

*Full Length Research Paper*

# Pharmacokinetic comparison of ferulic acid in healthy volunteers and patients with functional dyspepsia following oral administration of Chaihu-Shugan-San

Xin-Jian Qiu<sup>1,2</sup>, Xi Huang<sup>1,2,3\*</sup>, Ze-Qi Chen<sup>1,2</sup>, Ping Ren<sup>1</sup>, Wei Huang<sup>1</sup>, Feng Qin<sup>1</sup>, Si-Hang Hu<sup>1</sup>, Jun Huang<sup>1</sup>, Juan He<sup>1</sup>, Zhao-Qian Liu<sup>4</sup> and Hong-Hao Zhou<sup>4</sup>

<sup>1</sup>Laboratory of Ethnopharmacology, Institute of Integrated Traditional Chinese and Western Medicine, Xiangya Hospital, Central South University, 410008 Changsha, China.

<sup>2</sup>Key Unit of Traditional Chinese Medicine Gan of SATCM, Xiangya Hospital, Central South University, 410008 Changsha, China.

<sup>3</sup>Key Unit of the 11th Five-year Plan of SATCM in Cerebrosis, Xiangya Hospital, Central South University, 410008 Changsha, China.

<sup>4</sup>Institute of Clinical Pharmacology, Central South University, 410008 Changsha, China.

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Ferulic acid (FA) of Chuanxiong Rhizoma could promote gastrointestinal motor has been reported. However, its pharmacokinetic characteristics in patients with functional dyspepsia (FD) have never been investigated. The present study was designed to evaluate and compare the pharmacokinetics of FA in patients with FD and healthy volunteers following oral administration of Chaihu-Shugan-San (CSS). Eight healthy volunteers and seven patients with FD were enrolled and blood samples were obtained after oral administration of CSS. Quantification of FA in serum for pharmacokinetic study was achieved by using a simple and rapid UPLC-PDA method. After oral administration of CSS to healthy volunteers and patients, the maximum concentrations were  $355.53 \pm 65.31$  and  $199.69 \pm 41.04$  ng/ml at  $26.95 \pm 7.64$  and  $27.51 \pm 5.02$  min, respectively. Compared with the value of  $AUC_{0-480}$  ( $22.41 \pm 3.28$   $\mu\text{g/ml min}$ ) in healthy volunteers, a smaller value of  $AUC_{0-480}$  ( $14.83 \pm 2.48$   $\mu\text{g/ml min}$ ) was observed in patients with FD. There were statistically differences in the pharmacokinetic parameters of FA including the values for  $C_{\text{max}}$ ,  $AUC_{0-480}$ ,  $CL/F$  and  $MRT_{0-480}$  between healthy volunteers and patients. The pharmacokinetic parameters showed that FD reduced the absorption of FA after oral administration of CSS.

**Key words:** Functional dyspepsia, ferulic acid, pharmacokinetics, Chaihu-Shugan-San.

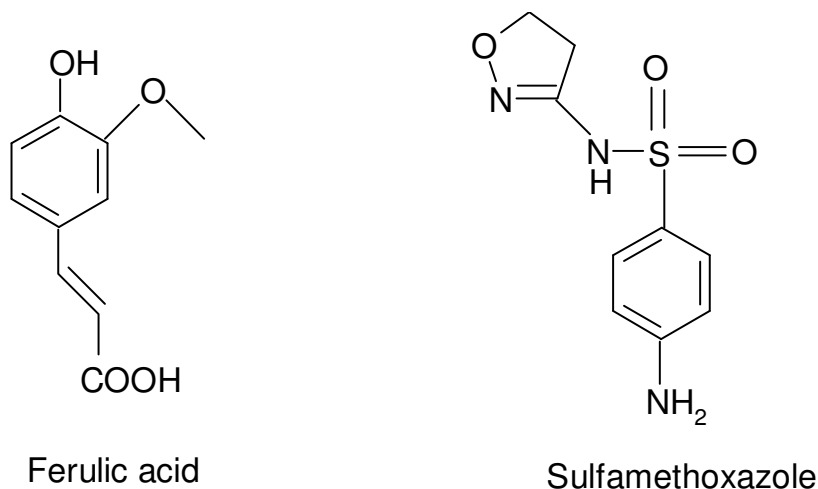
## INTRODUCTION

Functional dyspepsia (FD) is a clinical disorder defined by recurrent upper abdominal symptoms without organic diseases to explain the symptoms. Associated symptoms are early satiety, nausea, vomiting, abdominal distension, bloating and anorexia (Tack et al., 2006). The high incidence of FD (10 to 20%) (Olafsdottir et al., 2010) is a great burden to economy and quality of life of people worldwide (Talley, 2008). To date, prokinetic effect of

agents in clinical is still unsatisfactory (Tack, 2008; Sanger et al., 2008).

Chaihu-Shugan-San (CSS, from Jing-yue-quan-shu, Jing-yue Zhang, 1562 A.D, China), a popular traditional Chinese medicine (TCM) formula, has been successfully used to improve postprandial fullness and early satiation of FD in China for centuries (Wu and Li, 2009; Wang et al., 2005). CSS is made of seven herbal drugs Chuanxiong Rhizoma, bupleurum root, Pericarpium Citri Reticulatae, Rhizoma Cyperi, Fructus Aurantii, Paeonia and Radix Glycyrrhizae. Chuanxiong Rhizoma, as one of the major herbal drugs in this formula plays an important role in treating FD (Zhang, 2010). Many studies have

\*Corresponding author. E-mail: [tcmhuangx59@163.com](mailto:tcmhuangx59@163.com). Tel: +86 731 84327222. Fax: +86 731 84328386.



**Figure 1.** The chemical structures of ferulic acid and sulfamethoxazole.

demonstrated that ferulic acid (FA) of Chuanxiong Rhizoma could simultaneously induce enterokinetic effect, anti-oxidative and anti-inflammatory activities (Badary et al., 2006; Ou et al., 2003; Perluigi et al., 2006). FA has the similar effect as CSS of promoting gastrointestinal motor which contributes directly or indirectly to the therapeutic effect of CSS on FD. FA could be considered as one of the main active components of CSS.

Some pharmacokinetic studies of FA in rats or healthy volunteers have been reported after taking traditional Chinese medical formulas or herbal drugs (Li et al., 2007; Li and Bi, 2003; Gan et al., 2007). Recent reviews have indicated that the states of disease can modify the pharmacokinetics of drugs (Ventresca and Mariani, 1996) and the compatibility principle of TCM could also affect the pharmacokinetics of the prescription (Huang et al, 2000). However, there has been no paper simultaneously reporting the pharmacokinetic parameters of FA in patient with FD and healthy men following oral administration of CSS. It is necessary to evaluate and compare the pharmacokinetics of FA in healthy volunteers and patients with FD after oral administration of CSS.

A rapid, sensitive, simple and accurate UPLC- PDA method to determine FA in plasma of patients and healthy volunteers was developed and successfully applied to pharmacokinetic study. Information obtained in this study might be useful for the clinical applications of CSS to treat patients with FD.

## MATERIALS AND METHODS

### Crude drugs

The CSS decoction included seven crude drugs: bupleurum root, Pericarpium Citri Reticulatae, Chuanxiong Rhizoma, Rhizoma Cyperi, Fructus Aurantii, Paeonia and Radix Glycyrrhizae in a ratio

of 8 : 5 : 5 : 5 : 5 : 3 : 2. All the dry herbs were purchased from Xiangya Hospital (Changsha, China) and identified by director pharmacist Xinzhong Li (Xiangya Hospital, Central South University) with a voucher specimen (No. 200906). The herbs were immersed in distilled water (1:8, w/v) for 1 h and then boiled for 30 min. The boiling procedure was repeated twice. The filtrates were mixed and lyophilized to obtain the powder form of CSS which was stored at 4°C until used.

### Chemicals and reagents

FA and sulfamethoxazole (SMZ) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their purities were more than 99%. SMZ was used as internal standard (IS). The chemical structures of FA and SMZ are given in Figure 1. Acetonitrile and methanol (HPLC grade) were obtained from Tedia Company, Inc, Fairfield, Ohio (US). Other reagents were of analytical grade. House triple-distilled water from silica glass equipment was always used.

### Chromatographic conditions

The ACQUITY UPLC System consisted of a binary solvent manager, a sample manager, a column heater and a PDA detector which were from Waters Corp, Milford, MA, USA. The PDA optical detector was an ultraviolet spectrophotometer that operates between 190 and 500 nm. The analytical column was a Waters BEH C18 Column (2.1 × 100 mm id, particle size 1.7 μm). The mobile phase was acetonitrile-0.5% acetic acid water (17:83, v/v). The column temperature was maintained at 40°C and the auto sampler was conditioned at 25°C. The flow rate was 0.5 ml/min and the injection volume was 6 μl.

### The determination of FA in CSS decoction

The lyophilized powder of CSS was dissolved in distilled water and an aliquot (1.0 ml) of the solution was extracted by methanol (9.0 ml). The mixture solution was vortexed for 3 min and subsequently centrifuged for 10 min at 12000 × g. The supernatant solution was filtered through a 0.22 μm filter before UPLC analysis. The content

of FA in CSS was measured under the aforementioned chromatographic conditions.

### Volunteers

Seven patients with FD (three males, four females; mean age, 32.6 years, range 24–49 years) and eight healthy volunteers (four males, four females; mean age, 23.2 years, range 21–27 years) were recruited. The Medical Ethics Committee of Xiangya Hospital (Central South University, Changsha, China) approved the study protocols.

This study was performed according to Good Clinical Practice and International Conference on Harmonization guidelines. Patients were eligible to participate in this study if they had suffered from upper abdominal discomfort (including early satiety, postprandial fullness, bloating or nausea) for at least 12 weeks within the last 12 months, according to the Rome III criteria (Drossman, 2006).

Before participating in the trial, all the patients had had a physical examination, including laboratory testing (full blood count, sedimentation rate, fasting blood glucose and liver function tests), an abdominal ultrasound and upper gastrointestinal endoscopy. They were excluded if there was evidence of organic disease or surgery that was likely to explain the symptoms of FD.

### Preparation of standard and quality control samples

Standard stock solutions were prepared by dissolving FA and SMZ in methanol to yield a nominal concentration of 32 µg/ml and 100 µg/ml respectively and stored at 4°C. They were further diluted in methanol to make working standards. Calibration samples of FA (5, 10, 20, 40, 80, 160 and 320 ng/ml) were prepared by spiking 1 ml blank plasma with appropriate amounts of working standard solutions and SMZ (I.S.: 50 µl). Quality control (QC) samples of FA were independently prepared at three different concentration levels (10, 40 and 160 ng/ml) to determine the recovery, accuracy and the precision of the method. All the plasma samples were stored at -20°C prior to analysis.

### Plasma sample preparation

The blank plasma (1 ml) in a centrifuge tube (5 ml) was added different amounts of FA (5–320 ng, 50 µl), fixed amount of SMZ (5000 ng, 50 µl) and 850 µl of methanol. The mixture was mixed thoroughly by ultrasound vortexed for 30 s. Next, the denatured protein precipitated was separated by centrifuged and the supernatant was transferred to another tube and evaporated to dryness in a water bath at 50°C under a stream of nitrogen. The residues were reconstituted in methanol (50 µl) and then vortexed for 15 s. The centrifugation procedure was repeated as above and then 6.0 µl of the supernatant solution was injected to UPLC for analysis.

### Calibration curve and LOQ

Standard samples of FA (5–320 ng/ml) and 5000 ng of SMZ (I.S.) in plasma were prepared as previously mentioned. Standard curves were established following the extraction and UPLC analyses of the spiked plasma samples. After determining the peak-area ratios of FA to SMZ in the UPLC chromatograms, the calibration curve was established by least-squares linear fitting of the peak-area ratios of FA to the internal standard. The LOQ was defined as the lowest concentration.

### Precision and accuracy

The intra-day accuracy and precision were assessed by determining QC samples at three concentration levels of FA (10, 40 and 360 ng/ml) on the same day (n = 6). The inter-day accuracy and precision were also evaluated from the analysis of the QC samples on three consecutive days (n = 6). Precision was expressed as relative standard deviation (RSD) and accuracy was expressed as [(mean found concentration – added concentration)/(added concentration)] × 100%.

### Recovery

The relative recoveries of FA from human plasma were determined by QC samples (n = 6). The peak-area ratios (FA to SMZ) of the UPLC chromatograms were compared with those of reference solutions to calculate the relative recoveries of FA.

### Stability in human plasma

The short-term, long-term and freeze–thaw stabilities of FA in plasma were assessed using QC samples (n = 6). Short-term stability was assessed by analyzing QC plasma samples kept at room temperature for 4 h that exceeded the routine preparation time of samples. Long-term stability was determined by assaying QC plasma samples after storage at -20°C for 14 days. Freeze–thaw cycles (-20°C /room temperature) were also applied to QC samples to investigate the stability of FA. In each freeze–thaw cycle, the samples were frozen and stored at -20°C for 24 h, and subsequently thawed at room temperature.

### Pharmacokinetic study

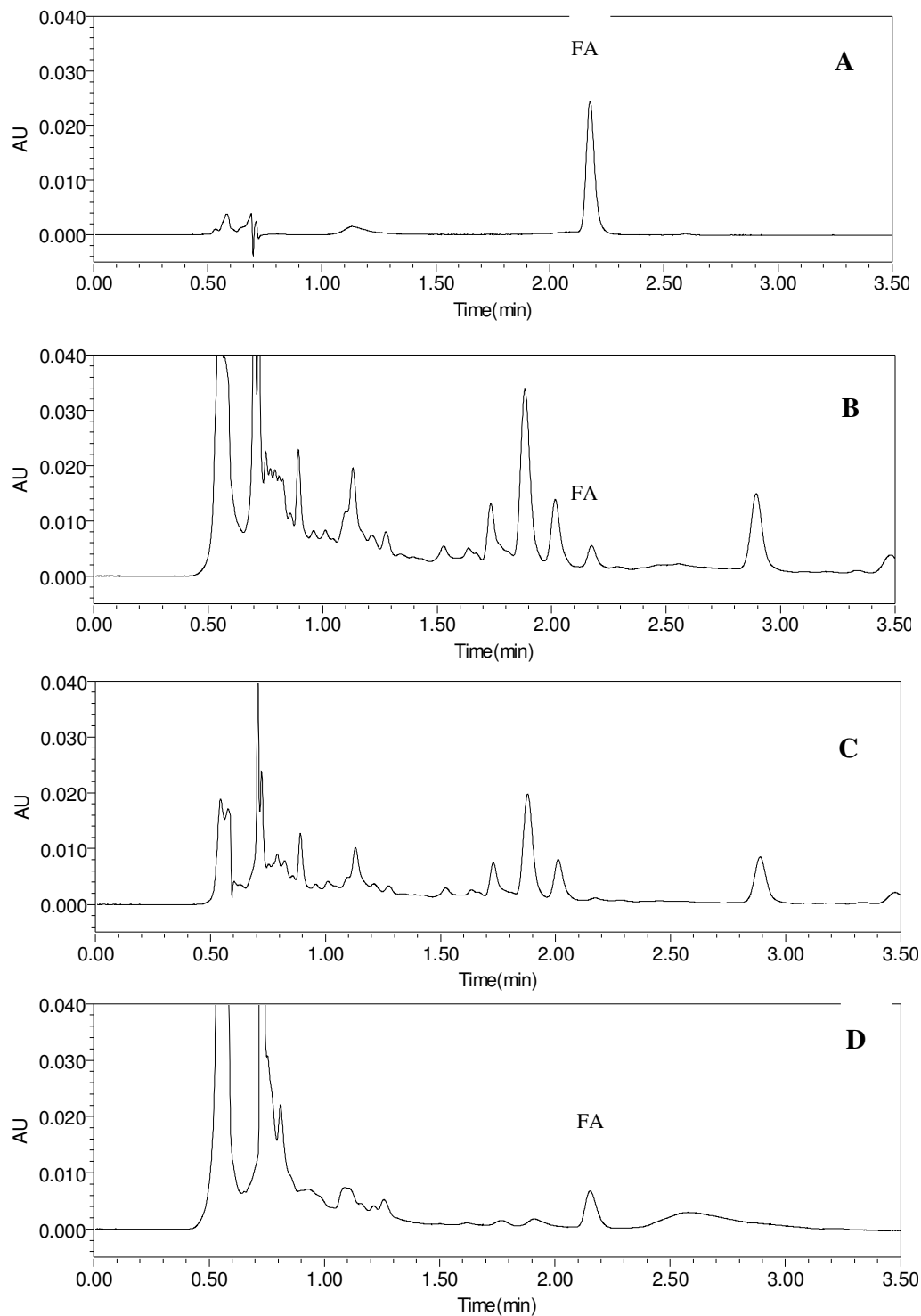
CSS, at a dose of 4 g/kg body weight (Xu, 2010; Li, 2010), was orally administered to volunteers. The volunteers had been fasting for 12 h before drug administration but they had free access to water. Blood samples (10 ml) were collected in Heparinized tubes at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 min after administration, placed for 30 min at room temperature and then centrifuged at 12000 × g for 10 min at 4°C to obtain plasma. The resulting plasma (1 ml) was then mixed with 950 µl methanol and 50 µl IS solution (SMZ, 5000 ng). The next steps were performed following the aforementioned method of plasma sample preparation. The concentrations of FA in patient plasma were determined at each time point.

### Statistical analysis

All data were expressed as mean ± SD. The database was set up with the SPSS 15.0 software package from SPSS Inc., Chicago, Illinois (US). Differences between the two groups were analyzed by one-way analysis of variance. A probability of less than 0.05 was considered to be statistically significant.

## RESULTS

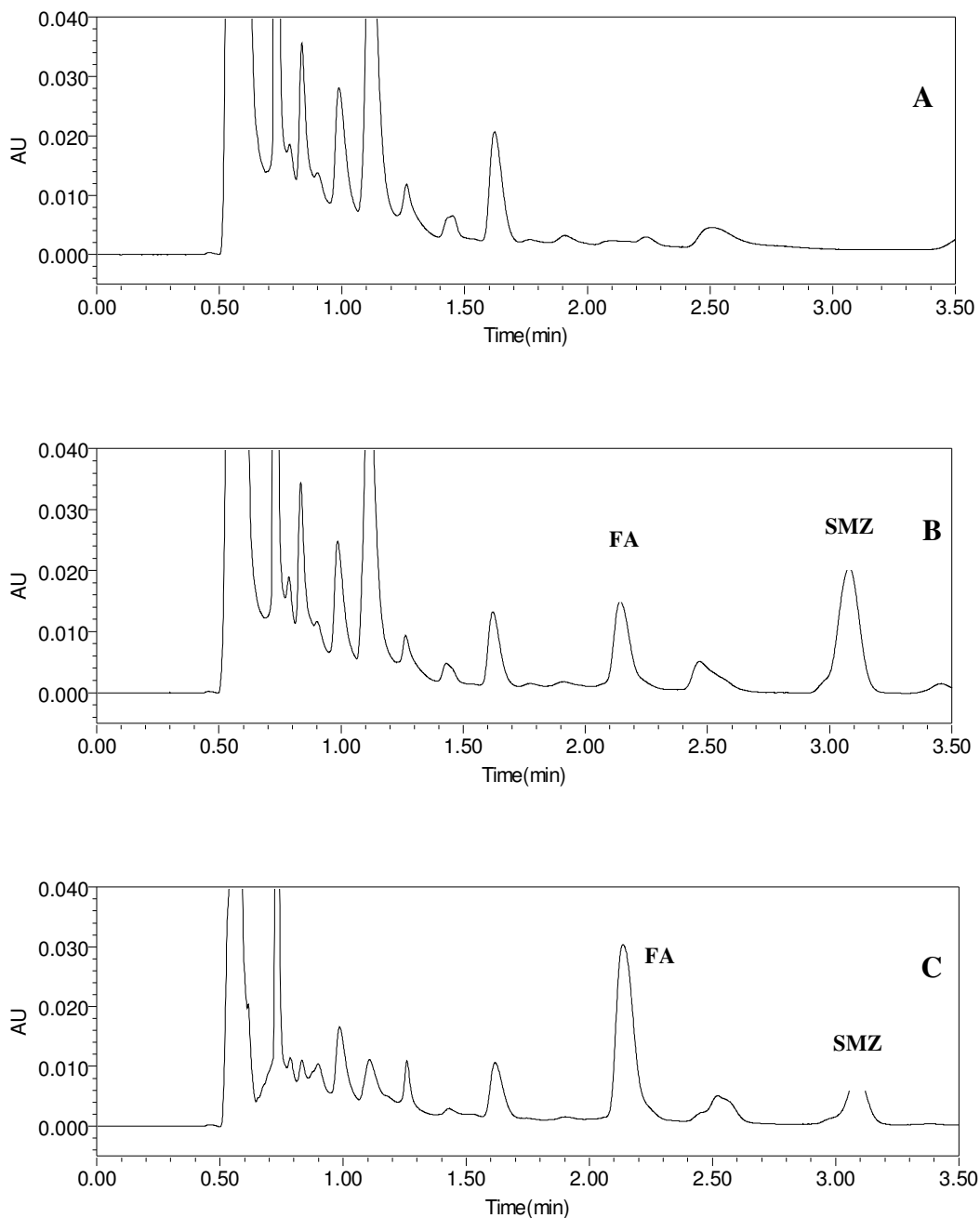
The content of FA (113.16 µg/g) in CSS decoction was calculated firstly. Typical chromatograms of authentic standards, CSS, CSS test sample without Chuanxiong Rhizoma and Chuanxiong Rhizoma were shown in Figure 2. Representative chromatograms of a control blank



**Figure 2.** Typical chromatograms for FA at 325 nm. (A) FA standard; (B) CSS test sample; (C) CSS test sample without Chuanxiong Rhizoma; (D) Chuanxiong Rhizoma.

sample, spiked plasmas and a subject sample were given in Figure 3. FA and SMZ in plasma were completely separated without significant interference. The retention times of FA and SMZ were 2.1 and 3.1 min, respectively.

The calibration curve was linear over the concentration range from 5 to 320 ng/ml in human plasma. The representative regression equation of calibration curve was  $y = 51770x - 0.57025$  ( $r^2 = 0.9995$ ,  $n = 6$ ), and the limit of



**Figure 3.** Typical chromatograms of FA in patient plasma samples. (a) chromatogram of a blank plasma sample; (b) chromatogram of a plasma sample spiked with FA and SMZ (IS); (c) chromatogram of a plasma sample of patient with FD taken 30 min after oral administration of CSS.

quantification the (LOQ) and limit of detection (LOD) were 3 ng/ml and 1 ng/ml. The precision and accuracy of the method were summarized in Table 1. The intra-day and inter-day precisions were less than 6.26 and 3.11%, and the RE was within  $\pm 4.42\%$ . The mean recovery ratios of FA at concentrations of 10, 40 and 160 ng/ml were found to be  $98.71 \pm 1.24$ ,  $95.58 \pm 2.51$  and  $102.09 \pm 3.18\%$  with  $RSD \leq 3.86\%$  (Table 2). Table 3 summarizes the results

of short-term stability, long-term stability and freeze-thaw stability of FA in human plasmas.

The serum FA concentration-time curves were analyzed using a DAS program (the Chinese Society of Mathematical Pharmacology) on a personal computer to determine the compartment model. The plasma concentration-time curve of FA was fitted with the two-compartment models. The mean plasma concentration

**Table 1.** Precision and accuracy of the UPLC method for FA in human plasma (n = 6).

Nominal concentration (ng/ml)	Intra-day(n=6)			Inter-day(n=6)		
	Mean±SD (ng/ml)	RSD (%)	RE (%)	Mean±SD (ng/ml)	RSD (%)	RE (%)
10	9.92±0.62	6.26	-0.77	9.87±0.12	1.26	-1.28
40	39.24±1.99	5.08	-0.19	38.23±1.01	2.62	-4.42
160	162.41±7.29	4.49	1.51	163.35±5.09	3.11	2.09

RSD, relative standard deviation; RE, relative error.

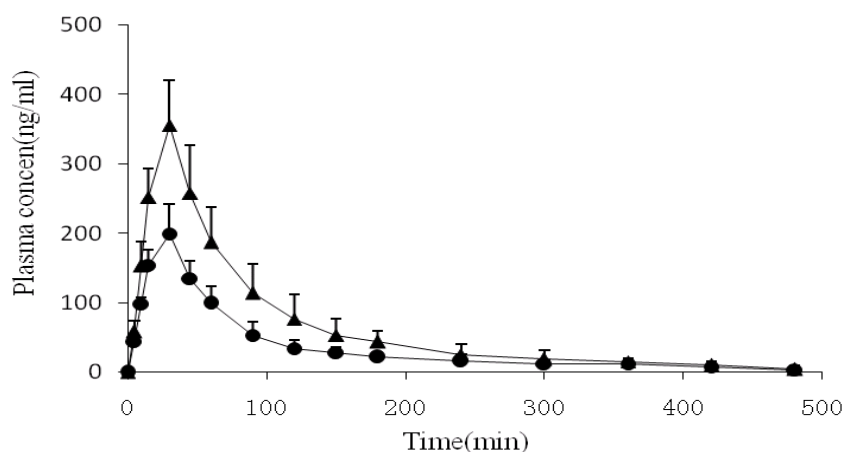
**Table 2.** Recovery of FA from human plasma.

+	Standard sample concentration (ng/ml)	Recovery (%) (Mean±SD)	RSD (%)
FA	10	98.71±1.24	1.74
	40	95.58±2.51	3.01
	160	102.09±3.18	3.86

n = 6. The standard sample concentrations represented low (10ng/ml), medium (40ng/ml) and high (160 ng/ml) concentrations of FA.

**Table 3.** Stability of FA in human plasma at three QC levels (n = 6).

Stability	FA (ng/ml)		
	10	40	160
Short-term stability	9.95±1.16	39.55±2.29	161.93±5.07
Long-term stability	9.79±1.54	39.38±3.36	159.36±5.28
Freeze-thaw stability	9.81±2.01	38.93±5.11	157.31±8.84



**Figure 4.** Serum concentration-time profiles of FA in patients with FD and healthy volunteers after oral administration of CSS (4 g/kg). Values are mean±SD, n=7 patients with FD (●) and n= 8 healthy volunteers (▲).

versus time profiles of FA in patients and healthy volunteer serum after oral administration of CSS was illustrated in Figure 4. The main pharmacokinetic parameters such as  $T_{max}$ ,  $C_{max}$ ,  $T_{1/2}$ ,  $k_a$ ,  $AUC_{0-480}$ ,  $MRT_{0-480}$  and  $CL/F$  were calculated and were listed in Table 4.

## DISCUSSION

In Chaihu-Shugan-San (CSS) decoction there are many components including ferulic acid, ligustilide, saikoside, gallic acid, liquiritin, naringin, peoniflorin and so on.

**Table 4.** Pharmacokinetic parameters of FA in serum after oral administration of CSS to healthy volunteers and patients with FD.

Parameter	Patients with FD	Healthy volunteers
T <sub>max</sub> (min)	27.51±5.02	26.95±7.64
C <sub>max</sub> (ng/mL)	199.69±41.04	355.53±65.31**
AUC (0-480) (µg/mL min)	14.83±2.48	22.41±3.28 *
T <sub>1/2</sub> (min)	65.49±16.06	58.37±13.74
K <sub>a</sub> (L/min)	0.132±0.072	0.116±0.055
CL/F (L/min/kg)	206.35±29.07	159.86±17.03*
MRT (0-480) (min)	131.88±16.07	110.09±18.92*

Values are mean ± SD, n= 7 patients with FD, n= 8 healthy volunteers. \**P*<0.05, \*\* *P*<0.01. T<sub>max</sub>, the time to reach peak concentration; C<sub>max</sub>, maximum plasma concentration; AUC, area under concentration–time curve; T<sub>1/2</sub>, apparent elimination half-life; K<sub>a</sub>, absorption constant; CL/F, apparent clearance; MRT, mean residence time.

However, according to the searching results of literatures, only ferulic acid of these components has been reported with an important effect on the gastrointestinal motility. Ferulic acid has the similar effect as CSS of promoting gastrointestinal motor which contributes directly or indirectly to the therapeutic effect of CSS on functional dyspepsia. Therefore, ferulic acid was chosen as the marker to compare the pharmacokinetic profile in healthy volunteers and patients with functional dyspepsia.

The pharmacokinetic analysis of FA in animal and human plasma by HPLC based on liquid–liquid extraction, boiling-water-bath and perchloric acid extraction has been reported (Li and Bi, 2006; Li et al., 2008; Qi et al., 2007). However, these extraction methods could not provide satisfactory recovery for FA. As shown in Table 2, the recovery of FA in human plasmas extracted by methanol was acceptable. SMZ was selected as the appropriate internal standard which was stable and did not exist in human plasma. It was eluted after FA and separated from endogenous compounds in the chromatogram. The ACQUITY UPLC BEH C<sub>18</sub> column (2.1× 100 mm, 1.7 µm), mobile phases consisted of acetonitrile–0.5% acetic acid water, isocratic elution and flow rates of 0.5 ml/min were optimized as the suitable chromatogram conditions. The total analysis time was 3.5 min and the retention time of FA was 2.1 min which was significantly shorter than that achieved in previous method with better resolution (Li et al., 2007; Zafra-Gómez et al., 2010). The acceptable peak shape and satisfactory separation of FA and SMZ from endogenous components were achieved in human plasma under the above chromatogram condition. The method was validated for linearity, accuracy, precision, LOQ and recovery and was successfully applied to the pharmacokinetic study of FA in healthy volunteers and patients with FD.

After oral administration of CSS by healthy volunteers, FA was absorbed at a fast absorption rate and reached a maximum plasma concentration (C<sub>max</sub>) value (355.53±65.31 ng/ml) within 26.95±7.64 min. The plasma

concentration of FA declined with a half-life (T<sub>1/2</sub>) value of 58.37±13.74 min. However, after oral administration of CSS by FD patients, the C<sub>max</sub> value of FA was 199.69±41.04 ng/ml within 27.51±5.02 min, and the plasma concentration of FA declined with a value of T<sub>1/2</sub> of 65.49±16.06 min. Compared with the AUC<sub>0-480</sub> (area under the curve) value (22.41±3.28 µg/ml min) after oral administration of CSS by healthy volunteers, a smaller AUC<sub>0-480</sub> value (14.83±2.48 µg/ml min, *P*<0.05) of FA in FD patients was obtained. There were statistically differences in the pharmacokinetic parameters of FA including the values for C<sub>max</sub>, AUC<sub>0-480</sub>, CL/F and MRT<sub>0-480</sub> between healthy volunteers and patients. The pharmacokinetic parameters showed that FD reduced the absorption of FA in human after oral administration of CSS.

Compared with Modified Xiao-Yao Decoction to healthy volunteers (Li et al, 2008), the T<sub>max</sub> of FA was advanced from 45 min to 26.95 and 27.5 min after oral administration of CSS to healthy volunteers and patient with FD. The other pharmacokinetic parameters including T<sub>max</sub>, C<sub>max</sub>, T<sub>1/2</sub>, AUC<sub>0-480</sub>, CL/F and MRT<sub>0-480</sub> were also different. Recent reviews have indicated that the compatibility principle of TCM (Huan et al., 2008) and the states of disease (Ventresca and Mariani, 1996) could affect the pharmacokinetics of drugs. Modified Xiao-Yao decoction given to healthy volunteers has fourteen herbs while CSS given to healthy volunteers and patients with FD has seven herbs. In addition, the pharmacokinetics of FA was often studied in some other experiments using rats, rabbits and dogs (Li et al., 2007; Li and Bi, 2003; Huang et al., 1996). Compared with the pharmacokinetic parameters of FA in patients and healthy men, the information obtained from animals was significantly different.

In conclusion, clinical pharmacokinetic study of herbs and TCM in patients is very useful to treat disease. In this study, the main pharmacokinetic parameters of FA between healthy volunteers and patient with FD following oral administration of CSS were significant different

suggested that the state of the disease of FD could modify the pharmacokinetics of FA. The result was very important to understand the absorption, distribution, metabolism and excretion of FA in patients with FD and provide information on the dosage of CSS to treat FD in clinical applications.

## ACKNOWLEDGMENTS

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