Simultaneous determination of ginkgolide A, B, C, bilobalide and rutin in rat plasma by LC-MS/MS and its application to a pharmacokinetic study

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Received: August 4, 2021 • Accepted: September 28, 2021

ABSTRACT

A reliable LC-MS/MS method for the determination of five bioactive constituents (bilobalide, BLL; ginkgolide A, GLA; ginkgolide B, GLB; ginkgolide C, GLC; rutin) of Ginkgo biloba leaf extracts (GBE) in rat plasma was established, fully validated and applied to an intragastric pharmacokinetic study of a preparation of GBE in rat. Samples were extracted with ethyl acetate. C18 column was selected as analytical column in this method. Mobile phase was water with 0.01% formic acid and acetonitrile. Quantification was performed in negative multiple-reaction monitoring mode. Matrix instability of terpene lactones was noticed and hydrochloric acid was used as a stabilizer. This method showed good precision and accuracy, recovery was reproducible and matrix effect was negligible. Among four terpene lactones, BLL had the highest exposure and the shortest terminal half-life, GLA and GLB had lower exposure and longer terminal half-life, the exposure of GLC was lowest and its terminal half-life was the maximum, and all of them showed rapid absorption. This study provides a reference for determination of terpene lactones and flavonol glycoside prototypes in GBE and offers pharmacokinetic data of flavonol glycoside prototype in GBE.

KEYWORDS

Ginkgo biloba, terpene lactones, flavonol glycosides, stabilizer, pharmacokinetic

INTRODUCTION

Ginkgo biloba leaf extracts (GBE) are clinically applied worldwide to cure cardiovascular diseases and central nervous system diseases, such as myocardial ischemia reperfusion injury [1], coronary heart disease [2], acute ischemic stroke [3] and Alzheimer’s disease [4].

Existing research showed that terpene lactones and flavonol glycosides were the main bioactive constituents of GBE [5]. Terpene lactones in GBE can be classified as diterpenes (ginkgolides) and sesquiterpene (bilobalide) [6]. Some determination methods for terpene lactones of GBE have been reported in previous researches [6–8], and there wasn’t any stability problem of terpene lactones in plasma been mentioned in these studies, however the instability of lactones in plasma can be predicted readily [9]. If the stability of analytes in the
whole life cycle of samples cannot be guaranteed, then the reliability of these studies is questionable. Therefore, it was necessary to develop a more reliable analytical method for determination of four terpene lactones in rat plasma.

Structural diversity of flavonol glycosides in GBE was observed. Nearly 30 flavonol glycosides were found, but most of them are derived from three aglycones [10, 11]. Previous, flavonol glycosides were indirectly monitored by their aglycones after hydrolysis [7, 12], due to the complexity of flavonol glycosides. However, different flavonol glycosides with same aglycone might have diverse pharmacokinetic properties, so the results based on aglycones might be biased.

Moreover, the pharmacokinetic data of flavonol glycoside prototypes in GBE is still scarce, hence an analytical method for determination of flavonol glycoside prototypes and a pharmacokinetic research of them were needed. Rutin was an abundant flavonol glycoside of GBE [13, 14] and was selected as a representative flavonol glycoside to be investigated in this study.

To quantify the rat plasma concentrations of bilobalide (BLL), ginkgolide A (GLA), ginkgolide B (GLB), ginkgolide C (GLC) and rutin, we established and validated a reliable LC-MS/MS method. Furthermore, an intragastro pharmacokinetic study of crushed Yinxing Tongzhi Dripping Pills, a preparation of GBE, in rat was conducted. In addition, the simultaneous dose proportionality assessment of $C_{\text{max}}$ and AUC for GLA, GLB, GLC and BLL was firstly reported in public.

**EXPERIMENTAL**

**Reagents and materials**

GLA (purity >97.4%), GLB (purity >95.6%), GLC (purity >94.0%), BLL (purity >99.8%), rutin (purity >91.7%) and chloramphenicol (CHL, purity >99.8%) were provided by National Institutes for Food and Drug Control (China).

Ethyl acetate was manufactured by TEDIA (USA). Methanol and acetonitrile were manufactured by Merck (Germany). Pure water was prepared by Cascade I Water Purification System of Pall Corporation (USA). Formic acid was manufactured by Anaqua Chemicals Supply (USA). Heparin sodium was manufactured by Sinopharm Chemical Reagent Co.,ltd. (China) and hydrochloric acid (guaranteed reagent) was manufactured by Beijing Leagene Biotech.co.,ltd. (China) and hydrochloric acid (guaranteed reagent) was manufactured by Sinopharm Chemical Reagent Co.,ltd. (China). Blank rat plasma was prepared in our laboratory before method validation.

Yinxing Tongzhi Dripping Pills were supplied by Zhejiang Jiuxu Pharmaceutical Co., Ltd. (China). Total content of four terpene lactones was 9.4%, and content of total flavonol glycosides was 29%.

**UPLC-MS/MS instruments and parameters**

An API 5500 Triple Quad UPLC-MS/MS System (AB SCIEX, USA) was employed for UPLC-MS/MS analysis.

Waters ACQUICY BEH C18 (2.1 × 100 mm, 1.7 µm) was selected as analytical column in this method. Mobile phase was water with 0.01% formic acid (A) and acetonitrile (B) and flow rate was 0.3 mL min$^{-1}$. The gradient elution was shown below: 0–0.5 min, 15% B; 0.5–2.5 min, 15–30% B; 2.5–4.5 min, 30–45% B; 4.5–5.0 min, 45–95% B; 5.0–6.5 min, 95% B; 6.5–6.6 min, 95–15% B; 6.6–8.0 min, 15% B. The temperature of column oven and autosampler were 40 and 4 °C, respectively.

Quantification was performed in electrospray ionization source negative mode. The scan mode was multiple-reaction monitoring. Dominating mass spectrometer parameters were exhibited in Table 1. Ion source temperature, ionspray voltage, curtain gas, nebulizing gas and auxiliary gas were 550 °C, −4500 V, 35 psi, 45 psi and 45 psi, respectively.

**Animals**

Specific pathogen-free SD Rats (9 males, 9 females, body weight 213–308 g) were produced by the Experimental Animal Center of Zhejiang Academy of Medical Sciences. All experiment schemes of animal study were permitted by Animal Ethics Committee of Zhejiang Academy of Medical Sciences (2019-274).

**Preparation of stock solutions and working solutions**

Stock solutions for calibration standard of BLL (1.00 mg mL$^{-1}$), GLA (1.10 mg mL$^{-1}$), GLB (0.99 mg mL$^{-1}$), GLC (1.01 mg mL$^{-1}$) and rutin (1.28 mg mL$^{-1}$) were yielded in methanol respectively. Meanwhile, five analytes were dissolved into methanol respectively to yield another series of stock solution for quality control (QC), the concentrations of analytes were as follows: 1.01 mg mL$^{-1}$ (GLA), 1.13 mg mL$^{-1}$ (GLB), 1.06 mg mL$^{-1}$ (GLC), 0.12 mg mL$^{-1}$ (BLL) and 1.00 mg mL$^{-1}$ (rutin).

Stock solution of internal standard (IS, 1.04 mg mL$^{-1}$) was yielded by dissolving standards of chloramphenicol into methanol.

All working solutions of five analytes and IS (25 ng mL$^{-1}$) was yielded through a dilution with methanol. The preservation temperature of all solutions was 4 °C.

**Sample preparation**

60 µL of sample (including 10 µL of stabilizer) was unfrozen in 25 °C water bath prior to processing. Then, sample and 10 µL of working solution of CHL was blended for 1 min and extracted with 400 µL of ethyl acetate immediately. Next,
blend sample by a multi-tube vortexer for 5 min and centrifuge for 5 min at 8,000 g. After that, dryness of organic phase was conducted at ambient temperature under nitrogen. Finally, 100 μL of water-methanol (1:1, v/v) was used to reconstitute residue and 10 μL of processed sample was detected.

Method validation

Blank rat plasma from six lots and lower limit of quantification (LLOQ) samples were processed in parallel. Selectivity was validated by computing the peak area ratio (blank samples/LLOQ sample).

Signal to noise (S/N) ratio of LLOQ sample was measured to assess sensitivity.

Through the detection of two blank plasma samples straight after upper limit of quantification (ULOQ) sample, carryover effect was appraised.

2 μL of working solutions of calibration standards was added into 58 μL of blank rat plasma (including 10 μL of stabilizer) to prepare calibration standards at seven concentration levels. Calibration curves were created depend on the nominal concentration ratio of analytes to CHL (X-axis) and the peak area ratio (Y-axis) in calibration standards and 1/(x²) was used as weighting factor.

2 μL of working solutions of LLOQ, low quality control (LQC), middle quality control (MQC) and high quality control (HQC) were added into 58 μL of blank plasma (including 10 μL of stabilizer) respectively to prepare QC samples. Then, QC samples were determined in three independent analytical runs to investigate the precision and accuracy.

Dilution quality control (DQC) samples with concentration above ULOQ were diluted with nine-fold volume of blank rat plasma. Based on precision and accuracy of diluted QC samples, dilution integrity was evaluated.

Working solutions of LQC, MQC and HQC were added into blank rat plasma post or before extraction. By calculated the peak area ratio (pre-extraction/post-extraction), extraction recovery of five analytes and CHL was obtained.

Working solutions of LQC and HQC were added into blank rat plasma from six lots or pure water post-extraction respectively. Matrix effect of five analytes and CHL was assessed based on the peak area ratio (rat plasma/water).

Prior to preparation, LQC and HQC samples were exposed to ambient temperature for short-range (3 h), −70 °C for long-term (20 d) and two freeze–thaw cycles to explore the matrix stability of analytes.

Prior to injection, processed LQC, MQC and HQC samples were exposed to autosampler temperature for 24 h to study the processed sample stability of analytes.

Pharmacokinetic study

Before dosing, eighteen rats (nine males and nine females) were fasted for more than 8 h but drank water freely. Rats were randomized into three dosage groups (50, 100, or 200 mg kg⁻¹) according to body weight and administered orally with a suspension of crushed Yinxing Tongzhi Dipping Pills (prepared with 0.5% carboxy methylcellulose sodium). At pre-dose and 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h post-dose, about 0.2 mL blood samples from jugular vein were obtained and were stored in heparinized centrifuge tubes on wet ice. Those harvest samples were centrifuged for 10 min at 8,000 g within 0.5 h. Then 10 μL of 0.2 mol L⁻¹ hydrochloric acid as a stabilizer was added into 50 μL of plasma. The preservation temperature of samples was −70 °C.

Data analysis

The UPLC-MS/MS data was obtained from Analyst software (version 1.6.3) and analyzed by MultiQuant software (version 3.0.2). Phoenix WinNonlin (version 8.1.0) was adopted to compute pharmacokinetic parameters of analytes based on non-compartment model. Dose proportionality assessment was performed with confidence interval approach [15–17] using Phoenix WinNonlin software (version 8.1.0).

RESULTS AND DISCUSSION

Method optimization

To realize an excellent MS response, MS parameters of five analytes and CHL were optimized. MS response of terpene lactones was rather poor in positive mode, but in negative mode, all of the analytes and CHL showed good response, so negative mode was chosen. MS/MS spectra of five analytes and CHL in negative mode were displayed in Fig. 1.

To develop an appropriate extraction method, protein precipitation and liquid-liquid extraction were assessed. When plasma samples (1 ng mL⁻¹) were processed with methanol protein precipitation, analytes showed poor MS response. When the same samples were handled with methyl tert-butyl ether (MTBE), terpene lactones showed high response, but the peak of rutin cannot be observed. Alternatively, ethyl acetate was used as extraction solvent, good response was achieved for all analytes. It might be related to the higher solubility of rutin in ethyl acetate than in MTBE.

As predicted, matrix instability of four terpene lactones was noticed during method development. When LQC and HQC samples were exposed to room temperature for 4 h, terpene lactones were reduced by 29–79%. In contrast, when samples were treated with 0.2 mol L⁻¹ hydrochloric acid, the degradation of terpene lactones was insignificant after the storage at room temperature for 4 h. Meanwhile, hydrochloric acid makes no difference to the plasma concentration of rutin. The result showed that 0.2 mol L⁻¹ hydrochloric acid was a suitable stabilizer for this study. Significantly, although hydrochloric acid was added into samples during samples preparation in some literature [7, 18], the stability of terpene lactones cannot be guaranteed before sample preparation.
Method validation

As exhibited in Fig. 2, in six lots of blank plasma, there was no conspicuous interference peak of five analytes and CHL.

In the chromatogram of LLOQ sample, the S/N ratio of analytes was greater than five (Fig. 2).

In blank rat plasma samples after ULOQ sample, carryover of analytes and IS was acceptable.

Within the concentration scope, calibration curves were investigated. Typical calibration curves were listed in Table 2. In all batches, the correlation coefficients of calibration curves and the back-calculated concentrations of calibrators at least six levels met the acceptance criteria.

Data of precision and accuracy was summarized in Table 3. Precision (RSD) and accuracy (RE) of diluted DQC samples were within 15.0%, as listed in Table 3, indicating
that samples at concentration exceeded ULOQ could be diluted with blank matrix and then be detected.

As summarized in Table 4, matrix effect was negligible and recovery was reproducible.

As exhibited in Table 5, when exposed to ambient temperature for short-term (3 h), −70 °C for long-term (20 d) and two freeze–thaw cycles, analytes were steady in rat plasma.

When kept at autosampler temperature for 1 d, all of analytes were stable in processed samples (Table 5).

**Pharmacokinetic study**

The pharmacokinetic profiles of GLA, GLB, GLC, BLL and rutin in rats after intragastric administration of crushed Yinxing Tongzhi Dripping Pills were presented in Fig. 3. Because the sensitivity of method is limited (LLOQ of rutin was 0.2 ng mL\(^{-1}\)), the concentration of rutin in most samples from 50.0 to 100 mg kg\(^{-1}\) dosage groups was below quantitation limit, corresponding concentration-time curves couldn’t be drawn, and relevant concentration data was listed in Table S1 and S2. The pharmacokinetic parameters of four terpene lactones were listed in Table 6 (Pharmacokinetic parameters of rutin cannot be calculated due to low plasma concentration). BLL had the highest C\(_{\text{max}}\) and AUC, and the shortest terminal half-life, indicating its good absorption and fast elimination. GLA and GLB exhibited lower exposure and longer terminal half-life. The exposure of GLC was lowest and its terminal half-life was the maximum, in other words, GLC showed poor absorption and slow elimination. Moreover, the T\(_{\text{max}}\) of BLL, GLA and GLB was within 1 h and the T\(_{\text{max}}\) of GLC was within 1.5 h, demonstrating their rapid absorption.

| Analyte | Regression equation | Correlation coefficient (r) | Linear range (ng mL\(^{-1}\)) |
|---------|---------------------|-----------------------------|------------------------------|
| GLA     | y = 5.47217x + 0.03364 | 0.99680                     | 1.00–600                     |
| GLB     | y = 11.51566x + 0.03959 | 0.99721                     | 0.500–100                    |
| GLC     | y = 5.94723x + 0.00459  | 0.99814                     | 0.200–20.0                   |
| BLL     | y = 6.38369x + 1.03455e\(^{-4}\) | 0.99953               | 0.500–300                    |
| rutin   | y = 1.99244x + 0.00812 | 0.99325                     | 0.200–10.0                   |

**Table 3. Accuracy and precision**

| Analyte | Theoretical concentration (ng mL\(^{-1}\)) | Average observed concentration (ng mL\(^{-1}\)) | RSD (%) | RE (%) | Average observed concentration (ng mL\(^{-1}\)) | RSD (%) | RE (%) |
|---------|------------------------------------------|---------------------------------------------|--------|------|---------------------------------------------|--------|------|
| GLA     | 1.00                                     | 0.929                                       | 4.8    | −7.1 | 1.01                                       | 8.0    | 0.7  |
|         | 3.00                                     | 3.06                                        | 3.5    | 1.9  | 3.11                                       | 4.0    | 3.8  |
|         | 40.0                                     | 42.2                                        | 1.9    | 5.4  | 41.9                                       | 3.1    | 4.9  |
|         | 500                                      | 448                                         | 2.2    | −10.4 | 446                                       | 2.1    | −10.8 |
|         | 5,000                                    | 4,373                                       | 0.9    | −12.5 |                                           |        |      |
| GLB     | 0.500                                    | 0.453                                       | 6.2    | −9.4 | 0.493                                       | 7.6    | −1.4 |
|         | 1.5                                       | 1.55                                        | 3.8    | 3.2  | 1.57                                       | 3.9    | 4.9  |
|         | 10.0                                     | 10.3                                        | 3.3    | 2.5  | 10.2                                       | 4.5    | 2.4  |
|         | 80.0                                     | 74.0                                        | 2.3    | −7.5 | 73.8                                       | 2.2    | −7.8 |
|         | 800                                      | 726                                         | 2.3    | −9.2 |                                           |        |      |
| GLC     | 0.200                                    | 0.174                                       | 9.8    | −12.8 | 0.192                                       | 10.0   | −4.2 |
|         | 0.600                                    | 0.581                                       | 4.2    | −3.3 | 0.590                                       | 4.5    | −1.6 |
|         | 3.00                                     | 3.03                                        | 1.3    | 0.9  | 2.99                                       | 3.2    | −0.4 |
|         | 16.0                                     | 15.6                                        | 2.9    | −2.3 | 15.7                                       | 3.1    | −2.2 |
|         | 160                                      | 149                                         | 2.4    | −6.7 |                                           |        |      |
| BLL     | 0.500                                    | 0.498                                       | 7.6    | −0.4 | 0.516                                       | 6.8    | 3.2  |
|         | 1.5                                       | 1.46                                        | 3.1    | −2.4 | 1.48                                       | 4.1    | −1.2 |
|         | 20.0                                     | 19.0                                        | 2.8    | −5.1 | 19.4                                       | 4.5    | −3.1 |
|         | 250                                      | 227                                         | 2.7    | −9.3 | 229                                        | 2.7    | −8.4 |
|         | 2,500                                    | 2,434                                       | 1.3    | −2.6 |                                           |        |      |
| rutin   | 0.200                                    | 0.193                                       | 17.7   | −3.6 | 0.194                                       | 15.0   | −2.9 |
|         | 0.600                                    | 0.623\(^{a}\)                               | 11.0\(^{a}\) | 3.8\(^{a}\) | 0.581\(^{b}\) | 8.7\(^{b}\) | −3.2\(^{b}\) |
|         | 2.00                                     | 2.25                                        | 5.1    | 12.7 | 2.04                                       | 10.7   | 2.1  |
|         | 8.00                                     | 9.02                                        | 6.1    | 12.7 | 8.50\(^{b}\)                                 | 8.9\(^{b}\) | 6.3\(^{b}\) |
|         | 80.0                                     | 91.1\(^{b}\)                                | 4.2\(^{b}\) | 13.9\(^{b}\) |                                           |        |      |

\(^{a}\)n = 5. One value among six replicates was abnormal and statistical analysis was calculated excluding it.  
\(^{b}\)n = 17. One value among eighteen replicates was abnormal and statistical analysis was calculated excluding it. 
RSD, Relative Standard Deviation; RE, Relative error.
The low plasma concentration of rutin indicated its poor oral bioavailability, which was consistent with previous studies [19–21]. As shown in Table 7, at the dosage ranging from 50.0 to 200 mg kg⁻¹, 90% confidence interval of GLA, GLB and GLC only partially overlap corresponding acceptance interval, showing that the dose proportionality was inconclusive. Similarly, inconclusive results of AUC₀−ₜ and AUC₀−∞ were observed for BLL. But for the slope ln(Cₘₐₓ) to ln(dose) of BLL, 90% confidence interval was completely within the acceptance interval, indicating that a proportional change in Cₘₐₓ across the dose range studied.

The number of experiment animals was limited in this study, so these results must be confirmed on a larger group of animals in the future.

CONCLUSIONS

To quantify the rat plasma concentrations of bilobalide, ginkgolide A, B, C and rutin, we established and validated a reliable LC-MS/MS method. Hydrochloric acid was a suitable stabilizer for this study. This method provides a reference for simultaneous determination of terpene lactones and

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### Table 4. Recovery and matrix effect

| Analyte | Theoretical concentration (ng mL⁻¹) | Recovery Mean | RSD (%) | IS normalized matrix factor Mean | RSD (%) |
|---------|-----------------------------------|---------------|---------|---------------------------------|---------|
| GLA     | 3.00                              | 79.1          | 2.8     | 88.8                            | 2.2     |
|         | 40.0                              | 92.8          | 1.3     |                                 |         |
|         | 500                               | 95.0          | 1.4     | 89.6                            | 3.7     |
| GLB     | 1.50                              | 86.2          | 1.7     | 95.3                            | 4.7     |
|         | 10.0                              | 91.3          | 3.2     |                                 |         |
|         | 80.0                              | 96.1          | 1.9     | 94.8                            | 3.3     |
| GLC     | 0.600                             | 87.7          | 4.7     | 100                             | 4.4     |
|         | 3.00                              | 91.3          | 2.9     |                                 |         |
|         | 16.0                              | 96.0          | 3.2     | 99.8                            | 3.8     |
| BLL     | 1.50                              | 83.0          | 1.6     | 98.2                            | 5.1     |
|         | 20.0                              | 92.8          | 2.2     |                                 |         |
|         | 250                               | 94.2          | 1.2     | 97.0                            | 3.2     |
| rutin   | 0.600                             | 86.4          | 11.2    | 92.3                            | 14.8    |
|         | 2.00                              | 73.5⁺         | 12.2⁺   | 119                             | 11.4    |
|         | 8.00                              | 60.1          | 2.4     | 119                             | 11.4    |
| CHL     | 25.0                              | 92.6          | 3.8     |                                 |         |

⁺n = 5. One value among six replicates of MQC samples was abnormal and statistical analysis was calculated excluding it.

### Table 5. Matrix stability and processed sample stability of five analytes (n = 6)

| Analyte | Theoretical concentration (ng mL⁻¹) | Preservation at ambient temperature for 3 h | RSD (%) | RE (%) | Preservation at −70°C for 20 d | RSD (%) | RE (%) | Two freeze–thaw cycles | RSD (%) | RE (%) | Processed sample stability kept for 1 d at autosampler | RSD (%) | RE (%) |
|---------|-----------------------------------|--------------------------------------------|---------|--------|--------------------------------|---------|--------|------------------------|---------|--------|-----------------------------------------------------|---------|--------|
| GLA     | 3.00                              | 4.5                                        | 13.8    |        | 0.9                                           | 14.9    |        | 2.6                                                | −6.2    | 3.8    | 14.2                                               |         |        |
|         | 40.0                              | 4.1                                        | −4.7    |        | 1.6                                           | −10.8   |        | 2.0                                                | −7.9    | 2.3    | −2.6                                               |         |        |
| GLB     | 1.50                              | 3.0                                        | 6.3     |        | 1.5⁺                                          | 14.7⁺   |        | 2.9                                                | 4.6     | 3.4    | 10.0                                               |         |        |
|         | 10.0                              | 2.6                                        | −2.5    |        | 2.1                                           | 3.3     |        | 2.8                                                | −5.3    | 4.1    | 0.0                                                |         |        |
| GLC     | 0.600                             | 5.5                                        | 0.8     |        | 2.0                                           | 8.1     |        | 2.6                                                | 1.2     | 3.9    | 7.1                                                |         |        |
|         | 3.00                              | 5.5                                        | −0.6    |        | 1.8                                           | 2.4     |        | 3.4                                                | −2.8    | 2.9    | 5.5                                                |         |        |
|         | 16.0                              | 4.7                                        | 3.7     |        | 1.8                                           | 14.9    |        | 2.7                                                | −10.0   | 3.2    | 7.7                                                |         |        |
|         | 20.0                              | 4.1                                        | −2.3    |        | 1.8                                           | −1.9    |        | 2.4                                                | −6.8    | 4.1    | 1.5                                                |         |        |
| BLL     | 1.50                              | 3.0                                        | −8.4    |        | 6.8                                           | −1.1    |        | 3.2                                                | 10.8    | 7.6    | 2.1                                                |         |        |
|         | 20.0                              | 8.3                                        | −10.0   |        | 3.5                                           | 1.1     |        | 9.3                                                | 9.9     | 5.8    | 4.2                                                |         |        |

⁺n = 5. One value among six replicates was abnormal and statistical analysis was calculated excluding it.
**Fig. 3.** Average plasma concentration-time curves of five analytes and CHL. (A) BLL, (B) GLA, (C) GLB, (D) GLC and (E) rutin in rats after intragastric administration of crushed Yinxing Tongzhi Dipping Pills

**Table 6.** Pharmacokinetic parameters of four terpene lactones in rats after intragastric administration of crushed Yinxing Tongzhi Dipping Pills (Mean ± SD, n = 6)

| Compound | Dose (mg kg⁻¹) | Parameter | \(\text{AUC}_{0-\text{t}}\) (h*ng mL⁻¹) | \(\text{AUC}_{0-\infty}\) (h*ng mL⁻¹) | MRTₜ₀⁻₄ | MRTₜ₀⁻∞ | t₁/₂ | \(T_{\text{max}}\) (h) | \(C_{\text{max}}\) (ng mL⁻¹) |
|----------|----------------|-----------|---------------------------------|---------------------------------|--------|--------|------|-----------------|-----------------|
| GLA      | 50.0           |           | 471 ± 159                       | 475 ± 159                       | 1.93 ± 0.24 | 2.05 ± 0.23 | 1.99 ± 0.73 | 0.75 ± 0.27 | 184 ± 44.6       |
|          | 100            |           | 861 ± 208                       | 871 ± 207                       | 2.01 ± 0.13 | 2.17 ± 0.14 | 2.24 ± 0.15 | 0.66 ± 0.13 | 316 ± 50.6       |
| GLB      | 50.0           |           | 1,560 ± 417                     | 1,601 ± 416                     | 2.22 ± 0.33 | 2.64 ± 0.74 | 3.24 ± 1.38 | 0.43 ± 0.19 | 562 ± 62.7       |
|          | 100            |           | 288 ± 45.1                      | 295 ± 44.9                      | 2.47 ± 0.18 | 2.83 ± 0.30 | 2.58 ± 1.03 | 0.80 ± 0.19 | 961 ± 25.3       |
| GLC      | 50.0           |           | 20.3 ± 4.49                     | 22.4 ± 3.96                     | 2.74 ± 0.30 | 5.05 ± 3.28 | 5.74 ± 5.52 | 1.38 ± 0.54 | 5.80 ± 1.41      |
|          | 100            |           | 33.1 ± 8.73                     | 36.2 ± 10.8                     | 2.84 ± 0.18 | 4.31 ± 2.22 | 4.29 ± 3.53 | 1.29 ± 0.46 | 9.19 ± 2.41      |
| BLL      | 50.0           |           | 52.6 ± 11.4                     | 60.6 ± 21.4                     | 2.98 ± 0.27 | 5.04 ± 2.98 | 4.97 ± 3.04 | 1.22 ± 0.31 | 15.4 ± 5.53      |
|          | 100            |           | 549 ± 106                       | 551 ± 107                       | 1.82 ± 0.14 | 1.85 ± 0.15 | 1.80 ± 0.21 | 0.83 ± 0.13 | 211 ± 34.3       |
|          | 200            |           | 1,103 ± 90.1                    | 1,105 ± 89.6                    | 1.90 ± 0.05 | 1.92 ± 0.05 | 1.34 ± 0.26 | 0.93 ± 0.13 | 404 ± 22.0       |
|          | 200            |           | 1,932 ± 290                     | 1,942 ± 290                     | 1.94 ± 0.22 | 2.01 ± 0.22 | 1.78 ± 0.45 | 0.68 ± 0.27 | 694 ± 60.6       |

**Table 7.** Dose proportionality assessment of GLA, GLB, GLC and BLL based on confidence interval method

| Analyte | Parameter | Slope | 90% Confidence interval | Acceptance interval | Dosage proportionality |
|---------|-----------|-------|-------------------------|---------------------|------------------------|
| GLA     | \(C_{\text{max}}\) | 0.82  | 0.70–0.94               | 0.74–1.26           | Inconclusive           |
|         | \(\text{AUC}_{0-\text{t}}\) | 0.87  | 0.67–1.07               | 0.84–1.16           | Inconclusive           |
|         | \(\text{AUC}_{0-\infty}\) | 0.89  | 0.69–1.08               | 0.84–1.16           | Inconclusive           |
| GLB     | \(C_{\text{max}}\) | 0.87  | 0.71–1.03               | 0.74–1.26           | Inconclusive           |
|         | \(\text{AUC}_{0-\text{t}}\) | 0.82  | 0.71–0.93               | 0.84–1.16           | Inconclusive           |
|         | \(\text{AUC}_{0-\infty}\) | 0.83  | 0.72–0.94               | 0.84–1.16           | Inconclusive           |
| GLC     | \(C_{\text{max}}\) | 0.68  | 0.46–0.90               | 0.74–1.26           | Inconclusive           |
|         | \(\text{AUC}_{0-\text{t}}\) | 0.69  | 0.52–0.87               | 0.84–1.16           | Inconclusive           |
|         | \(\text{AUC}_{0-\infty}\) | 0.70  | 0.50–0.90               | 0.84–1.16           | Inconclusive           |
| BLL     | \(C_{\text{max}}\) | 0.86  | 0.78–0.94               | 0.74–1.26           | Proportional           |
|         | \(\text{AUC}_{0-\text{t}}\) | 0.91  | 0.80–1.02               | 0.84–1.16           | Inconclusive           |
|         | \(\text{AUC}_{0-\infty}\) | 0.91  | 0.81–1.02               | 0.84–1.16           | Inconclusive           |
flavonol glycoside prototype in GBE. An intragastric pharmacokinetic study of crushed Yinxing Tongzhi Dripping Pills in rat was conducted based on the analytical method. These results provide data about the pharmacokinetic property of flavonol glycoside prototype in GBE and the dose proportionality of $C_{\text{max}}$ and AUC for GLA, GLB, GLC and BLL.

Conflicts of interest: The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by Zhejiang Jiuxu Pharmaceutical Co., Ltd., National Major Scientific and Technology Special Project for “Significant New Drugs Development” (2019ZX09301-161) and Youth Foundation of Zhejiang Academy of Medical Sciences (2020Y002).

SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at https://doi.org/10.1556/1326.2021.00962.

ABBREVIATIONS

| Abbreviation | Description |
|--------------|-------------|
| IS | internal standard |
| LLOQ | lower limit of quantification |
| ULOQ | upper limit of quantification |
| QC | quality control |
| LQC | low quality control |
| MQC | middle quality control |
| HQC | high quality control |
| DQC | dilution quality control |
| RE | relative error |
| RSD | relative standard deviation |
| BLL | bilobalide |
| GLA | ginkgolide A |
| GLB | ginkgolide B |
| GLC | ginkgolide C |
| GBE | *Ginkgo biloba* leaf extracts |
| CHL | chloramphenicol |
| MTBE | methyl tert-butyl ether |

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