—Regular Articles—

Reverse-phase HPLC Determination and Pharmacokinetic Study of Vanillic Acid in the Plasma of Rats Treated with the Traditional Chinese Medicinal Preparation Di-Gu-Pi Decoction

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A sensitive, simple, and accurate method for the determination and pharmacokinetic study of vanillic acid in rat plasma was developed using reverse-phase HPLC with UV detection after oral administration of the traditional Chinese medicine preparation of the Di-Gu-Pi decoction. Plasma samples taken from rats were extracted with methanol. The constituent vanillic acid was separated on a C_{18} stationary phase and a mobile phase of acetonitrile-water (15:85, v/v) (adjusted to pH 3.0 using phosphoric acid), with a UV detector setting at 260 nm. The validated HPLC method developed was used to determine the pharmacokinetic profile of vanillic acid in rat plasma after administration of the Di-Gu-Pi decoction.

Key words——Di-Gu-Pi decoction; vanillic acid; pharmacokinetics

INTRODUCTION

Traditional Chinese medicine (TCM) uses natural therapeutic agents under the guidance of TCM theory and has played an indispensable role in the prevention and treatment of diseases in China. Traditionally, Di-Gu-Pi is prepared from the root bark of *Lycium Chinese Mill* or *Lycium barbarum L.* and has been used for the treatment of diabetes, hemorrhagic inflammation, hypertension, ulcers, and fever in TCM.[1,2] The Di-Gu-Pi decoction is officially listed in the *Chinese Pharmacopoeia* and is often made into preparations for the treatment of diabetes in China. It has shown remarkable hypoglycemic activity in experiments in alloxan-diabetic mice.[3] Vanillic acid is used as a marker compound to characterize the decoction. Pharmacokinetic studies of ingredients in Chinese herbs contribute to understanding their mechanisms of action and to promoting the development of TCM. TCM compound preparations are often administered by the oral route. Methods are available for determination of vanillic acid in biosamples.[4] The chemical constituents of the Di-Gu-Pi decoction are complex, and there are few reports on its pharmacokinetics. We report a pharmacokinetic study of the constituent vanillic acid in the Di-Gu-Pi decoction to determine a limited pharmacokinetic profile.

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EXPERIMENTAL

Materials and Reagents Di-Gu-Pi [*Cortex Lycii Radicis* (CLR)] was purchased from the Tianyitang TCM shop (Shenyang, China) and identified by Professor Qishi Sun (Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, China). Vanillic acid and p-hydroxybenzoic acid were both ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their purity was more than 98.0%. Phosphoric acid was of analytical grade, and acetonitrile was of chromatographic grade.

Chromatographic System The essential parts of the LC 2010A consisted of a high-speed autosampler, column oven, UV detector set at 260 nm, LC workstation for data collection, and a 200×4.6 mm i.d. column. The stationary phase of the column was a Diamonsil C_{18} (5-μm particle size). The mobile phase containing acetonitrile: water (15:85, v/v) (adjusted to pH 3.0 using phosphoric acid) was delivered at a flow rate of 0.8 ml/min. The experiments were conducted at 30°C.

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

Sample Preparation Plasma samples (0.1 ml) with the internal standard added to each were shaken with methanol 1.0 ml for 2 min and centrifuged at 3000 rpm for 10 min. The methanol extract was transferred to a dry tube. The procedure was repeated twice and the methanol extracts collected were dried at 40°C under a nitrogen stream. The residue was dissolved in methanol 100 (μl and the sample preparation was then injected into the chromatographic system.

Calibration Procedure Various amounts of vanillic acid and the internal standard were added to 200 μl of blank plasma. Solutions prepared as described above were injected and peak area ratios of vanillic acid and the internal standard were regressed against the concentration of vanillic acid. The calibration was accomplished via a standard curve by chromatographing known weight ratios of the sample constituent vanillic acid and the internal standard *p*-hydroxybenzoic acid. A plot was made of ratio A_{van}/A_{phy} versus W_{van}/W_{phy} (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 (1.0 ml), the concentration could be used as the abscissa of the plot. The concentration range of vanillic acid (approximately 48.8—1952.0 ng/ml) was determined.

Recovery, Precision, and Accuracy The recovery rate was determined using the standard addition method at concentrations of 122.0, 488.0, and 1464.0 ng/ml. The precision (within-day and between-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 accurary was determined by comparing the measured concentration with its true value. The values are presented in Table 1.

Table 1. Precision and Accuracy of the LC Method in Determining Vanillic Acid Levels in Rat Plasma

Run	Added concentration (ng/ml)		
	122.0	488.0	1464.0
1	122.08	486.59	1458.16
	118.78	486.29	1459.88
	118.74	490.74	1461.75
	124.14	487.01	1469.91
	122.16	496.15	1468.33
	124.76	489.67	1469.52
2	122.06	486.59	1455.56
	122.07	493.93	1456.55
	119.61	486.69	1461.99
	123.34	487.86	1465.37
	120.85	494.67	1467.53
	119.59	483.27	1467.48
3	120.76	490.54	1466.94
	120.89	485.64	1457.20
	120.96	489.18	1456.01
	121.93	490.19	1460.39
	119.44	482.46	1448.11
	118.23	482.39	1466.25
n	18	18	18
Mean (ng/ml)	121.13	488.33	1462.05
SD	1.86	3.96	6.03
Relative error (%)	−0.71	0.07	−0.13
Between-day RSD (%)	2.51	2.44	1.00
Within-day RSD (%)	2.91	3.32	2.41

RESULTS AND DISCUSSION

During the development of the method, the ethyl acetate to extracted vanillic acid ratio from rat plasma and deproteinization by acetonitrile and 5% trichloroacetic acid were investigated. A low recovery rate (60—75%), poor resolution, and poor plots resulted using the above samples. The methanol to extracted vanillic acid from rat plasma ratio was found to be the optimal sample preparation procedure and it produced good resolution and high recovery rates.

To ensure sufficient selectivity and suitable analytical time for vanillic acid in rat plasma, the chromatographic system was optimized. Methanol-water and acetonitrile-water columns and adjustment to pH 3.0 using phosphoric acid were tested. An acetonitrile-water column adjusted to pH 3.0 using phosphoric acid yielded suitable sensitivity and selectivity in this method, and the separation of vanillic acid and internal standard was improved. Phosphoric acid was added to the mobile phase to reduce tailing peaks. The sample plasma was extracted with methanol, allowing removal of excess interference and efficient extraction of the constituent from the sample plasma. The wavelength of 260 nm was used as the detection wavelength at which vanillic acid shows the greatest absorption.

The chemical properties of vanillic acid and *p*-hydroxybenzoic acid are similar, and thus *p*-hydroxybenzoic acid can be used as the internal standard. It was separated well from vanillic acid and showed little endogenous interference in this assay.

Typical chromatograms of the blank and spiked

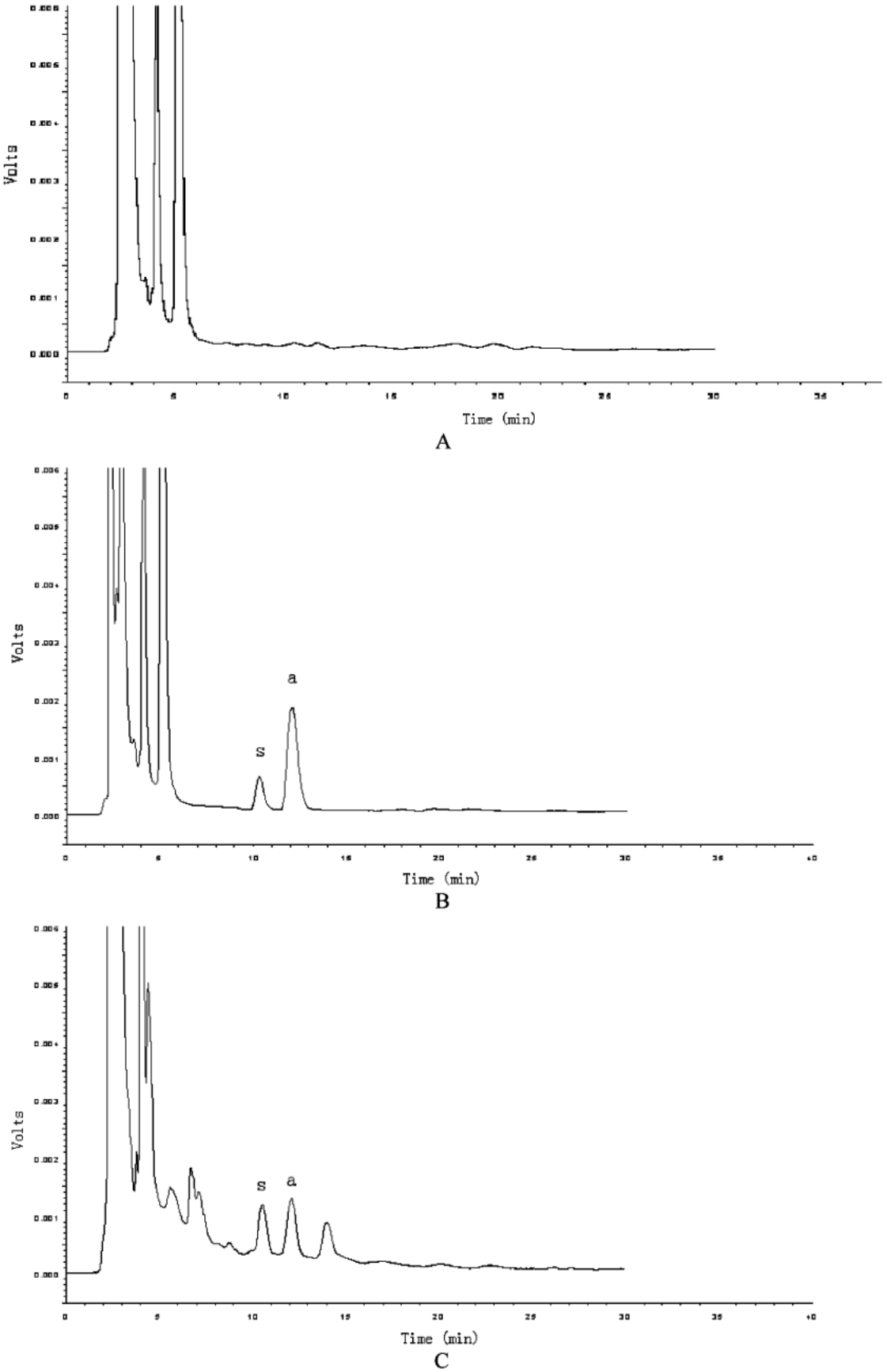

Fig. 1. (A) Chromatogram of a Blank Plasma Sample, (B) Chromatogram of a Plasma Sample Spiked with Vanillic Acid (a) and Internal Standard (s), and (C) Chromatogram of a Plasma Sample of a Rat Taken 3 h after the Oral Administration of the Di-Gu-Pi Decoction

Table 2. Pharmacokinetic Parameters of Vanillic Acid in the TCM Preparation of the Di-Gu-Pi Decoction

Rat no.	$t_{1/2}$ (h)	Ke (1/h)	AUC_{0-12} μg·h/ml	$AUC_{0-\infty}$ (μg·h/ml)	MRT_{0-12} (h)	$MRT_{0-\infty}$ (h)	$T_{otal}CL$ (ml/min)	V_Z (l/kg)
1	1.32	0.525	4234.39	4234.39	3.31	3.31	3150	360
2	2.11	0.328	5053.74	5210.47	4.04	4.38	2560	468
3	2.23	0.311	4620.40	4804.49	4.06	4.49	2780	535
4	1.72	0.403	4219.72	4219.72	2.90	2.90	3160	471
5	1.25	0.552	4122.77	4122.77	3.07	3.07	3230	351
Mean	1.84	0.376	4450.56	4508.33	3.51	3.65	2960	471

plasma are shown in Fig. 1(A) and 1(B), in which the retention time was 12.1 min for vanillic acid and 10.5 min for *p*-hydroxybenzoic acid. There were no coeluting disturbance peaks in the vicinity of the two peaks on the chromatogram of the blank plasma. Chromatograms of rat plasma taken 3 h after the oral administration of the Di-Gu-Pi decoction (800 mg/kg) are shown in Fig. 1(C).

The calibration curve of the peak-area ratio (y) versus the concentration (x, ng/ml) was linear: $y=0.0008x+0.1713$, $R^2=0.9977$, $n=7$. The linear range for the determination of vanillic acid was 48.8—1952.0 ng/ml, which will permit the use of this method in future pharmacokinetic studies of this drug. The quantitation limit was 48.8 ng/ml. The precision (percent relative standard deviation, %RSD) was 2.41% to 3.32% and the between-day precision (%RSD) was 1.00% to 2.51%. The accuracy was −0.71% to 0.07%. The recovery of vanillic acid was obtained from the corresponding spiked plasma. The mean recovery rate was 92.3% ($n=6$). The accuracy, precision, and recovery rate were thus favorable.

The assay was applied to the pharmacokinetic study of vanillic acid in the TCM preparation of the Di-Gu-Pi decoction. Plasma samples from rats were taken at 0.0 (before administration), 0.17, 0.50, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 12.0 h after the oral administration of the decoction. Figure 2 shows the mean plasma concentration-time plot of vanillic acid, and its pharmacokinetic parameters are presented in Table 2.

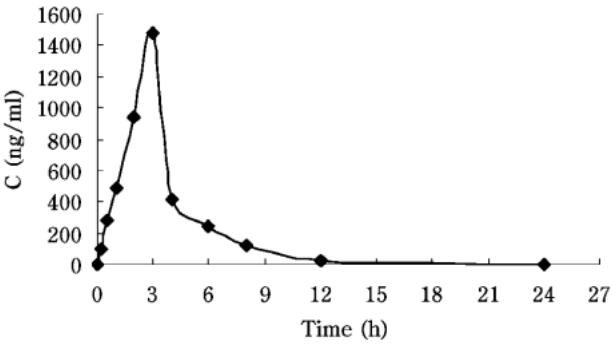

Fig. 2. Plot of the Mean Concentration of Vanillic Acid in the Plasma of Rats versus Time after Oral Administration of the Di-Gu-Pi Decoction

CONCLUSION

This study describes a sensitive, specific, and rapid method that has been demonstrated to be suitable for the pharmacokinetic study of vanillic acid in the TCM Di-Gu-Pi decoction, with UV detection for the determination of vanillic acid levels in rat plasma.

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