JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 12083–12088 12083
DOI:10.1021/jf103191a

LC−MS Determination and Pharmacokinetics of *p*-Coumaric Acid in Rat Plasma after Oral Administration of *p*-Coumaric Acid and Freeze-Dried Red Wine

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A sensitive and efficient liquid chromatography−mass spectrometry (LC−MS) method was developed and validated for the determination of *p*-coumaric acid (CA) in rat plasma. After addition of the internal standard (IS) hydrochlorothiazide and acidification with 2 M hydrochloric acid, plasma samples were extracted by ethyl acetate and separated on a Kromasil C_{18} column (200 mm × 4.6 mm, 5 μm) using a mobile phase composed of methanol−0.5‰ acetic acid (60:40, v/v) within a runtime of 6.0 min. Analysis was performed in selected ion monitoring (SIM) mode with a negative electrospray ionization (ESI) interface. The target ions were *m*/*z* 163.15 for CA and *m*/*z* 295.95 for IS. The linear range was 0.01−15 $\mu g \cdot mL^{-1}$, and the lower limit of quantification (LLOQ) was 0.01 $\mu g \cdot mL^{-1}$. The intraday and interday precision (RSD %) were lower than 10% and accuracy (RE%) ranged from 97.1 to 103.2%. The validated method was successfully applied to the comparative pharmacokinetic study of CA in rat plasma after oral administration of CA and freeze-dried red wine, respectively. It was found that both AUC and $T_{1/2}$ of CA in freeze-dried red wine were increased significantly ($p < 0.05$) compared with that in monomer. In addition, a double-peak profile could be observed from the concentration−time curve after oral administration of freeze-dried red wine.

KEYWORDS: *p*-Coumaric acid; freeze-dried red wine; LC−MS; pharmacokinetics

INTRODUCTION

Red wine is a commonly consumed beverage worldwide. Epidemiological data indicates that a moderate intake of red wine is associated with many well-known health-promoting properties such as free-radical scavenging and inflammatory modulation, metal chelating and enzyme modulation (*1*−*3*), reduction of the susceptibility of low-density lipoproteins (LDL) to oxidation both *in vitro* (*4*) and *in vivo* (*5*), and inhibition of platelet aggregation, vasorelaxing activity and modulation of lipid metabolism (*6*, *7*). The protection observed as a result of a moderate red wine consumption may partly be explained by the intake of polyphenols. Polyphenols, as efficient antioxidant compounds, are especially abundant in red wine (*8*−*11*).

p-Coumaric acid (CA, **Figure 1A**), one of the most abundant and bioactive polyphenols in red wine, is an antioxidant which plays indispensable roles in the prevention of colon cancer (*12*,*13*), inhibiting the growth of bacteria (*14*), regulating the estrus cycle of cyclic female mice (*15*), and most importantly, protecting low-density lipoprotein cholesterol from oxidation (*16*, *17*) and preventing cardiovascular diseases (*18*). It has been reported that there is a highly efficient intestinal absorption of CA *in vivo* (*19*). In addition, studies have demonstrated that CA is transported across human intestinal Caco-2 cells (*20*, *21*).

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However, as far as we are aware, there have been still few reports on the pharmacokinetic study of the active polyphenols in red wine while pharmacokinetics is essential to clarify the mechanism of action and enable instructions in practical applications. In addition, only a few analytical methods have been described for determination of CA in biological fluids, including HPLC-ECD (*18*, *19*, *21*) and HPLC−UV methods (*22*−*24*), but not LC−MS methods, whereas LC−MS methods can provide highly confident analyte identification in complex biological specimens and allow for consistent time saving in routine analyses (*25*).

In this paper, a selective and sensitive LC−MS method for determination of CA in rat plasma was developed for the first time. The method was validated and successfully applied to the comparative pharmacokinetic study of CA in rat plasma after oral administration of CA and freeze-dried red wine, respectively. This is also the first report on the pharmacokinetics of active components in red wine, which could provide an experimental basis for daily consumption.

MATERIALS AND METHODS

Materials, Chemicals and Reagents. Red wine made of *cabernet sauvignon*, which was purchased from Greatwall (Cofco wines and spirits company Ltd., China, Dec. 2009), was freeze-dried. The freeze-dried sample was kept at −20 °C, and then dissolved in water just before administration. CA was purchased from Sigma (98.0%, St. Louis, MO, USA).

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Published on Web 11/11/2010

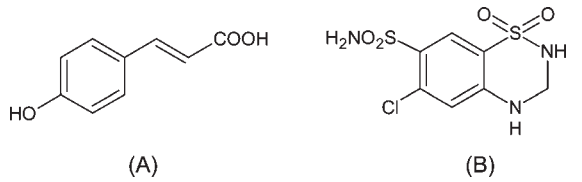

Figure 1. Chemical structures of *p*-coumaric acid and hydrochlorothiazide for the analysis: (**A**) *p*-coumaric acid (CA); (**B**) hydrochlorothiazide (IS).

Hydrochlorothiazide (99.8%, IS, **Figure 1B**), used as internal standard, was purchased from the National Institute for Control of Biological and Pharmaceutical Products (Beijing, China). Methanol and acetic acid of HPLC grade were purchased from Fisher Scientific (Pittsburgh, PA, USA) and Concord Tech. Co. (Tianjin, China), respectively. All the other reagents were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the experiment.

Animals. Male pathogen-free Wistar rats (220−250 g) were provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Animal study was carried out in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University (Shenyang, China), and the protocol was approved by the Animal Ethics Committee of the institution.

LC−MS Instruments and Analytical Conditions. The assay was performed on a Shimadzu (Japan) LC−MS 2010A system. Liquid chromatographic separation was achieved on a Kromasil C_{18} column (200 mm × 4.6 mm, 5 μm) and preceded by a C_{18} guard column (4.0 mm × 2.0 mm, Phenomenex, Torrance, CA, USA). The column and autosampler tray temperature were kept constant at 25 and 4 °C, respectively. The mobile phase was methanol−0.5‰ acetic acid (60:40, v/v). The flow rate was set at 0.8 mL·min^{-1} with 25% of the eluent splitted into the inlet of the mass spectrometer. The injection volume was 10 μL. The total analysis time was 6.0 min. The analytes were ionized with an ESI source in negative ion mode under the following source conditions: nebulizing gas 1.5 L·min^{-1}; drying gas 2.0 L·min^{-1}; CDL temperature 200 °C; heat block temperature 200 °C; detector voltage 1.60 kV; the other parameters were fixed as for the tuning file. Analysis was carried out by selected ion monitoring at *m*/*z* 163.15 for CA and at *m*/*z* 295.95 for IS.

Preparation of Standard Solutions and Quality Control Samples. The stock solutions of CA and IS were prepared in methanol at concentration levels of 0.2 and 1 mg·mL^{-1}, respectively. Standard solutions were prepared by diluting the stock solutions above with methanol. The concentration of working solution for internal standard was 10 μg·mL^{-1}. Since CA was reported to be sensitive to light (*24*), all CA and IS solutions were stored at 4 °C away from light. Calibration standards of CA at concentrations of 0.01, 0.02, 0.04, 0.1, 0.2, 1, 5, and 15 μg·mL^{-1} were prepared by spiking appropriate amounts of the standard solutions in blank plasma. Three levels of quality control (QC) samples (0.02, 0.8, and 12 μg·mL^{-1}) were prepared separately in the same fashion.

Preparation of Plasma Samples. 100 μL plasma samples were spiked with 10 μL of IS (10 μg·mL^{-1}), 10 μL of methanol and 50 μL of 2 M hydrochloric acid, and extracted for 5 min with 2 mL of ethyl acetate. After centrifugation (15,000 rpm, 4 °C, 5 min), the organic phase was transferred to another vial and evaporated to dryness in a thermostatic controller at 30 °C under a slight stream of nitrogen. Then the residue was dissolved in 100 μL of mobile phase; 10 μL of it was used for LC−MS analysis. During the extraction procedure, samples were kept protected from light.

Method Validation. *Selectivity*. Selectivity was assessed by comparing chromatograms of six different batches of blank plasma obtained from six rats with those of corresponding standard plasma samples spiked with CA and IS, and a plasma sample after intravenous administration.

Linearity, LLOQ and LOD. Calibration curves were prepared by assaying standard plasma samples at eight concentrations of CA ranging from 0.01 to 15 μg·mL^{-1}. The linearity of each calibration curve was determined by plotting the peak area ratio (y) of CA/IS vs the nominal concentration (x) of the analyte. The calibration curves were constructed by weighted ($1/x^2$) least-squares linear regression. The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curve at which the signal-to-noise ratios (S/N) were greater than 10. The acceptable accuracy within ±20% and the precision below

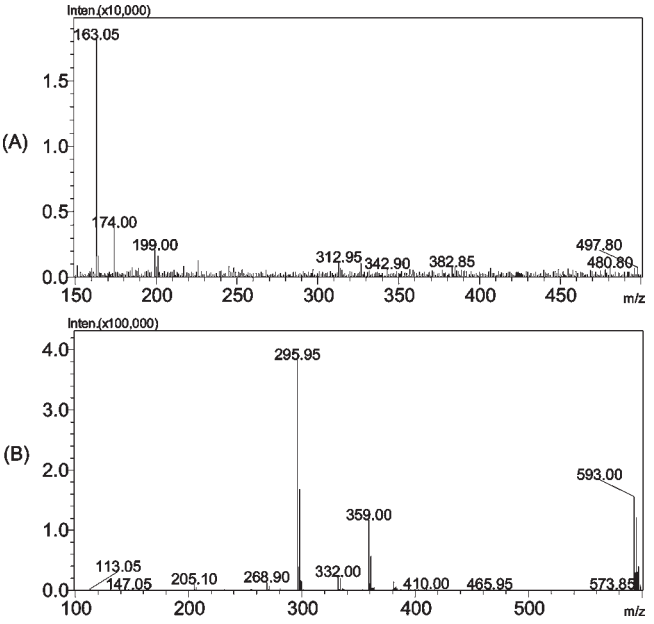

Figure 2. Full-scan mass spectra of CA (**A**) and IS (**B**).

20% were obtained. The limit of detection (LOD) was estimated as the amount of CA which caused a signal three times to noise.

Precision and Accuracy. The precision and accuracy of the method were assessed by six replicate analyses of QC samples at low, medium and high concentrations on three separate days. Precision was defined as the relative standard deviation (RSD %) while accuracy was defined as relative error (RE %).

Extraction Recovery and Ionization. The extraction recovery of CA was determined at three QC levels on six occasions by comparing the peak areas of CA from extracted samples with those in postextracted blank plasma samples spiked with CA at the same concentration. Recovery of IS was determined in the same way at a concentration of 10 μg·mL^{-1}. Suppression of ionization was evaluated by comparing the absolute peak area from a control plasma sample extracted and then spiked with a known amount of analyte with that from the neat standard prepared in the same reconstitution solvent.

Stability. The QC samples (six replicates at each concentration) were assayed under several different conditions to assess the stability of CA in rat plasma. The resulting concentrations were compared with their theoretical concentrations, and the percentage of concentration deviation was calculated. Stability samples were to be concluded stable if the relative errors were within ±15%.

Pharmacokinetic Application. The method was used to determine CA in rat plasma after oral administration of CA (2.35 mg·kg^{-1}) and freeze-dried red wine (at a dose containing CA 2.35 mg·kg^{-1}), respectively. Animals were divided into two groups, with six rats in each. Blood samples (about 0.25 mL) were collected from the abdominal vein before dosing and at 0.033, 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 6 h after dosing of CA, and before dosing and at 0.033, 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h after dosing of freeze-dried red wine. Then the blood samples were immediately transferred to heparinized tubes and centrifuged at 4,000 rpm for 5 min. The plasma obtained was stored at −20 °C until analysis. The plasma concentrations of CA at different times were expressed as mean ± SD, and the mean concentration−time curve was plotted. All data were processed by noncompartmental analysis using the DAS 2.1 software package (Chinese Pharmacological Society). The comparison of pharmacokinetic parameters between administration of CA and freeze-dried red wine was calculated by SPSS 16.0 (Statistical Package for the Social Science).

RESULTS

Mass Spectral Analysis. Under the electrospray ionization conditions, CA and IS all exhibited higher sensitivity in the negative ion mode than in the positive ion mode, with the deprotonated molecular ion $[M - H]^-$. In the negative ESI mode,

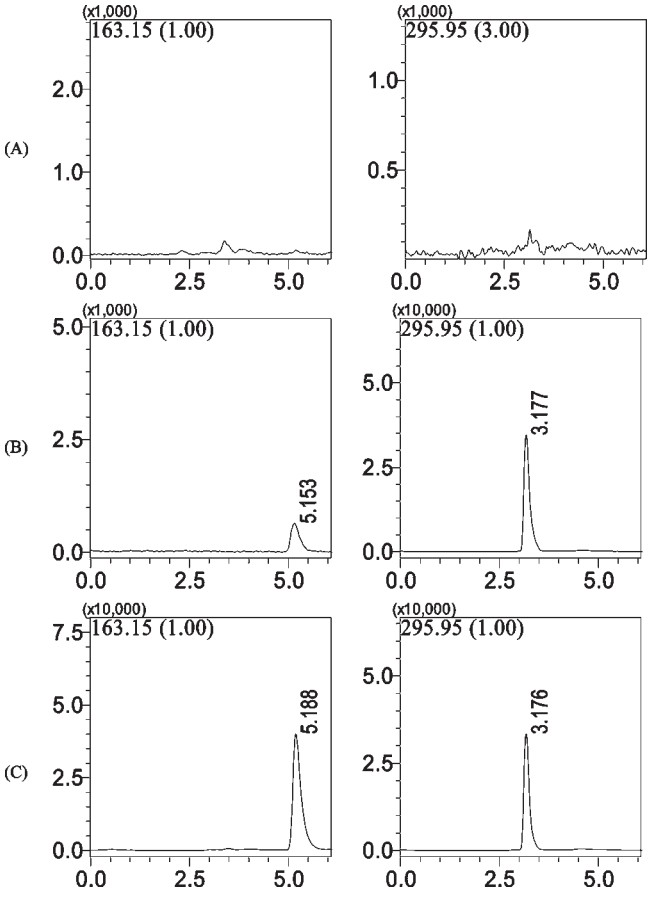

Figure 3. Typical SIM chromatograms of bland plasma (**A**), blank plasma spiked with CA and IS (LLOQ, 0.01 $\mu g \cdot mL^{-1}$) (**B**), and a rat plasma sample (0.25 h after oral administration of freeze-dried red wine) (**C**). (The left is the SIM chromatogram of 163.15 for CA, while the right is the SIM chromatogram of 295.95 for IS.)

the full-scan mass spectra of CA and IS after injection in the mobile phase were as shown in **Figure 2**. The quantitative analysis was carried out by SIM at m/z 163.15 for CA and m/z 295.95 for IS, respectively.

Method Validation. *Specificity*. No endogenous interference was observed at retention times of CA (5.2 min) and internal standard (3.2 min) because of the high selectivity of SIM mode. Typical chromatograms of blank plasma, spiked plasma sample and subject sample are shown in **Figure 3**.

Matrix Effect. Three replicate analyses of samples at 0.02, 0.8, and 12 $\mu g \cdot mL^{-1}$ in plasma and three replicate analyses of samples at 0.02, 0.8, and 12 $\mu g \cdot mL^{-1}$ in methanol were analyzed. The ratios of the absolute peak areas at the same concentrations were within the range 0.92−1.06. These results showed that matrix effects could be ignored.

Linearity, LLOQ and LOD. The calibration curves, which related to the concentrations of CA to the area ratios of CA to IS, showed good linearity in the range of 0.01−15 $\mu g \cdot mL^{-1}$. A typical equation of the calibration curve was $y = 0.5970x + 0.02331$ ($r = 0.9963$, $n = 8$). The LLOQ for CA in plasma was 0.01 $\mu g \cdot mL^{-1}$ (lowest standard level, signal-to-noise > 20:1) with coefficient of variation of 13.53% and accuracy of 99.2% ($n = 6$). The LOD of CA was 0.002 $\mu g \cdot mL^{-1}$ which produced a signal-to-noise of 3:1.The data above showed that the assay was sensitive enough for pharmacokinetics study of CA *in vivo*.

Precision and Accuracy. The intra- and interday precision and accuracy for CA from QC samples are summarized in **Table 1**. The intra- and interday precisions (RSD%) were measured to be below 9.66%, while the accuracy (RE%) ranged from −2.93% to 3.19%. The results, calculated by one-way ANOVA, were within the acceptable range, and the method was indicated to be accurate and precise.

Table 1. Precision and Accuracy of the LC−MS Method To Determine CA in Rat Plasma (n = 3 days, Six Replicates per Day)

concn ($\mu g \cdot mL^{-1}$)		RSD (%)		
added	found	intraday	interday	rel error (%)
0.02	0.0206 ± 0.0015	7.9	4.0	3.2
0.8	0.815 ± 0.067	8.3	8.3	1.9
12	11.65 ± 1.11	9.7	9.3	−2.9

Table 2. Stability of CA in Rat Plasma (n = 6)

	spiked concn ($\mu g \cdot mL^{-1}$)		
measd concn ($\mu g \cdot mL^{-1}$)	0.02	0.8	12
Stability for 7 Days at −20 °C			
mean ± SD	0.0205 ± 0.0026	0.793 ± 0.088	11.65 ± 1.07
RSD (%)	12.4	11.1	9.2
3 Freeze and Thaw Stability			
mean ± SD	0.0197 ± 0.0026	0.774 ± 0.087	12.22 ± 1.02
RSD (%)	13.3	11.2	8.4
Stability at Room Temperature for 4 h Kept Protected from Light			
mean ± SD	0.0204 ± 0.0019	0.831 ± 0.077	13.32 ± 0.20
RSD (%)	9.5	9.2	1.5
Postpreparative Stability (12 h in the Autosampler Tray)			
mean ± SD	0.0189 ± 0.0024	0.836 ± 0.018	11.71 ± 1.11
RSD (%)	12.7	2.2	9.5

Extraction Recovery and Stability. The extraction recoveries of CA at concentrations of 0.02, 0.8, and 12 $\mu g \cdot mL^{-1}$ ($n = 6$) were determined to be 87.5 ± 3.4%, 84.6 ± 8.0%, and 84.3 ± 1.2%, respectively. The mean recovery of IS was investigated as 89.3 ± 1.7% at the concentration of 10 $\mu g \cdot mL^{-1}$.

The stability of CA in rat plasma under different conditions was summarized in **Table 2**. CA was stable at room temperature for 4 h, at −20 °C for at least 7 days, and the samples' concentrations did not show significant change after 3 freeze and thaw cycles. In addition, the postpreparative samples in the mobile phase with IS in the autosampler were also stable at 4 °C for at least 12 h.

Application of the Method in Pharmacokinetic Studies. The developed method has been successfully applied for the pharmacokinetic study of CA in rat plasma after oral administration of CA and freeze-dried red wine sample, separately. The concentration−time curves (mean ± SD) of CA after oral administration of CA (2.35 $mg \cdot kg^{-1}$) and freeze-dried red wine (at a dose containing CA 2.35 $mg \cdot kg^{-1}$) in rat plasma are shown in **Figure 4**, and the corresponding pharmacokinetic data are shown in **Table 3**.

After oral administration of CA and freeze-dried red wine, CA was absorbed rapidly and reached a maximum concentration of 3.15 ± 0.49 and 1.71 ± 0.10 $\mu g \cdot mL^{-1}$ at 0.17 ± 0.06 and 0.27 ± 0.14 h, while the plasma concentration of CA declined with a $T_{1/2}$ of 1.28 ± 0.59 h and 3.09 ± 1.27 h ($p < 0.05$), respectively. Compared to the $AUC_{0-\infty}$ (2.32 ± 0.57 $\mu g \cdot h \cdot mL^{-1}$) after oral administration of CA, a larger $AUC_{0-\infty}$ (6.37 ± 0.93 $\mu g \cdot h \cdot mL^{-1}$, $p < 0.05$) after oral administration of freeze-dried red wine was obtained. There were statistically significant differences ($p < 0.05$) in pharmacokinetic parameters of CA including the $T_{1/2}$,

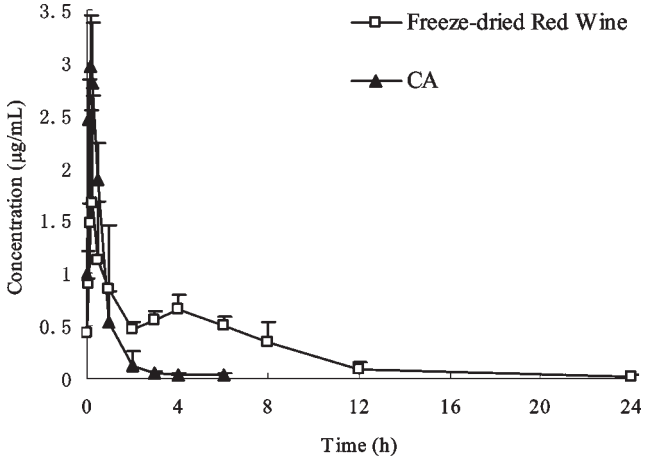

Figure 4. Mean plasma concentration−time curves of CA in rat plasma after oral administration of CA (2.35 mg·kg^{-1}) and freeze-dried red wine (at a dose containing CA 2.35 mg·kg^{-1}).

Table 3. The Main Pharmacokinetic Parameters of CA after Oral Administration of CA (2.35 mg·kg^{-1}) and Freeze-Dried Red Wine (at a Dose Containing CA 2.35 mg·kg^{-1})

		value (mean ± SD)		
parameter	unit	CA	CA in freeze-dried red wine	*P*
T_{max}	h	0.17 ± 0.06	0.27 ± 0.14	0.142
C_{max}	μg·mL^{-1}	3.15 ± 0.49	1.71 ± 0.10	0.055
$T_{1/2}$	h	1.28 ± 0.59	3.09 ± 1.27	0.028
AUC_{0-t}	μg·h·mL^{-1}	2.29 ± 0.55	6.26 ± 0.93	0.000
$AUC_{0-\infty}$	μg·h·mL^{-1}	2.32 ± 0.57	6.37 ± 0.93	0.000

AUC_{o-t} and $AUC_{0-\infty}$ between two groups. In particular, a double-peak profile could be observed from the concentration−time curve of CA in rat plasma.

DISCUSSION

Method Development. Several analytical assays have been reported for determination of CA in biological fluids, which included the HPLC−UV method (*22*−*24*) and the HPLC-ECD method (*18*). However, the HPLC−UV method suffered from low sensitivity and long analysis time while the HPLC-ECD method engaged in gradient elution with long analysis time and quantization without internal standard. The HPLC−MS method proposed overcame the aforementioned issues associated with previous assays. Furthermore, the HPLC−MS/MS method in multiple reaction monitoring (MRM) mode was tested for a more sensitive method for CA analysis. Since CA was a conjugated molecule, it was difficult to find a suitable and selective fragment for detection in the MRM mode and the sensitivity was lower. The HPLC−MS method in the SIM mode proposed was proved to be more rapid, sensitive, specific and accurate.

The column and mobile phase played critical roles in achieving good chromatographic behavior (including peak symmetry and short analysis time) and appropriate ionization. Referring to the reports on HPLC determination of CA (*22*−*24*, *26*, *27*), a Kromasil C_{18} column (200 mm ×4.6 mm, 5 μm) was chosen as the analytical column, and methanol−0.5‰ acetic acid (60:40, v/v) was adopted as the mobile phase for proper retention time, good peak symmetry and sufficient ionization response for the analyte and IS.

Although protein precipitation has been reported for extraction of CA in biological samples (*19*, *22*, *23*), liquid−liquid extraction (LLE) usually offers a much cleaner sample that in turn makes the method more robust and scalable. Therefore, the LLE method was investigated in our study. Among the solvents such as ether, ethyl acetate and ethyl acetate−isopropanol (95:5, v/v), ethyl acetate gave the highest recovery. The adoption of hydrochloric acid was necessary because it could improve the recovery greatly. Therefore, ethyl acetate accompanied with 2 M hydrochloric acid as pH modifier were chosen for a good cleanup of plasma samples and adequate extraction recovery for extracting CA and IS.

An internal standard should be used when performing pharmacokinetic study. The selection of the internal standard was an arduous and hard process since many available compounds of phenolic structure were present in red wine. An appropriate internal standard will control for extraction, HPLC injection and ionization variability. In this method hydrochlorothiazide was chosen as IS for its coincident extraction recovery, ionization response in ESI mass spectrometry with the analyte and proper chromatographic retention time. It was also stable during the period of pretreatment and assaying of the plasma samples. Moreover, hydrochlorothiazide was not a component of red wine.

Pharmacokinetic Comparison. The pharmacokinetic results of rapid absorption and elimination obtained after oral administration of CA were in good agreement with the pharmacokinetic studies reported previously (*19*, *22*). However, it was different from the pharmacokinetic process of CA in freeze-dried red wine. After oral administration of freeze-dried red wine, CA was absorbed rapidly as well but the C_{max} was lower, and CA underwent slow elimination as the plasma concentration could still be detected at 24 h. In particular, the AUC_{0-t} and $AUC_{0-\infty}$ of CA was significantly larger, and a double-peak profile was observed from the concentration−time curve. These differences might result from the interactions of other constituents or transformation from cinnamates in freeze-dried red wine, hepatoenteral circulation, or other reasons. Further studies should be performed to discover the exact reasons.

The absolute oral bioavailability (*F*) of CA in monomer and freeze-dried red wine was investigated. Six rats were given CA monomer (2.35 mg·kg^{-1}) by intravenous administration, and the $AUC_{0-\infty}$ was obtained as 4.48 ± 0.57 μg·h·mL^{-1}. By calculating the ratios of the mean values of $AUC_{0-\infty}$ at the same dose, the *F* values of CA in monomer and freeze-dried red wine were 51.84% and 142.09%, respectively. In view of the large *F* value (> 100%) of CA in freeze-dried red wine, there might be precursors of CA in freeze-dried red wine which could metabolize into CA *in vivo*. There were many kinds of *p*-coumarates in red wine which could transform into CA under certain circumstances *in vitro* (*28*−*30*). Some coumarates might metabolize to CA *in vivo* (*23*). *E*-6-*O*-*p*-Coumaroyl scandoside methyl ester, an ester of CA, was hydrolyzed to CA *in vivo*. CA as a metabolite was absorbed and eliminated more slowly (T_{max}, 1.2 h; $T_{1/2}$, 1.3 h) after administration of the prodrug. These *p*-coumarates were the potential ingredients for the precursors of CA in freeze-dried red wine. The time course of CA in freeze-dried red wine might be conjunct behavior of CA and its precursors. It was similar to the pharmacokinetic process of isofraxidin in rat plasma after oral administration of the extract of *Acanthopanax senticosus* (*31*). Some precursor of isofraxidin metabolized into it and caused the second peak in the concentration−time curve. However, further investigations are needed to find out which are the precursors and how the transformation takes place.

Experiment of enterohepatic recirculation was conducted in the linked-rat model (*32*−*34*) (three groups composed of six rats). It was a system consisting of a hepatoduodenal shunt in which the bile of a drug-treated donor rat was diverted to the duodenum of

an untreated recipient rat via a bile cannula. Blood samples were collected from the abdominal vein of recipient rats before and at 1, 2, 4, 8 h after intragastric admistration of freeze-dried red wine to the donor rats twice (at a dose containing CA 1.18 $mg \cdot kg^{-1}$, at an interval of 0.25 h). A small amount of CA was detected in plasma samples. The result of detection of CA in the recipient rats indicated that CA underwent slight enterohepatic recirculation. Enterohepatic recirculation of CA might contribute to the larger AUC and the double-peak profile of CA in freeze-dried red wine.

It was reported that CA was taken up by monocarboxylic acid transporter (MCT) in human intestinal Caco-2 cells (*21*). Hydroxylation of the substrate of MCT such as benzoic and cinnamic acids would decrease the affinity for MCT because hydrogen bonding between the hydroxyl group of the substrate and MCT might interfere with the molecular recognition (*35*). In addition, benzoic acid was proved to inhibit the absorption of CA (*19*). The absorption of CA in freeze-dried red wine might be inhibited by benzoic and cinnamic acids which were present in red wine. As a result, the C_{max} was lower in the group of freeze-dried red wine. The inhibition might interfere with both the absorption and the elimination of CA. The change of fluidity might also inhibit the absorption of CA.

All of the circumstances mentioned above could be the reasons for the differences between the pharmacokinetic process of CA monomer and freeze-dried red wine. The coexistence of free CA, its precursors and many other constituents in freeze-dried red wine might be the naturally controlling releaser of CA. It could be concluded that oral adminitation of freeze-dried red wine had priority to administration of CA monomer due to its larger bioavailability and slower elimination of CA.

In conclusion, a sensitive and efficient LC−MS method was developed and validated for the determination of CA in rat plasma for the first time. The LLOQ of this method was 0.01 $\mu g \cdot mL^{-1}$, and the runtime was within 6.0 min. The method was successfully applied to the comparative pharmacokinetic study of CA after oral administration of CA and freeze-dried red wine, respectively. Compared with CA monomer, larger AUC, longer $T_{1/2}$ and a double-peak profile were found after oral administration of freeze-dried red wine. Further investigations were conducted to find out the exact reasons for the differences. This was the first time that the pharmacokinetic profile of CA in freeze-dried wine was revealed, which indicated the priority of red wine to active component monomer. It would be helpful in understanding of health-promoting properties of red wine.

ABBREVIATIONS USED

LC−MS, liquid chromatography−mass spectrometry; CA, *p*-coumaric acid; IS, internal standard; SIM, selected ion monitoring; ESI, electrospray ionization; LOD, limit of detection; LLOQ, lower limit of quantification; QC, quality control; RSD, relative standard deviation; RE, relative error; SD, standard deviation; MRM, multiple reactions monitoring; *F*, absolute bioavailability; MCT, monocarboxylic acid transporter.

LITERATURE CITED

(1) Pietta, P.; Simonetti, P.; Gardana, C.; Brusamolino, A.; Morazzoni, P.; Bombardelli, E. Relationship between rate and extent of catechin absorption and plasma antioxidant status. *Biochem. Mol. Biol. Int.* **1998**, *46*, 895−903.

(2) Rahman, I.; Biswas, S. K.; Kirkham, P. A. Regulation of inflammation and redox signaling by dietary polyphenols. *Biochem. Pharmacol.* **2006**, *72*, 1439−1452.

(3) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: History, production, and role in disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287−313.

(4) Bertelli, A. A.; Giovannini, L.; Stradi, R.; Bertelli, A.; Tillement, J. P. Plasma, urine and tissue levels of trans-and cis-resveratrol (3,4′,5-trihydroxystilbene) after short-term or prolonged administration of red wine to rats. *Int. J. Tissue React.* **1996**, *18*, 67−71.

(5) Nigdikar, S. V.; Williams, N. R.; Griffin, B. A.; Howard, A. N. Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation in vivo. *Am. J. Clin. Nutr.* **1998**, *68*, 258−265.

(6) Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.

(7) Demrow, H. S.; Slane, P. R.; Folts, J. D. Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. *Circulation* **1995**, *91*, 1182−1188.

(8) Waterhouse, A. L. Wine phenolics. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 21−36.

(9) Bradlow, H. L.; Telang, N. T.; Sepkovic, D. W.; Osborne, M. P. Phytochemicals as modulators of cancer risk. *Adv. Exp. Med. Biol.* **1999**, *472*, 207−221.

(10) Yang, C. S.; Landau, J. M.; Huang, M. T.; Newmark, H. L. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu. Rev. Nutr.* **2001**, *21*, 381−406.

(11) George, J. S.; Linda, G.; Josephy, P. D.; Goldberg, D. M.; Eleftheros, P. D. A comparison of the anticarcinogenic properties of four red wine polyphenols. *Clin. Biochem.* **2006**, *39*, 492−497.

(12) Ferguson, L. R.; Zhu, S. T.; Harris, P. J. Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29 cells. *Mol. Nutr. Food. Res.* **2005**, *49*, 585−593.

(13) Guglielmi, F.; Luceri, C.; Giovannelli, L.; Dolara, P.; Lodovici, M. Effect of 4-coumaric and 3, 4-dihydroxybenzoic acid on oxidative DNA damage in rat colonic mucosa. *Br. J. Nutr.* **2003**, *89*, 581−587.

(14) Aziz, N. H.; Farag, S. E.; Mousa, L. A.; Abozaid, M. A. Comparative antibacterial and antifungal effects of some phenolic compounds. *Microbios* **1998**, *93*, 43−54.

(15) Pakrashi, A.; Pakrasi, P. L.; Kabir, S. N. Effect of *p*-coumaric acid on immature estrogen treated and cyclic female mice. *Experientia* **1979**, *35*, 843−844.

(16) Zang, L. Y.; Cosma, G.; Gardner, H.; Shi, X.; Castranova, V.; Vallyathan, V. Effect of antioxidant protection by *p*-coumaric acid on low-density lipoprotein cholesterol oxidation. American Journal of Physiology. *Cell Physiol.* **2000**, *279*, C954−960.

(17) Vieira, O.; Laranjinha, J.; Madeira, V.; Almeida, L. Cholesteryl ester hydroperoxide formation in myoglobin-catalyzed low density lipoprotein oxidation: concerted antioxidant activity of caffeic and *p*-coumaric acids with ascorbate. *Biochem. Pharmacol.* **1998**, *5*, 333−340.

(18) Wallerath, T.; Li, H.; Gödtel-Ambrust, U.; Schwarz, P. M.; Förstermann, U. A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO syntheses. *Nitric Oxide* **2005**, *12*, 97−104.

(19) Konishi, Y.; Hitomi, Y.; Yoshioka, E. Intestinal absorption of *p*-coumaric and gallic acids in rats after oral administration. *J. Agric. Food Chem.* **2004**, *52*, 2527−2532.

(20) Konishi, Y.; Shimizu, M. Transepithelial transport of ferulic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 856−862.

(21) Konishi, Y.; Kobayashi, S.; Shimizu, M. Transepithelial transport of *p*-coumaric acid and gallic acid in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 2317−2324.

(22) Meng, Z.; Wang, W.; Xing, D. M.; Lei, F.; Lan, J. Q.; Du, L. J. Pharmacokinetic study of *p*-coumaric acid in mouse after oral administration of extract of Ananas comosus L. leaves. *Biomed. Chromatogr.* **2006**, *20*, 951−9555.

(23) Liu, K.; Yan, L. Q.; Yao, G. C.; Guo, X. J. Estimation of *p*-coumaric acid as metabolite of E-6-O-*p*-coumaroyl scandoside methyl ester in rat plasma by HPLC and its application to a pharmacokinetic study. *J. Chromatogr. B* **2006**, *831*, 303−306.

(24) Ran, G. M.; He, B.; Yao, G. C.; Bao, L. D; Guo, X. J. Study of pharmacokinetics of *p*-coumaric acid in Baihuasheshecao injection. *Chin. J. Pharm. Anal.* **2005**, *25*, 759−761.6.

(25) Achlile, C.; Giorgio, F.; Fabrirlo, B. Determination of acidic and basic/neutral pesticides in water with a new microliter flow rate LC/MS particle beam interface. *Anal. Chem.* **1994**, *66*, 1416−1423.
(26) Tian, S.; Nakamur, K.; Cui, T.; Kayahara, H. High-performance liquid chromatographic determination of phenolic compounds in rice. *J. Chromatogr. A* **2005**, *1063*, 121−128.
(27) Cai, R.; Hettiarachchye, N.; Jalaluddin, M. High-performance liquid chromatography determination of phenolic constituents in 17 varieties of cowpeas. *J. Agric. Food Chem.* **2003**, *51*, 1623−1627.
(28) Clifford, M. N. Chlorogenic acids and other cinnamates-nature, occurrence and dietary burden. *J. Agric. Food Chem.* **1999**, *79*, 362−372.
(29) Torres, J. L.; Rosazza, J. P. N. Reaction of *p*-coumaric acid with nitrite: product isolation and mechanism studies. *J. Agric. Food Chem.* **2001**, *49*, 1486−1492.
(30) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, No. suppl., 2073S−2085S.
(31) Sun, H.; Lv, H. T.; Zhang, Y. M.; Wang, X. J.; Bi, K. S.; Cao, H. X. Pharmacokinetics of isofraxidin in rat plasma after oral administration of the extract of *Acanthopanax senticosus* using HPLC with solid phase extraction method. *Chem. Pharm. Bull.* **2007**, *55*, 1291−1295.
(32) Tsai, T. H.; Shum, A. Y. C.; Chen, C. F. Enterohepatic circulation of chloramphenicol and its glucuronide in the rat by microdialysis using a hepatoduodenal shunt. *Life Sci.* **2000**, *66*, 363−370.
(33) Marier, J. F.; Vachon, P.; Gritsas, A.; Zhang, J.; Moreau, J. P.; Ducharme, M. P. Metabolism and disposition of resveratrol in rats: extent of absorption, glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 369−373.
(34) Chen, Y. J.; Huang, S. M.; Liu, C. Y.; Yeh, P. H.; Tsai, T. H. Hepatobiliary excretion and enterphepatic circulation of colchicines in rats. *Int. J. Pharm.* **2008**, *350*, 230−239.
(35) Konishi, Y.; Kubo, K.; Shimizu, M. Structural effects of phenolic acids on the transepithelial transport of fluorescein in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 2014−2017.

Received for review May 15, 2010. Revised manuscript received October 13, 2010. Accepted October 14, 2010.