospray ionization (ESI) source and operated with Agilent MassHunter B.01.03 software (Agilent Technologies, USA).

2.3. Chromatographic and mass spectrometric conditions

Chromatographic separation was performed on a Diamonsil® C18 column (150 mm × 4.6 mm, 5 μm; Beijing, China) protected by a Phenomenex C18 guard column (Torrance, CA, USA). The mobile phase consisted of water (A) and methanol (B) using an isocratic elution with a mixture of 30 volumes of solvent A and 70 volumes of solvent B. The flow rate was 0.6 mL/min, and the injection volume was 20 μL. The time for each analysis run was only 3.5 min, and a divert valve was used to divert the eluent to waste from 0 to 1.9 min, and to MS from 1.9 min to 3.5 min.

The mass spectrometer was operated in negative ESI scan mode for bergenin. Quantification was obtained using multi-reaction monitoring (MRM) transition, which was selected as m/z 327.3 → m/z 192.0 for bergenin and m/z 431.1 → m/z 311.1 for IS (Fig. 2). MS/MS conditions were optimized as follows: gas temperature, 300 °C; gas flow, 10 L/min; nebulizer, 30 psi; collision gas (N2), 0.35 Pa. The fragment energy was set at 110 V and 160 V for the analyte and IS, respectively. The collision energy was optimized to be 15 eV and 18 eV for the analyte and IS, respectively.

2.4. Preparation of B. purpurascens extract

Ten grams B. purpurascens shattered into powder was extracted three times with 50% ethanol under reflux. After the removal of the major solvent in vacuum, the aqueous residue was then diluted with water to get the B. purpurascens extract with a concentration equivalent to 1.58 g/mL of the raw B. purpurascens material.

To calculate the administration dose, the content of bergenin in aqueous B. purpurascens extract was quantitatively determined using the LC–MS/MS method described above. The content of bergenin in the extract was 16.3 mg/mL.

2.5. Preparation of standard and quality control (QC) samples

Standard stock solution of 100 μg/mL bergenin was accurately prepared by dissolving appropriate amounts of the standard in acetonitrile. A 1000 ng/mL isovitexin solution as IS was also prepared in acetonitrile. All solutions were stored at 4 °C.

Calibration standards were prepared by spiking appropriate amount of the standard solutions in 100 μL of blank rat plasma to yield final concentrations of 1.00, 3.00, 10.0, 30.0, 100, 300, 1000, and 2000 ng/mL for bergenin. Three concentration levels of QC samples were prepared containing bergenin (3.00, 30.0, 1800 ng/mL) in the same manner.

Fig. 1 Chemical structure of bergenin and isovitexin (IS).
2.6. Sample preparation

100 μL of rat plasma samples (Calibration standards, QC samples, and pharmacokinetic plasma samples) was mixed with 100 μL of acetonitrile and 100 μL of IS working solution. The mixture was vortexed for 1 min and then centrifuged at 10,000 rpm for 5 min. Then about 100 μL of the supernatant was transferred to the autosampler vials ready for injection into the LC–MS/MS system.

2.7. Method validation

The method performance was validated according to the FDA recommendations proposed in 2001 [16,17]. Several validation characteristics were investigated, such as selectivity, linearity, lower limit of quantification (LLOQ), precision, accuracy, matrix effect, extraction recovery and stability.

2.8. Pharmacokinetic study

Six male Wistar rats (200±20 g) were purchased from the Experimental Animal Center of Guangdong Medical College (Dongguan, China). The rats were housed in an air-conditioned room at temperature of 22±2 °C and a relative humidity of 50±10% with a 12 h dark–light cycle and allowed food and water spontaneously. Rats were fasted for 12 h before the experiment with water freely available.

After an oral administration of aqueous *B. purpurascens* extract at a dose of 6 mL/kg (equivalent 97.8 mg bergenin/kg body weight), blood samples were harvested into 1.5 mL heparinized eppendorf tubes from each rat via the orbital vein before dosing and at 0.083, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 5.0, 8.0, 12.0 and 24.0 h. After centrifugation at 3000 rpm/min for 10 min, plasma samples were transferred to neat tubes and stored at −20 °C until analysis. All pharmacokinetic parameters were evaluated by noncompartmental analysis using DAS 2.0 software (Chinese Pharmacological Society, China).

3. Results and discussion

3.1. Method development

In the preliminary experiment the ESI and atmospheric pressure chemical ionization (APCI) sources were chosen to obtain good and stable MS response. Bergenin and IS could be ionized under negative ESI or APCI conditions. It was found that ESI can provide higher sensitivity for the analyte and IS than that of APCI.

Different chromatographic conditions, especially the composition of mobile phase, were assessed to achieve good resolution and strong MS intensity, as well as short run time. Various combinations of acetonitrile, methanol and water were tested and compared to identify the appropriate mobile phase. As a result, a mixture consisting of water and methanol (30:70, v/v)
was finally adopted as the mobile phase. Flow rate of 0.6 mL/min produced good peak shapes and short run time (3.5 min).

3.2. Assay validation

3.2.1. Selectivity

Under the chromatographic conditions described above, bergenin and IS were eluted at 2.62 and 2.68 min (Fig. 3), respectively. No obvious interfering endogenous substances were observed at the retention times of bergenin and IS in blank plasma sample.

3.2.2. Linearity and LLOQ

The linear regressions of the peak area ratios versus concentrations were fitted over the concentration range 1.00–2000 ng/mL for bergenin in rat plasma. A typical equation of the calibration curve was $y = 3.9781 \times 10^{-5}x + 6.1424 \times 10^{-5}$, $r^2=0.9936$, where $y$ represents the ratio of bergenin peak area to that of IS and $x$ represents the plasma concentration. The LLOQ of bergenin in plasma was 1.00 ng/mL. The present method exhibited a good linearity and sensitivity.

3.2.3. Precision and accuracy

The intra- and inter-day precision and accuracy of bergenin are summarized in Table 1. In the present assay, the intra- and

| Added (ng/mL) | Found (ng/mL) | Precision (RSD%) | Accuracy (RE%) |
|--------------|--------------|------------------|---------------|
|              |              | Intra-day        | Inter-day     |
| 3.00         | 3.00 ± 0.15  | 5.4              | 0.6           |
| 30.0         | 31.0 ± 1.2   | 4.0              | 3.4           |
| 1800         | 1878 ± 124   | 4.0              | 4.4           |

Table 2 Extraction recoveries and matrix effects of bergenin in rat plasma ($n=3$).

| Added (ng/mL) | Extraction recovery (%) | Matrix effect (%) |
|--------------|-------------------------|------------------|
| Bergenin     |                         |                  |
| 3.00         | 100.5 ± 2.7             | 102.1 ± 0.8      |
| 30.0         | 99.3 ± 2.6              | –                |
| 1800         | 99.1 ± 5.6              | 99.0 ± 3.2       |
| Isovitexin (IS) |                    |                  |
| 1000         | 101.3 ± 1.9             | 105.1 ± 2.3      |
inter-day precision (RSD) were within 5.4% and 11.8%, respectively; the accuracy did not exceed 4.4%. The obtained data were within acceptable criteria and conformed to the guidelines for bioanalytical method validation [16], which indicated that the present method was reproducible and reliable for the determination of bergenin in rat plasma samples.

3.2.4. Matrix effect (ME)

The MEs for bergenin at concentrations of 3.00 and 1800 ng/mL were measured to be 102.1 ± 0.8% and 99.0 ± 3.2% (n = 3), respectively. The ME for IS was 105.1 ± 2.3% (n = 3). As a result, the ME from plasma was negligible in this method (Table 2).

3.2.5. Extraction recovery

The extraction recoveries of bergenin at three QC levels (3.00, 30.0, 1800 ng/mL) were determined by comparing the peak area of the analyte to which the analyte was added post-protein precipitation at equivalent concentrations. The recovery of the IS (1000 ng/mL) was determined in a similar way. At three concentration levels of the analyte, the recoveries were between 99.1 ± 5.6% and 100.5 ± 2.7% (Table 2).

3.2.6. Stability

The freeze–thaw stability was determined after three freeze–thaw cycles at −20 °C with a minimal interval of 24 h. The long-term stability was assessed after keeping the samples at ambient condition for 2 h. Post-preparative stability was evaluated by analyzing post-protein precipitation samples kept in the autosampler at ambient condition for 12 h. The results of the stability study are presented in Table 3, which confirm the high stability of bergenin throughout the determination.

3.3. Pharmacokinetics application

After oral administration of B. purpurascens extract at a dose of 6 mL/kg (equivalent 97.8 mg bergenin/kg body weight) to six Wistar rats, their plasma concentration of bergenin was determined by the described LC–MS/MS method. The mean plasma concentration–time profile is represented in Fig. 4 and the main pharmacokinetic parameters are listed in Table 4. Plasma concentration of bergenin was detectable as early as 10 min after administration, with a mean peak concentration of 275 ± 164 ng/mL at 0.292 ± 0.102 h.
in plasma ($C_{\text{max}}$) of 275 ± 164 ng/mL and the time to peak ($T_{\text{max}}$) of 0.29 ± 0.102 h. The elimination half life ($t_{1/2}$), mean residence time (MRT) and area under the concentration–time curve from time 0 to 24 h (AUC$_{0-24}$) were 8.03 ± 3.24 h, 4.99 ± 0.66 h and 548 ± 408 ng h/mL, respectively.

4. Conclusion

In general, a rapid and sensitive LC–MS/MS method was developed and validated for the determination of bergenin in rat plasma after oral administration of $B$. purpurascens extract. The pharmacokinetics obtained from this study and the method developed herein might provide useful information for clinical application of this TCM.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jpha.2013.01.005.

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