Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 549–553
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Quantitative determination of puerarin in dog plasma by HPLC and study on the relative bioavailability of sustained release tablets

Fuzheng Ren, Qiufang Jing *, Yongjia Shen, Hongmei Ma, Jingbin Cui

Department of Pharmaceutical Engineering, School of Chemistry and Pharmaceutics, East China University of Science and Technology, Shanghai 200237, China

Received 7 October 2005; received in revised form 30 November 2005; accepted 30 November 2005
Available online 18 January 2006

Abstract

To evaluate the bioavailability of puerarin sustained release tablet (SR-Tab.) and Yufengningxin tablet (YU-Tab.), a liquid chromatography method was developed and validated to determine puerarin in dog plasma. Chromatographic separation was performed on Diamonsil C_{18} column using a mixture of methanol–acetic acid–water (25:6:69, v/v/v) delivered at a flow rate of 1.0 ml/min and detected by UV. 4-Hydroxybenzaldehyde was used as the internal standard. The linear range for puerarin was from 60 to 1800 ng/ml ($r = 0.9991$) with a limit of quantitation of 60 ng/ml. Within-day accuracy and precision ranged from −3.0 to 2.2% and from 1.2 to 4.3%, between-day accuracy and precision ranged from −4.1 to 2.6% and from 1.3 to 5.7%, respectively. The mean extraction recoveries of puerarin determined over the three concentrations were (90.3 ± 5.2)%, (95.7 ± 1.4)% and (93.1 ± 3.5)%. A significant difference was observed in main pharmacokinetic parameters of T_{max}, C_{max} and $AUC_{0-\infty}$ between puerarin SR-Tab. and YU-Tab. in dogs. The smoother plasma concentrations were obtained from SR-Tab. in dogs and the results were as expected.

Keywords: Puerarin; Sustained release tablets; Yufengningxin tablets; Bioavailability; HPLC

1. Introduction

Puerarin (structure shown in Fig. 1, chemical name is 7,4′-dihydroxyisoflavone-8β-glucopyranoside) is one of the bioactive components isolated from the root of *Pueraria lobata* (*Willd.*) *Ohwi* and *P. thomsonni Benth* which is a traditional Chinese medicinal herb. It has been reported that puerarin exhibits many pharmacological effects of cardiac/cerebral blood vascular diseases such as anti-hypertension, anti-arteriosclerosis, dilating coronary arteries, decreasing myocardial oxygen consumption and improving microcirculation in both animals and humans suffering from cardiovascular disease [1–3]. Yufengningxin tablet (marketed in China and included in Chinese pharmacopeia) is a formulation of total isoflavones (mainly consisting of puerarin) from *P. lobata*, and have been used for the treatment of hypertension, senile schemic cerebrovascular disease and angina pectoris. Patients were given three to five Yu-Tab. for three times within 1 day in the clinic. In earlier literature [4,5], it was also reported that the elimination half-life period of puerarin in the volunteers and patients was so short that the plasma concentration was fairly low after a single dose of 5 mg/kg was given by intravenous injection for about 4 h. Therefore, it is necessary to prepare puerarin sustained release dosage forms to reduce dosing frequency, maintain a more even blood level and hopefully improve patient compliances [6]. Puerarin sustained release tablets (SR-Tab.) were prepared in our laboratory, which consisted of a mixture of drug, chintosan, lactose, alginate-Na, magnesium stearate and PVP K30 [7,8]. The oral bioavailability of SR-Tab. has not been reported in either animals or humans until now. Thus far, a number of HPLC methods with ultraviolet, fluorescence detection have been reported for the determination concentration of puerarin [9–12]. However, all those methods either lacked internal standard to quantify, or lacked selectivity and took longer analysis time. This study was to develop and validate a rapid and accurate liquid chromatography method to evaluate the relative bioavailability of SR-Tab. and YU-Tab. in dogs.

* Corresponding author. Tel.: +86 21 64252241; fax: +86 21 64253255.
E-mail address: jingqf@sohu.com (Q. Jing).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.11.041

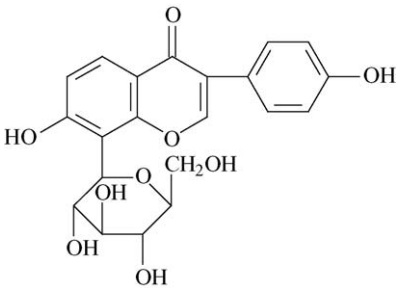

Fig. 1. Structure of puerarin.

2. Experimental

2.1. Chemicals and reagents

Puerarin reference standard was provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Yu-Tab. (*P. lobata* isoflavone, containing 13 mg puerarin per tablet) used as reference preparation was a commercial product from Beijing Tongrentang Pharmaceutical Co. (Beijing, China). Puerarin material was provided by Zhongce Pharmaceutical Co. (Yantai, China). Puerarin SR-Tab. was prepared in our laboratory. 4-Hydroxybenzaldehyde was purchased from Beijing second Chemical Reagents Co. HPLC-grade methanol, glacial acetic acid were obtained from Tedia Company Inc. (Fairfield, USA). All other chemicals used were of analytical grade unless otherwise indicated. Double-distilled water was used for all preparations.

2.2. Apparatus and chromatographic conditions

Chromatographic separation was performed with a chromatographic system (Shimadzu, Japan) equipped with a LC-10AD pump and a SPD-M10A UV–vis detector. An analytical column, Diamonsil C_{18} column (200 mm × 4.6 mm, 5 μm) from Dikma Technologies (Beijing, China) and a DL-II type guard column packed with YWG-C_{18} (10 mm × 4.0 mm, 10 μm) from Tianjin Chromatographic Science and Technology Company (Tianjin, China) were used for chromatographic separation. Mobile phase consisted of a mixture of methanol–acetic acid–water (25:6:69, v/v/v) delivered at a flow rate of 1.0 ml/min. The injection volume was 20 μl. Detection was performed at 250 nm at a constant temperature (25 ± 1 °C).

2.3. Sample preparation

To 1 ml of dog plasma, 50 μl of internal standard solution (4-hydroxybenzaldehyde, 9 μg/ml in methanol) and 1 ml methanol were added and vortex mixed for 5 min, 1.0 ml of perchloric acid solution (0.58 M) was added to precipitate protein and vortex mixed for 2 min and centrifuged (3000 rpm) for 15 min. The separated supernatant was evaporated to dryness in a water bath at 50 °C under the protection of nitrogen. The residue was reconstituted with 200 μl of the mobile phase by vortexing and a 20 μl volume was injected into the LC system.

2.4. Method validation

Plasma samples were quantified using the peak area ratio of puerarin to 4-hydroxybenzaldehyde. To evaluate linearity, serum calibration standards at concentrations of 60, 200, 500, 1000 and 1800 ng/ml were prepared and assayed in triplicate on 3 consecutive days. The accuracy and precision were assessed by determining quality control (QC) samples at three concentration levels on 3 different days. The accuracy (RE) was expressed as ((mean observed concentration − spiked concentration)/(spiked concentration)) × 100% and the precision as relative standard deviation (R.S.D.). Concentrations of puerarin in plasma samples were determined by back-calculation of the observed peak area ratios of the analyte and internal standard from the best-fit calibration curve using a weighted ($1/x^2$) linear regression. The extraction recoveries of puerarin at three QC levels (60, 500 and 1800 ng/ml, $n = 3$ at each concentration) were determined by comparing the peak area ratio of puerarin to internal standard from plasma samples spiked prior to extraction with those from plasma samples spiked post-extraction. The internal standard was added to both sets of samples. The stability of puerarin in the reconstituted solution under 20 °C for 24 h and in serum stored at −20 °C for 1 month was assessed by placing QC samples at three concentrations in triplicate.

2.5. Application of the developed LC method

The study was based on a single-dose, randomized, two-period crossover design at intervals of 1 week. Six dogs (13.6 ± 2.1 kg) were from the Laboratory Animal Center of Shanghai Medical University. In the morning of phase I, following an overnight fast (10 h), six dogs were given single dose of either one SR-Tab. (a dose equivalent to 130 mg puerarin) or 10 YU-Tab. (a dose equivalent to 130 mg puerarin) with 50 ml water. No other food was allowed until 4 h after dose administration while water intake was free. About 2 ml of blood samples was collected from the foreleg vein into heparinized test tubes at predose and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 16 h after dosing. Plasma was separated by centrifugation and kept frozen at −20 °C until analysis. After a washout period of 7 days, the study was repeated once again.

Pharmacokinetic analysis was performed by non-compartmental analysis. The maximum puerarin concentration (C_{max}) and corresponding peak time (T_{max}) were determined by the inspection of the individual drug serum concentration–time profiles. The elimination rate constant (K_e) was obtained from the least-square fitted terminal log-linear portion of the serum concentration–time profile. The elimination half-life ($t_{1/2}$) was calculated by $0.693/K_e$. The area under the serum concentration–time curve of puerarin from time zero to infinity ($AUC_{0-\infty}$) was determined by the trapezoidal rule to the last measurable concentration (C_t) plus the additional area from time t to infinity, calculated as C_t/K_e. The relative bioavailability was calculated as $(AUC_{0-\infty})_{SR\text{-}Tab.}/(AUC_{0-\infty})_{YU\text{-}Tab.}$.

3. Results and discussion

3.1. Optimization of analytical condition

Under the HPLC conditions used the analyte puerarin showed adequate separation from the interfering peaks. Acetic acid in the mobile phase was important for good peak shape probably by preventing the ionization of puerarin. A trial test indicated that perchloric acid was suitable for protein precipitation due to less volume and methanol was used for the extraction of puerarin in this study to obtain a relatively high recovery of puerarin from the dog serum.

3.2. Selectivity

The method selectivity was assessed by comparing the chromatograms of blank dogs serum with the corresponding spiked serum. Fig. 2 shows the typical chromatograms of a blank serum sample, dog serum sample collected after oral administration of YU-Tab. without the addition of the internal standard, a blank serum sample spiked with puerarin (500 ng/ml) and 4-hydroxybenzaldehyde (9 μg/ml) and a serum sample from a dog 180 min after an oral administration of one SR-Tab. No significant interferences of endogenous substances from the blank dog plasma with puerarin or 4-hydroxybenzaldehyde were detected and no matbolite(s) of puerarin interfered with the internal standard. Typical retention time for puerarin and 4-hydroxybenzaldehyde was 5.4 and 6.2 min, respectively. Therefore, the described LC method is selective for the determination of puerarin in dog plasma.

3.3. Linearity

Calibration standards were prepared in blank dog serum to give serum concentrations 60, 200, 500, 1000 and 1800 ng/ml for puerarin. The linear regression of the peak area ratio versus concentration was fitted over the concentration range of 60–1800 ng/ml in dog serum. A typical equation of the calibration curve was as follows: $R = 0.00113C - 0.0372$ ($r = 0.9991$), where R is the peak area ratio of puerarin to 4-hydroxybenzaldehyde and C is the concentration of puerarin.

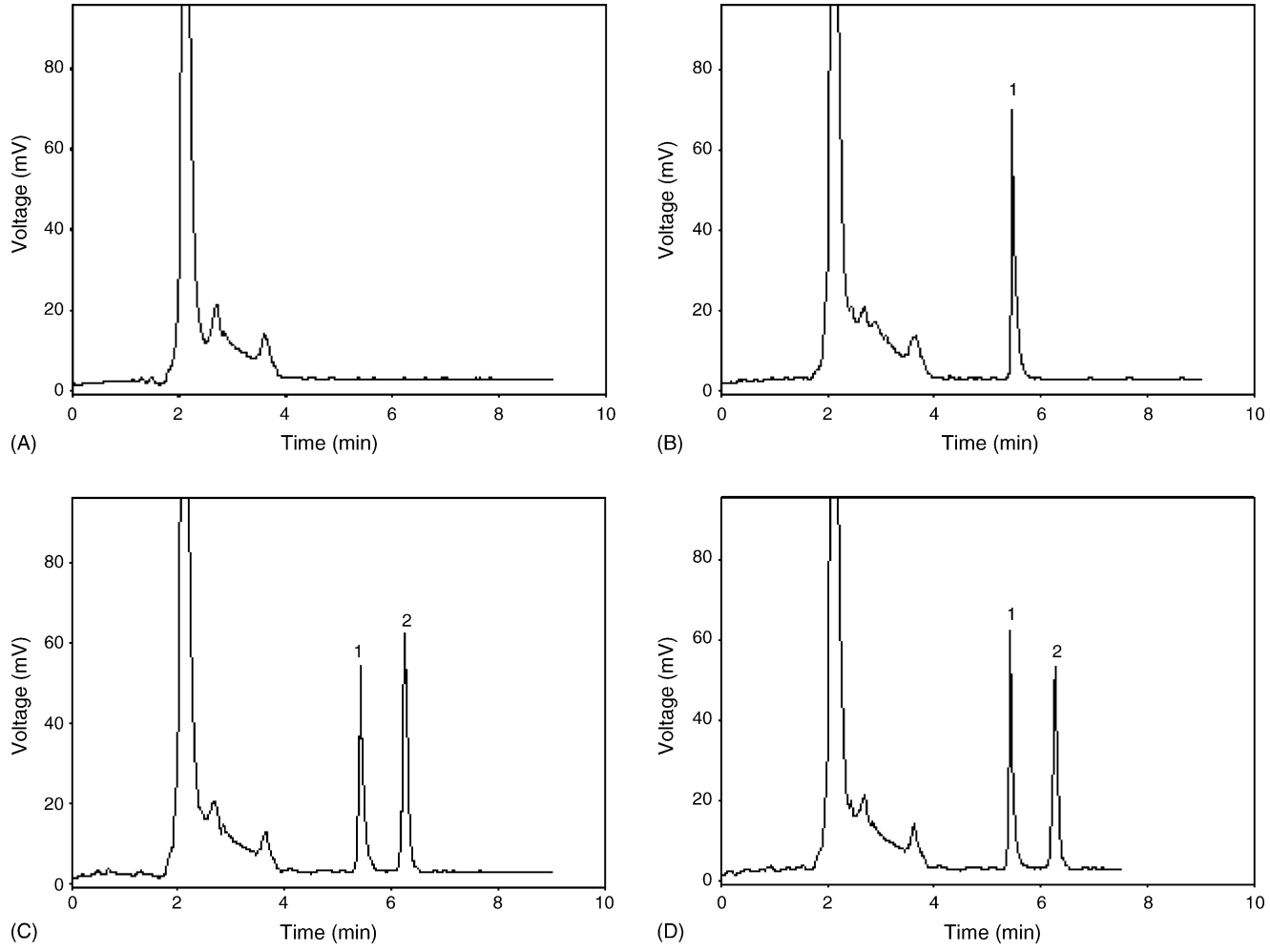

Fig. 2. Chromatograms of puerarin and 4-hydroxybenzaldehyde in serum samples. (A) Blank serum sample; (B) dog serum sample collected after oral administration of YU-Tab. without the addition of the internal standard. (C) Blank serum sample spiked with puerarin (500 ng/ml) and 4-hydroxybenzaldehyde (9 μg/ml); (D) dog serum sample collected at 180 min after oral administration of one SR-Tab. to a dog. Peak 1, puerarin ($t_R = 5.4$); peak 2, 4-hydroxybenzaldehyde ($t_R = 6.2$).

Table 1
Accuracy and precision of the developed LC method for the determination of puerarin in dog plasma

	Concentration (ng/ml)		
	60	500	1800
Within-day ($n=6$)			
Mean ± S.D. (ng/ml)	58.18 ± 2.51	496.35 ± 6.18	1839.41 ± 44.83
R.S.D. (%)	4.3	1.2	2.4
RE (%)	−3.0	−0.7	2.2
Between-day ($n=6$)			
Mean ± S.D. (ng/ml)	57.55 ± 3.43	494.17 ± 6.27	1847.65 ± 39.42
R.S.D. (%)	5.7	1.3	2.2
RE (%)	−4.1	−1.2	2.6

S.D.: standard deviation; R.S.D.: relative standard deviation; RE: relative error. RE (%) = 100 × ((mean concentration − nominal concentration)/nominal concentration).

3.4. Accuracy and precision

The limit of quantification for determination of puerarin in dog plasma, defined as the smallest sample concentration above which quantitation could be carried out with adequate accuracy and precision (Table 1), was found to be 60 ng/ml, which was sufficient for pharmacokinetic studies of puerarin preparations in dogs. Within-day accuracy and precision ranged from −3.0 to 2.2% and from 1.2 to 4.3%, between-day accuracy and precision ranged from −4.1 to 2.6% and from 1.3 to 5.7%, respectively.

3.5. Extraction recovery and stability

The extraction recoveries of puerarin, determined at three concentrations (60.0, 500.0 and 1800.0 ng/ml), were (90.3 ± 5.2)%, (95.7 ± 1.4)% and (93.1 ± 3.5)% ($n=3$). The extraction recovery of 4-hydroxybenzaldehyde was (94.5 ± 2.1)% ($n=3$).

Puerarin prepared samples were stable in the reconstituted solution of methanol–acetic acid–water (25:6:69, v/v/v) for at least 24 h at room temperature (20 °C). The mean relative error of puerarin between the initial concentrations and the concentrations stored at −20 °C for 1 month ranged from 4.2 to 1.9%, which indicated that puerarin was stable for at least 1 month at storage condition in dog serum.

3.6. Bioavailability study and pharmacokinetic analysis

The mean serum concentration–time profiles for the two formulations are presented in Fig. 3 and the pharmacokinetic parameters are given in Table 2.

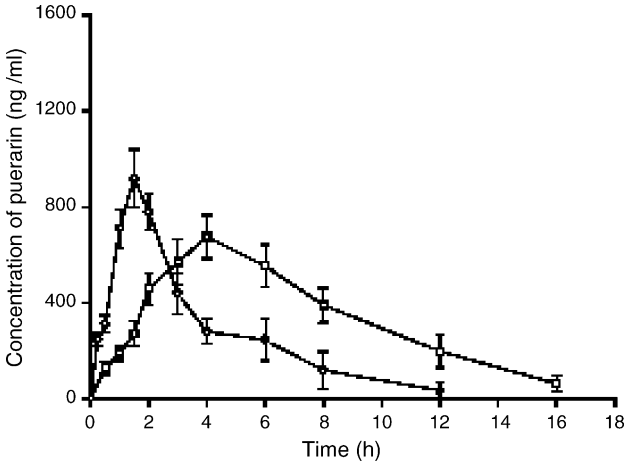

Fig. 3. Mean serum concentration–time curves of puerarin after oral administration of one SR-Tab. (130 mg) and YU-Tab. (130 mg). Each point represents the mean and standard deviation of six dogs. Formulation type: SR-Tab. (□); YU-Tab. (◯).

The $AUC_{0-\infty}$, t_{max} and C_{max} values showed significant difference between SR-Tab. and YU-Tab. The absorption of puerarin from SR-Tab. resulted in a 1.3-fold increase in bioavailability compared with YU-Tab. It was demonstrated that the absorption of puerarin from SR-Tab. was enhanced a little according to bioequivalence requirement (80–125%). The increased oral bioavailability following administration of SR-Tab. was most likely due to the enhancing effect of chitosan on the permeability of monolayers of intestinal epithelial cells because chitosan was used as a sustained release matrix in the formulation of SR-Tab. [13]. For two formulations of a single drug, the true terminal elimination half-life should be the similar. Because the concentrations of the SR-Tab. fell below the LOQ by 16 h and there were large individual differences, the true elimination half-life was not captured. The result showed apparent differences in half-life between YU-Tab. and SR-Tab.

4. Conclusions

A rapid, precise, accurate and reliable HPLC method for determination the bioavailability of SR-Tab. and YU-Tab. in dogs has been developed and validated. This method greatly simplified the process of determination and could be used for the pharmacokinetic study of pharmaceutical preparations in human. Moreover, this study also indicated that puerarin SR-Tab. might provide the smoother plasma concentration and better compliance for patients than YU-Tab. in clinical practice. The results were as expected.

Table 2
Pharmacokinetic parameters for puerarin in dogs following oral administration of SR-Tab. and YU-Tab. formulations ($n=6$)

	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (h ng/ml)	K_e (h^{-1})	$t_{1/2}$ (h)
YU-Tab.	1.50 ± 0.32	917.9 ± 123.2	3749.0 ± 565.8	0.4813 ± 0.1232	1.52 ± 0.37
SR-Tab.	4.00 ± 0.98**	674.9 ± 91.0**	4847.8 ± 877.3*	0.2654 ± 0.0828*	2.83 ± 0.84*

* $p<0.05$.
** $p<0.01$.

References

[1] X.P. Song, P.P. Chen, X.S. Chai, Acta Pharmacol. Sinica 9 (1988) 55–58.
[2] Y. Li, Y. Yang, Chin. Pharm. J. 32 (1997) 776–777.
[3] Q. Liu, Z. Lu, L. Wang, J. Tongji Med. Univ. 20 (2000) 43–45.
[4] X.L. Jin, G.F. Cheng, X.Y. Zhu, China J. Clin. Pharmacol. 7 (1991) 115–118.
[5] J.R. Robinson, Sustained and Controlled Release Drug Delivery Systems, Marcel Dekker, New York, 1978, pp. 71–121.
[6] X.Y. Li, P.R. Wang, D.H. Shao, Chin. J. Cardiol. 13 (1985) 175–178.
[7] Q.F. Jing, F.Z. Ren, Y.J. Shen, Chin. Traditional Herb. Drugs 33 (2002) 991–993.
[8] Q.F. Jing, F.Z. Ren, Y.J. Shen, J. East China Univ. Sci. Technol. 29 (2003) 173–176.
[9] X.L. Jin, X.Y. Zhu, Acta Pharmacol. Sinica 13 (1992) 284–288.
[10] Z. Zhang, X. You, Q. He, G. Liao, Chin. Pharm. J. 32 (1997) 104–106.
[11] B.S. Yu, X.P. Yan, G.B. Zhen, Y.P. Rao, J. Pharm. Biomed. Anal. 30 (2002) 843–849.
[12] B. Yan, D.M. Xing, Y. Ding, J.L. Tao, L.J. Du, J. Pharm. Biomed. Anal. 37 (2005) 297–301.
[13] P. Artursson, T. Lindmark, S.S. Davis, Pharm. Res. 11 (1994) 1358–1361.