

# An HPLC Method for the Determination and Pharmacokinetic Study of Cinnamic Acid in the Plasma of Rats Having Taken the Traditional Chinese Medicinal Preparation Ling-Gui-Zhu-Gan Decoction

Zonghua Song, Kaishun Bi\*, and Xu Luo

Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, Liaoning Province, P.R. China

## Abstract

A high-performance liquid chromatographic method for the determination and pharmacokinetic study of cinnamic acid in the plasma of rats after having been administered orally with the traditional Chinese medicinal (TCM) preparation Ling-Gui-Zhu-Gan decoction is established. Plasma samples taken from rats are acidified with hydrochloric acid and extracted with ethyl acetate. Separation of the main effective constituent cinnamic acid is accomplished on a C<sub>18</sub> stationary phase and a mobile phase of methanol-acetonitrile-2% glacial acetic acid (10:22:70, v/v) with a UV detector setting at 254 nm. After validation, the method is used to take a limited view of pharmacokinetic profiles of the TCM preparation of the Ling-Gui-Zhu-Gan decoction.

## Introduction

Traditional Chinese medicines (TCMs), or more specifically Chinese materia medica, is the natural therapeutic agent used under the guidance of the theory of TCM science and has played an indispensable role in the prevention and treatment of disease in China. Herbal medicines are used mostly in combinations in China and are made into certain preparations for easy and efficient use.

The Ling-Gui-Zhu-Gan decoction was reported originally in treatise on cold-induced febrile diseases and in synopsis of the golden chamber, both written by Zhang Zhongjing in the last years of the Han dynasty in China (approximately 206 B.C. to 200 A.D.). The decoction is prepared by boiling *Poria*, *Ramulus Cinnamomi*, *Rhizoma Atractylodis Macrocephalae*, and *Radix Glycyrrhizae* together. In the clinical practice of TCM science, the decoction has been used to treat arrhythmia,

cardiac failure, angina pectoris, and other cardiovascular diseases over a very long period of time and has produced quite a favorable effect (1).

*Ramulus Cinnamomi* is the main medicine of the decoction, and cinnamic acid is the major effective constituent of *Ramulus Cinnamomi*, thus cinnamic acid is used as one of the marker compounds to characterize the decoction tentatively (2,3). There are some determination methods for cinnamic acid in biosamples (4–6). Owing to the complexity of chemical constituents in TCM formulas, there are scarcely any reports of their pharmacokinetic studies. This study reports a pharmacokinetic study accomplished on the constituent cinnamic acid of the Ling-Gui-Zhu-Gan decoction to take a limited view of its pharmacokinetic profiles.

## Experimental

### Materials and reagents

Fuling (*Poria cocos* (Schw.) Wolf), Guizhi (*Cinnamomum cassia* Presl), Baizhu (*Atractylodes macrocephala* Koidz.), and Gancao (*Glycyrrhiza uralensis* Fisch.) were all purchased at the Tianyitang TCM shop (Shenyang, China); and cinnamic acid and *p*-dimethylaminobenzaldehyde were both ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ethanol, hydrochloric acid, and ethyl acetate were all analytical grade and methanol and acetonitrile were chromatographic grade.

### Chromatographic system

The essential parts of the high-performance liquid chromatographic (HPLC) system consisted of (a) a Shimadzu LC-10AD pump, (b) a SPD-10A UV-vis detector set at 254 nm, (c) a 20- $\mu$ L injection loop, (d) an LC workstation for data collection, and (e) a 200- $\times$  4.6-mm-i.d. column. This column's sta-

\* Author to whom correspondence should be addressed; email ksbi@mail.sy.ln.cn.

tionary phase was a hypersil ODS C<sub>18</sub> (5- $\mu$ m particle size), its mobile phase was methanol–acetonitrile–2% glacial acetic acid (10:22:70, v/v) at a flow rate of 0.8 mL/min, and it was operated at room temperature.

### Standard solutions

Stock solutions of standard cinnamic acid and the internal standard *p*-dimethylaminobenzaldehyde were prepared with methanol. These solutions were spiked into drug-free plasma samples of rats to determine the recovery, precision, accuracy, and detection limit of the HPLC method. All standards were kept at 4°C before use.

### Sample preparation

Aliquot portions (2.0 mL) of the plasma sample were acidified with approximately 600  $\mu$ L of 0.1 mol/L HCl with the internal standard added to each of them. Then, each portion was shaken with 6 mL of ethyl acetate for 10 min and centrifuged at 2500 rpm for 10 min, and the organic layer was transferred into an empty tube. This procedure was repeated

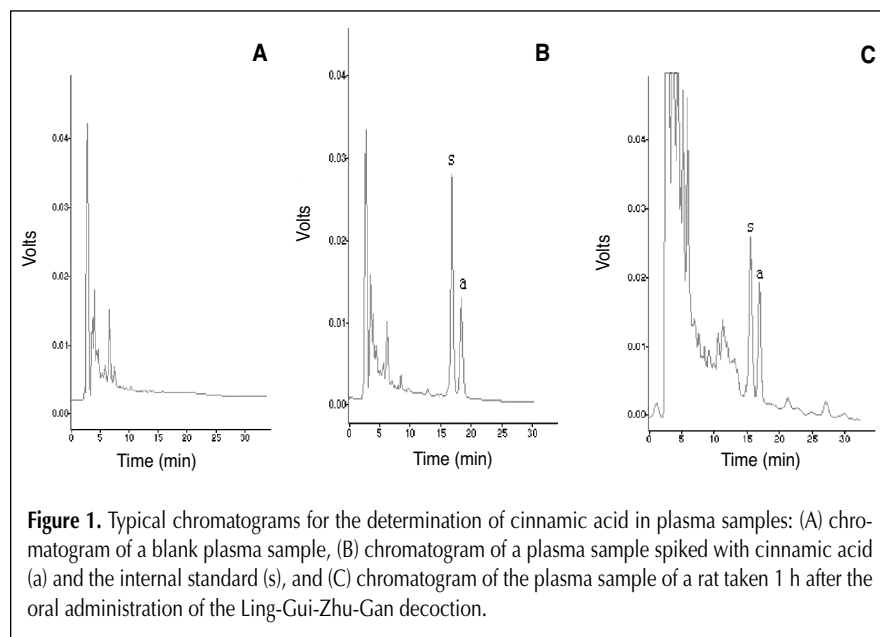
four times and the organic layer collected was dried at 40°C under a nitrogen stream. The residue was dissolved in 100  $\mu$ L methanol, and the sample solution was ready for injection into the chromatographic system aliquot by aliquot.

### Calibration procedure

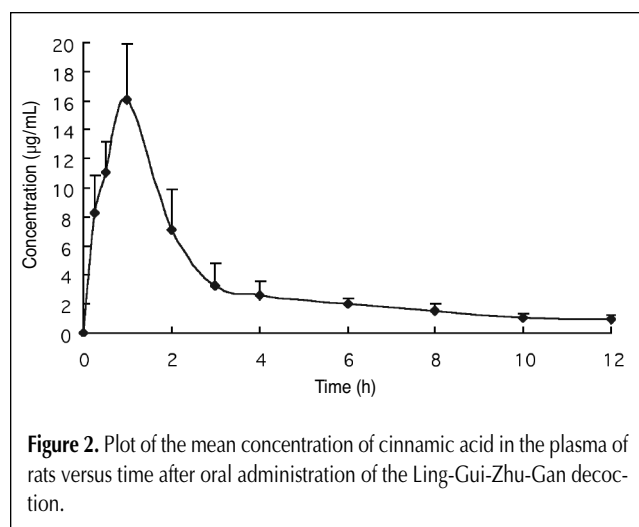
The calibration was accomplished via a standard curve by chromatographing known weight ratios of the sample constituent cinnamic acid and the internal standard *p*-dimethylaminobenzaldehyde. A plot was made of the ratio  $A_{cin}/A_{pdi}$  versus  $W_{cin}/W_{pdi}$  (where *A* is the peak area and *W* the weight). By adding the same weight of internal standard to the sample and fixing the aliquot portion (2.0 mL in this case), concentration could be used as the abscissa of the plot. The concentration range of cinnamic acid (approximately 0.84 to 67.2  $\mu$ g/mL) was studied.

### Recovery, precision, and accuracy

The recovery was determined by the standard addition method at their concentrations (1.68, 8.40, and 67.20  $\mu$ g/mL), and the precision (within-day and day-to-day) of the method was calculated at the same three concentrations. The variability of the peak-area ratio at each concentration was determined as a measure of the precision of the assay. The accuracy was determined by comparing the measured concentration with its true value.



**Figure 1.** Typical chromatograms for the determination of cinnamic acid in plasma samples: (A) chromatogram of a blank plasma sample, (B) chromatogram of a plasma sample spiked with cinnamic acid (a) and the internal standard (s), and (C) chromatogram of the plasma sample of a rat taken 1 h after the oral administration of the Ling-Gui-Zhu-Gan decoction.



**Figure 2.** Plot of the mean concentration of cinnamic acid in the plasma of rats versus time after oral administration of the Ling-Gui-Zhu-Gan decoction.

## Results and Discussion

Typical chromatograms of the blank and spiked plasma are given in Figures 1A and 1B in which the retention time was 18 min for cinnamic acid and 16 min for *p*-dimethylaminobenzaldehyde. There were no coeluting disturbing peaks in the vicinity of the two peaks on the chromatogram of the blank plasma. A chromatogram of the plasma sample for the rat taken 1 h after the oral administration of the Ling-Gui-Zhu-Gan decoction (18 mL/kg) is given in Figure 1C.

The calibration curve for the determination of cinnamic acid in rat plasma is linear over the range of approximately 0.84 to 67.2  $\mu$ g/mL with a coefficient of determination ( $r^2$ ) of 0.9948 ( $n = 7$ ). This linearity range will permit the use of this method in future pharmacokinetic studies of this drug. The quantitation limit was 0.84  $\mu$ g/mL, the within-day precision (percent relative standard deviation, %RSD) was approximately 3.7% to 9.3% ( $n = 18$ ), and the day-to-day precision (%RSD) was approximately 2.4% to 5.8% ( $n = 18$ ). The accuracy was approximately  $-0.2\%$  to  $2.0\%$  ( $n = 18$ ). The recovery of cinnamic acid was obtained through the comparison of concentrations of its methanol extracts with those of the corresponding spiked plasma. The mean recovery was 75.1% ( $n = 6$ ). In all instances,

the accuracy, precision, and recovery showed satisfactory levels (7,8).

Ideally, an internal standard should display similar physico-chemical properties as the analyte. For this reason, *p*-dimethylaminobenzaldehyde (which has a similar chemical structure as cinnamic acid) was chosen as the internal standard, and its retention time allowed for a correct separation of the analyte. The acetonitrile has a lower background and shows better selectivity to cinnamic acid and the internal standard than the methanol, thus it was added to the mobile phase to optimize the separation. The plasma sample was acidified with hydrochloric acid and extracted to ethyl acetate. The sort and amount of the added acid was reviewed. We once used H<sub>3</sub>PO<sub>4</sub> and HCl, and the amount of HCl used was 0.1, 0.3, 0.5, and 1.0 times that of the volume of plasma samples. Also, the pre-treatment method adopted showed a strong ability to remove excessive interference and efficiently extract the drug interest from the plasma sample.

The assay was applied to the pharmacokinetic study of cinnamic acid in the TCM preparation of the Ling-Gui-Zhu-Gan decoction. Plasma samples from rats were taken at 0.0 (before administration), 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, and 12.0 h after the oral administration of the decoction (18 mL/kg). Figure 2 is the mean plasma concentration versus time plot of cinnamic acid in which its pharmacokinetic parameters are obtained ( $t_{\max} \approx 1$  h,  $C_{\max} = 16.1 \pm 3.8$   $\mu\text{g/mL}$ , and  $t_{1/2} = 5.4$  h).

Several pharmacological studies of the Ling-Gui-Zhu-Gan decoction on myocardial ischemia and arrhythmia have been attempted. There have been some indications that it could reduce the myocardial consumption of oxygen and the preload of heart and increase heart blood perfusion flow. Also, all of these pharmacological indices reached a maximum at approximately 60 min after the oral administration of the Ling-Gui-Zhu-Gan decoction. This was consistent with the case that the  $C_{\max}$  appeared at approximately 1 h in the pharmacokinetic curve. Traditionally, the Ling-Gui-Zhu-Gan decoction has been taken orally 3 times a day since the ancient times of Zhang Zhongjing. According to the study mentioned previously,  $t_{1/2}$  was 5.4 h, thus the decoction should be taken approximately 4 times a day (the two recipes are similar). The feasible phar-

macokinetic parameters  $C_{\max}$  and  $t_{1/2}$  of cinnamic acid suggest that it may be used as a marker compound to characterize some profiles of a TCM formula.

## Conclusion

This study describes a sensitive, specific, and rapid HPLC method with UV detection for the determination of cinnamic acid in rat plasma. This method has been demonstrated to be suitable for use in pharmacokinetic studies of cinnamic acid in the TCM Ling-Gui-Zhu-Gan decoction.

## References

1. Y.L. Fu and W.J. Zhang. The effect of "Ling Gui Shu Gan Tang" on cardiovascular system. *J. Beijing Coll. Trad. Chin. Med.* **13(4)**: 47–49 (1990).
2. Y. Nunoura, S. Jwagami, and T. Okamura. Rapid analysis of Chinese medicines in quality evaluations. 1. Components of Cinnamon cortex and Moutan cortex in "Hachimigan". *Osaka-furitsu Koshu Eisei Kenkyusho kenkyu Hokoku, Yakuji Shido Hen.* **17**: 17–23 (1983).
3. K. Sagara, T. Oshima, and T. Yoshida. Determinations in Cinnamon cortex by high-performance liquid chromatography. *J. Chromatogr.* **409**: 365–70 (1987).
4. J. Yuan, J.R. Bucher, T.J. Goehl, M.P. Dieter, and C.W. Jameson. Quantitation of cinnamaldehyde and cinnamic acid in blood by HPLC. *J. Anal. Toxicol.* **16(6)**: 359–62 (1992).
5. B.P. Nutley, P. Farmer, and J. Caldwell. Metabolism of trans-cinnamic acid in the rat and the mouse and its variation with dose. *Food Chem. Toxicol.* **32(10)**: 877–86 (1994).
6. V.S. Ferreira, C.B. Melios, M.V. Zanoni, and N.R. Stradiotto. Determination of cinnamic acid in human urine by differential-pulse polarography. *Analyst* **121(2)**: 263–67 (1996).
7. P.S. Vinod, K.M. Kamal, and D. Shrikant. Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies. *J. Pharm. Sci.* **81(3)**: 309–12 (1992).
8. R. Causon. Validation of chromatographic methods in biomedical analysis viewpoint and discussion. *J. Chromatogr. B* **689**: 175–80 (1997).

Manuscript accepted March 6, 2002.