Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 787 (2003) 421–425
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of spinosin in rat plasma by reversed-phase high-performance chromatography after oral administration of Suanzaoren decoction

Yujuan Li[a], Xinmiao Liang[b], Hongbin Xiao[b], Kaishun Bi[a,*]

[a]*School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, 110016, China*
[b]*Dalian Institute of Chemical Physics, the Chinese Academy of Science, Dalian, 116012, China*

Received 16 July 2002; received in revised form 26 November 2002; accepted 4 December 2002

Abstract

A sensitive, simple, and accurate method for determination of spinosin in rat plasma with sulfamethoxazole (SMZ) as internal standard was developed using RP-HPLC with UV detection. Sample preparations were carried out by protein precipitation with acetonitrile, followed by the evaporation of the acetonitrile to dryness. The resultant residue was then reconstituted in mobile phase and injected onto a Hypersil C_{18} (200×4.6 mm I.D., 5 μm) analytical column. The mobile phase consisted of acetonitrile–water (15:85, v/v) with 1% glacial acetic acid. The assay was shown to be linear over the range of 18.07–903.5 ng/ml ($R^2=0.995$). Mean recovery was determined as 93.6%. Within- and between-day precisions were ≤8.9% RSD. The limit of quantitation was 18.07 ng/ml. The HPLC method developed has been applied to determine the pharmacokinetics of spinosin in rat plasma after having taken Suanzaoren decoction.

Keywords: Spinosin

1. Introduction

Traditional Chinese medicine (T.C.M.) consists of natural therapeutic agents used under the guidance of the theory of traditional Chinese medical science. It has been widely used for the prevention and treatment of disease since antiquity in China. Herbal medicines are used mostly in combinations in China and are made into preparations for easy and efficient use.

Suanzaoren decoction is a famous T.C.M. preparation with the effects of hypnosis, sedation and anticonvulsion [1,2]. It has been widely used for the treatment of insomnia in the clinical practice [3,4]. The Suanzaoren decoction is made of five herbal medicines Suanzaoren (*Semen ziziphi spinosae*), Fuling (*Poria*), Chuanxiong (*Rhizoma chuanxiong*), Zhimu (*Rhizoma anemarrhenae*) and Gancao (*Radix glycyrrhizae*). All the herbs are included in Chinese Pharmacopoeia (Version 2000).

Semen ziziphi spinosae is the main medicine of Suanzaoren decoction, and spinosin (Fig. 1a) is the major effective constituent of *Semen ziziphi spinosae*. It plays an important role on the effect of sedation and hypnosis [5–8]. Thus, spinosin is used

*Corresponding author. Tel.: +86-24-2392-8487; fax: +86-24-2389-6050.
E-mail address: ksbi@mail.sy.ln.cn (K. Bi).

1570-0232/02/$ – see front matter
doi:10.1016/S1570-0232(02)01001-2

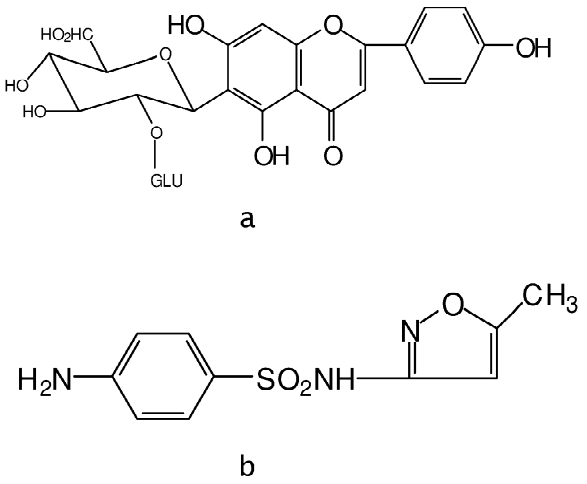

Fig. 1. Chemical structures of spinosin (a) and internal standard SMZ (b).

as one of the marker compounds to characterize the Suanzaoren decoction.

Pharmacokinetic studies of active ingredients in Chinese herbs will have considerable impact on illustrating their action mechanism and promoting the development of T.C.M.. To our knowledge, no report is available regarding high-performance liquid chromatographic assay of spinosin in rat plasma after oral administration of the Suanzaoren decoction. In order to support preclinical pharmacokinetic studies requiring the quantitation of spinosin in biological matrices, a sensitive, simple and accurate method for the determination of spinosin in rat plasma after oral administration of Suanzaoren decoction was developed.

2. Experimental

2.1. Materials

Suanzaoren, Fuling, Chuanxiong, Zhimu and Gancao were purchased from Tianyitang T.C.M.shop, Shenyang, China, and all the herbs were identified by senior engineer Chunquan Xu (Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang); spinosin was supplied by Shenyang Pharmaceutical University (98.8% purity); sulfamethoxazole (SMZ, Fig. 1b) used as internal standard (I.S.) was supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); glacial acetic acid was of analytical grade and acetonitrile was of chromatographic grade from Beijing Xingzhi Chemical Factory (Beijing, China). Healthy Wistar rats were supplied by the Shenyang Pharmaceutical University.

2.2. Preparation of Suanzaoren decoction

Suanzaoren (18 g), Fuling (10 g), Chuanxiong (5 g), Zhimu (10 g) and Gancao (3 g) were chopped into pieces and refluxed three times with 460 ml of 95% ethanol for 1 h. After removing the ethanol under reduced pressure, the residue was dissolved in water to get the Suanzaoren decoction with a Suanzaoren concentration of 1.8 g/ml.

2.3. Preparation of standard and internal standard stock solutions

The standard stock solution was prepared by dissolving 12.0 mg of spinosin in 25 ml methanol to obtain a nominal concentration of 0.480 mg/ml. The I.S. stock solution was prepared by dissolving 11.5 mg of SMZ in 50 ml methanol to obtain a nominal concentration of 0.230 mg/ml. All the stock solutions were kept at 4 °C before use.

2.4. Preparation of assay standard samples

Spinosin standard samples (18.07, 36.14, 90.35, 180.7, 361.4, 722.8 and 903.5 ng/ml) were prepared by spiking control rat plasma with appropriate amounts of the standard stock solution prepared above. Quality control (QC) samples to determine accuracy and precision of the method were independently prepared at low (18.07 ng/ml), medium (180.7 ng/ml) and high (903.5 ng/ml) concentrations in the same manner. The I.S. was added to each standard sample just prior to sample processing. All the samples were stored at −20 °C until analysis.

2.5. Extraction procedure

Each rat ($n=6$) was fasted for 12 h with free access to water during the experiment and administered an oral dose of 36 g/kg of Suanzaoren

decoction. A blood sample (0.5 ml) was collected at 0, 1, 2, 4, 5, 6, 7, 8, 10, 12, 15 and 24 h immediately transferred into heparinized tubes and centrifuged for 10 min at 3000 *g*. To 200 μl plasma 100 μl of I.S. solution (18.4 μg/ml) and 400 μl acetonitrile were added. Each tube was mixed thoroughly by vortexing for 90 s. After centrifugation for 10 min at 3000 *g*, the supernatant was transferred into labelled clean test tubes and evaporated to dryness in a water bath at 50 °C under a stream of nitrogen. The residues were reconstituted in 100 μl of mobile phase with vortexing for 90 s and the centrifugation procedure was repeated. Standard and QC samples were prepared following the above method. A 20-μl aliquot was injected onto the chromatographic column.

2.6. Assay conditions

A Hypersil C_{18} (200×4.6 mm, I.D., 5 μm) analytical column from Elite (Dalian, China) was used. As mobile phase a binary mixture of acetonitrile–water (15:85, v/v) containing 1% glacial acetic acid was delivered by a LC-10AD pump (Shimadzu, Kyoto, Japan) in isocratic mode at a flow-rate of 0.7 ml/min. The SPD-10A (Shimadzu, Kyoto, Japan) detector was set at 334 nm and all the measurements were performed at 35 °C. An LC-10 AD workstation was used for data acquisition.

3. Results and discussion

3.1. Specificity

The degree of interference by endogenous plasma constituents with spinosin and I.S. was assessed by inspection of chromatograms derived from processed blank plasma sample. Typical chromatograms of blank plasma, blank plasma spiked with spinosin and I.S., and rat plasma sample after administration of Suanzaoren decoction were presented in Fig. 2. Spinosin and the I.S. were eluted at 14.9 and 21.0 min, respectively. The total run time was less than 30 min. A good separation of the I.S. and spinosin was obtained under the specified chromatographic conditions. No interfering peaks were found at the retention times of spinosin and the I.S.

3.2. Calibration and validation

Various amounts of spinosin and I.S. (18.4 μg/ml) were added to 200 μl blank plasma. The solutions were prepared as above method. The calibration curve for the determination of spinosin in rat plasma was linear over the range of 18.07–903.5 ng/ml. The R^2 values for the standard curves were 0.995. The linear regression of the curve for peak area ratio versus concentration was weighted by $1/x^2$ (the

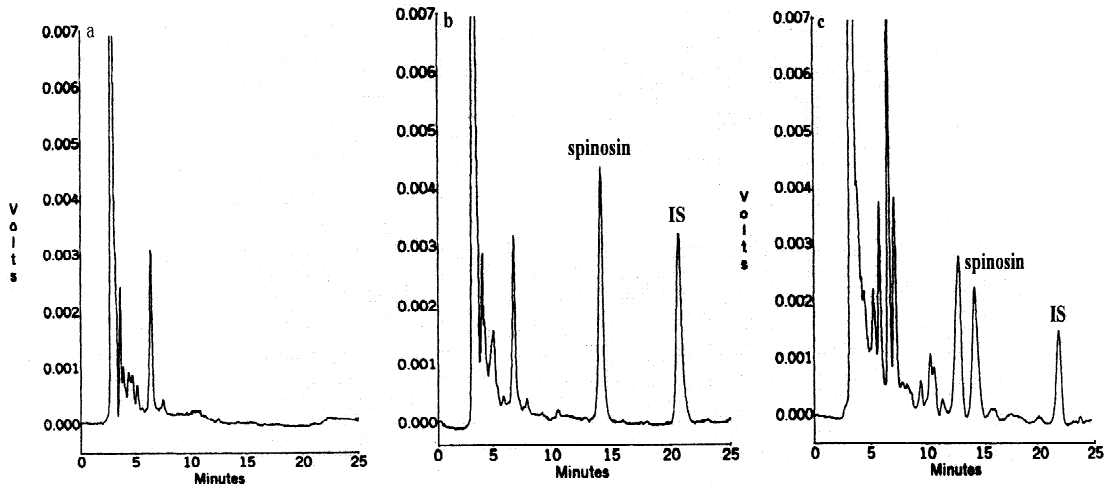

Fig. 2. HPLC chromatograms of blank plasma (a), blank plasma spiked with spinosin (361.4 ng/ml) and I.S. (18.4 μg/ml) (b) and plasma sample (6 h) after oral administration of Suanzaoren decoction (c).

reciprocal of the square of the spinosin concentration).

The recoveries of spinosin from the spiked plasma samples were calculated at low (18.07 ng/ml), medium (180.7 ng/ml) and high (903.5 ng/ml) concentrations. The spiked samples were processed as above. Recovery was calculated by comparing the observed concentrations with the spiked concentrations. The mean recovery was 93.6%. The accuracy and precision are summarized in Table 1. The results showed that both the precision and accuracy were satisfactory. The limit of quantitation (LOQ) was defined as the lowest drug concentration which can be determined with a relative error (RE) and precision (RSD) of $<20\%$. The LOQ of this method was 18.07 ng/ml.

The stability of spinosin in rat plasma was performed through three freeze–thaw cycles of the QC samples. The percent deviation from the concentration observed were 1.4%, −4.0% and −2.9%, respectively. Results of the stability experiments indicated that spinosin was very stable in rat plasma.

3.3. Pharmacokinetic applicability

The assay was applied to a preliminary pharmacokinetic experiment in rat. The Suanzaoren decoction was administered orally to the rat at a single dose and blood samples were collected at scheduled intervals. The pharmacokinetic parameters were estimated by the 3P87 program (the Chinese Society of Mathematical Pharmacology). The plasma spinosin concentration–time curve was fitted to the one-compartment with the first-order absorption model. The mean plasma concentration–time profile is illustrated in Fig. 3. The pharmacokinetic parameters are listed in Table 2. These parameters indicated that spinosin was cleared slowly from the body.

Table 1
Accuracy and precision of the HPLC method to determine spinosin in rat plasma

Concentration (ng/ml)		RSD (%)		RE (%)
$C_{nominal}$	$C_{observed}$	Within-day	Between-day	
18.7	17.40	4.1	8.9	−3.7
180.7	176.3	2.5	2.7	−2.4
903.5	897.3	1.7	3.6	−0.7

$C_{nominal}$, nominal concentration; $C_{observed}$, observed concentration.

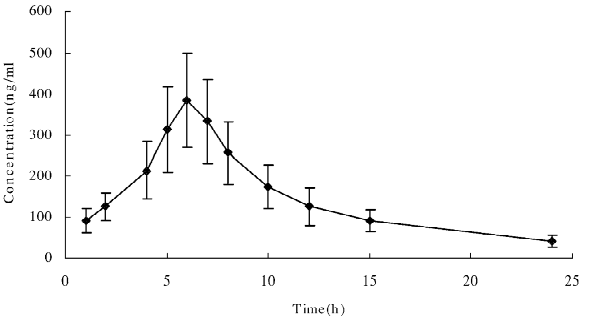

Fig. 3. Mean plasma concentration–time profile for spinosin in rat plasma after oral administration of Suanzaoren decoction.

3.4. Method development

During development of the method, ethyl acetate was tested to extract spinosin in rat plasma, however, the recovery of spinosin was unsatisfactory ($<30\%$). As we know, spinosin is one of the flavone-C-glycosides in Suanzaoren, and the low recovery of spinosin should be attributed to its high aqueous solubility. Other methods for sample preparation, such as deproteinization by acetonitrile, methanol, 6% perchloric acid, acetone or 10% trichloroacetic acid were tested. The results showed that deproteinization by acetonitrile gave good resolution and a high recovery in this method.

Spinosin possesses three strong absorption wavelengths (215, 272 and 334 nm). If the detector was set at 215 or 272 nm, spinosin and the I.S. were not well separated from the compounds of the herbs and endogenous substances. Under the above detection conditions, different separation conditions were

Table 2
Mean pharmacokinetic parameters of spinosin after oral administration Suanzaoren decoction

Parameters	Values
K_e (h^{-1})	0.1389±0.01154
C_{max} (ng/ml)	0.4122±0.1378
T_{max} (h)	6.000±0.6325
$T_{0.5}$ (h)	5.0211±0.4257

K_e, the apparent elimination rate constant; C_{max}, the maximum plasma concentration; T_{max}, the time to reach peak concentration; $T_{0.5}$, the apparent elimination half-life.

tested, while changing the composition of mobile phase still led to poor resolution or a long analytical time. A detection wavelength of 334 nm proved to be the most suitable and was selected for the assay.

4. Conclusions

In conclusion, a new HPLC method for the determination of spinosin in the plasma of rat after oral administration of Suanzaoren decoction has been developed. The within- and between-day precisions of the QC samples deviated from the nominal concentration by less than 8.9%. Recovery evaluations showed that spinosin was recovered from rat plasma at a rate of 93.6%. The method is simple, accurate, specific and precise for the determination of spinosin in rat plasma.

Herbal medicines are used mostly in combinations in China, and the presence of other herbs can play an important role in the effectiveness of the combinations. In this study, when the herb suanzaoren alone was administered to the rat, spinosin was scarcely detected under the same experimental conditions. An interesting conclusion was drawn that the presence of the other four herbs promoted the absorption of spinosin and affected the pharmacokinetic action of spinosin significantly. The developed method also can be applied to determine spinosin from the herb of *Semen ziziphi spinosae*. The pharmacokinetic results are useful for the further study of the clinical applications of the Suanzaoren decoction.

References

[1] Z.J. Zhang, in: Prescription of Jinkuiyaolue, People's Medical Publishing, Beijing, 1972, p. 21.
[2] J.Q. Xu, W.M.Z. Wang, in: Science of T.C.M. Prescriptions, People's Medical Publishing, Beijing, 1995, p. 306.
[3] Q.H. Meng, J. Snd. Mili. Med. Colg. 4 (1983) 75.
[4] X.Y. Ge, X.Y. Zhu, J. Henan Traditional Chin. Med. 19(6) (1991) 9.
[5] W.S. Woo, Phytochemistry 18 (1978) 353.
[6] K.H. Shin, C.K. Lee, W.S. Woo, S.S. Kang, Arch. Pharm. Res. 7 (1978) 1.
[7] C.L. Yuan, Z.B. Wang, Y. Jiao, Chin. J. Chin. Mater. Med. 12 (1987) 34.
[8] K. Kawashima, K. Saito, A. Yamada, S. Obara, T. Ozaki, Biol. Pharm. Bull. 20 (1997) 1171.