Determination of Geniposide in Rat Urine after Oral Administration of the Traditional Chinese Medicinal Preparation Yin-Zhi-Ku Decoction by High-Performance Liquid Chromatography

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Abstract

A high-performance liquid chromatography method with solid-phase extraction is introduced for the determination of geniposide in rat urine after oral administration of Yin-Zhi-Ku decoction. Geniposide and an internal standard (paeoniflorin) are extracted from urine using Strata cartridges. Analysis of the extract is then performed on a reversed-phase C_{18} column using acetonitrile–water (14:86, v/v) as eluting solvent system. UV detection is set at 238 nm. The calibration curve for geniposide is linear (r = 0.9996) in the concentration range of 2.0–240 µg/mL. Both intra- and interday precision of the geniposide are determined, and their relative standard deviation does not exceed 10%. The validated method is successfully applied to determine geniposide from rat urine after oral administration of Yin-Zhi-Ku decoction.

Introduction

Traditional Chinese medicine (TCM) is the natural therapeutic material used under the guidance of the theory of traditional Chinese medical science and has been applied by TCM practitioners for thousands of years. It is used mostly in combination, in which the composite formulae will produce a synergistic effect or antagonistic action. This medical approach has played an important role in the prevention and treatment of diseases.

Yin-Zhi-Ku (YZK) decoction has been used to treat hepatitis and has produced a favorable effect. The formula consists of *Herba Artemisiae Scopariae*, *Fructus Gardeniae*, and *Radix Sophorae Flavescentis* (2:1:1, w/w/w). Geniposide (Figure 1), a water-soluble iridoid glycoside purified from gardenia fruit, has been reported to treat hepatic and inflammatory conditions (1,2). Thus, geniposide can be used as one of the marker compounds to characterize the YZK. High-performance liquid chromatography (HPLC) (3–6) and thin-layer chromatography (7) are the two major methods that have been used for the determination of geniposide contained in medicinal preparations. The quantitative studies on geniposide in biosamples have been reported (8,9). However, there have been no studies on the excretion of geniposide. This paper describes a simple solid-phase extraction (SPE)–HPLC method for the determination of geniposide in rat urine after oral administration of the YZK decoction.

Experimental

Materials and reagents

Yin Chen (*Artemisia scoparia* Waldst. et Kit.), Zhi Zi (*Gardenia jasminoides* Ellis), and Ku Shen (*Sophora flavescens* Ait) were purchased from Yang He Tang Pharmcetical Co. Ltd. (Shanghai,

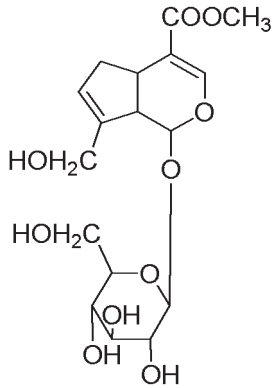

Figure 1. The chemical structure of geniposide.

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China). The herbal materials were extracted twice by refluxing in water (1:8, g/mL) for 1 h, and the water extract was concentrated and lyophilized. The dried powder was stored at 4°C prior to use.

Geniposide and the internal standard, paeoniflorin, were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and methanol were of HPLC grade (Fisher, Leics, U.K.). In-house triple-distilled water from silica glass equipment was always used.

HPLC conditions

The HPLC system consisted of a Waters 600E pump (Milford, MA), Waters 2487 UV–vis detector set at 238 nm, 20-µL injection loop, LC workstation for data collection, and Diamonsil C_{18} reversed-phase column (5 µm, 200 × 4.6 mm), which was protected by RP18 (5 µm) guard column (Dikma, Beijing, China). The mobile phase was water–acetoniltrile (86:14, v/v) filtered through a 0.45-µm Milipore filter (Billerica, MA) and degassed prior to use. The flow rate was 1 mL/min.

Content of geniposide in YZK

To calculate the administered dose of geniposide, its content in YZK decoction was calculated first, and the lyophilized extract of YZK was dissolved in distilled water and diluted to a concentration of 0.5 mg/mL. The mixture was centrifuged at 2500 rpm for 10 min, and the supernatant solution was obtained. Then, 20 µL of this solution was injected into the HPLC system for analysis. The content of geniposide in the lyophilized extract of YZK was determined, from the peak area ratios using the equation for linear regression obtained from the calibration curve, to be 0.98%.

SPE procedure

Cartridges with 3-mL capacity (Strata, Phenomenex, Torrance, CA) and liquid phase C_{18} chemically bound to silica gel (200 mg) were applied to isolate geniposide from rat urine. The columns were conditioned before use by means of 3 mL methanol and then 3 mL triple distilled water. Rat urine with geniposide and paeoniflorin (1 mL) was transferred into an SPE column cartridge. Columns with absorbed geniposide were purified by two amounts of 3 mL distilled water. The compounds were washed with 4 mL of 60% methanol under low vacuum. The resulting solution was evaporated to dryness at 40°C in vacuo. The evaporated residue was dissolved in 1 mL of mobile phase, and 20 µL of the solution was injected into the HPLC for analysis.

Calibration procedure

Stock solutions of geniposide and paeoniflorin were prepared with triple-distilled water. Geniposide was prepared at concentrations of 20, 100, 200, 400, 800, 1600, and 2400 µg/mL and paeoniflorin at 30 µg/mL. Then, 100 µL of each solution was added together to blank rat urine so that the resulting urine contained 2.0, 10, 20, 40, 80, 160, and 240 µg/mL geniposide and 3.0 µg/mL paeoniflorin. The serum was then processed according to the SPE procedure specified previously. The limit of quantitation (LOQ) in serum was defined as the lowest concentration on the calibration curve for which assay precision [relative standard deviation (RSD)] was lower than 10%.

Recovery

The recovery for geniposide at concentrations of 10, 80, and 160 µg/mL was calculated. The first group consisted of five 1-mL urine samples, each spiked with 100 µL of 100 µg/mL geniposide standard solution and 100 µL of 30 µg/mL internal standard solution, which contained 10 µg/mL of geniposide and 3.0 µg/mL of the internal standard in the end. The samples were processed according to the previously mentioned SPE procedure. The second group consisted of five urine samples with internal standard only, and geniposide was added into the eluting solution after the SPE procedure. Recoveries were calculated as the area ratio of geniposide to internal standard from the spiked samples and unextracted standard solution. The recovery for 80 and 160 µg/mL of geniposide were calculated in the same manner.

Animal, drug administration, and urine sampling

Male Sprague-Dawley rats (200–220 g) were obtained from the Laboratory Animal Center of Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Science (Shanghai, China). They were kept in an environmentally controlled breeding room for 3 days before starting the experiments. They were fed with standard laboratory food and water *ad libitum* and fasted overnight before the test.

An aqueous solution of YZK was orally administrated to rats at a dose of 310 mg/kg of geniposide and held in metabolic cages that allowed for the collection of urine samples. Urine samples were collected for a 96-h period after dosing. As soon as the collection was finished, the urine was centrifuged at 3500 rpm for 10 min at 10°C, and the supernatant was separated and stored in polypropylene tubes at –20°C prior to analysis.

Results and Discussion

Selectivity

Figure 2 shows the chromatograms obtained following the analysis of drug-free urine, drug-free urine spiked with geniposide and internal standard, and urine sample collected between 12 and 24 h after oral administration of the YZK decoction. The retention times of geniposide and internal standard were 9.76 and 17.27 min, respectively, and no interfering peaks were detected. This indicated the selectivity of the elaborated procedure was satisfactory.

Calibration curve

The calibration curve was prepared for geniposide in the range of 2.0 to 240 µg/mL, which covered the levels following the administration of a single dose of 310 mg/kg geniposide. The standard curve was described by the following equation:

$$y = 0.5968x - 0.0764 \qquad \text{Eq. 1}$$

where $r = 0.9996$, y is the peak area, x is the concentration, and r the correlation coefficient. The LOQ in serum was defined as the lowest concentration on the standard curve for which the assay precision was reflected by RSD ≤ 10%, and it amounted to 2.0 µg/mL.

Recovery and reproducibility

The recoveries for geniposide from rat urine were 93.8%, 92.1%, and 93.2% at the concentrations of 10, 80, and 160 µg/mL, respectively (Table I). The intra- and interday accuracies were estimated, and the studied concentrations (20, 40, 80, 160 µg/mL) were lower than 10%, as indicated by the respective values of RSD (Table II). This shows that the method was quite precise. Moreover, the small difference (≤ 10%) noted between added levels and the estimated concentrations have documented an appropriate accuracy of the elaborated method.

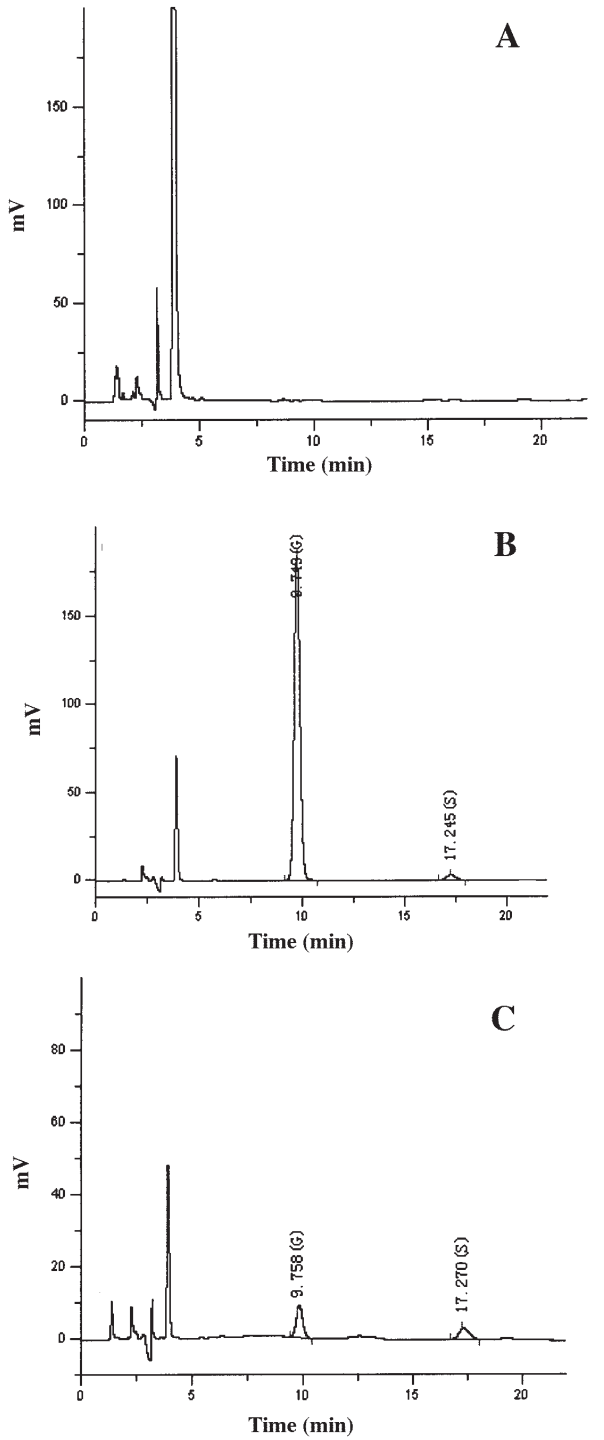

Figure 2. Typical chromatograms for the determination of geniposide in urine samples: blank urine sample (A); urine sample spiked with geniposide (G) and internal standard (S) (B); and urine sample collected between 12 and 24 h after oral administration of the YZK decoction (C).

Table I. Recovery of Geniposide from Rat Urine*

Spiked concentration (µg/mL)	%Recovery ± SD†	Average (%)
10	93.8 ± 2.1	
80	92.1 ± 2.3	93.0
160	93.2 ± 2.2	

* $n = 5$.
† Standard deviation.

Table II. Intra- and Interday Precision and Accuracy of Geniposide in Rat Urine

Concentration added (µg/mL)	Concentration measured (µg/mL) ± SD*	Accuracy (%)	RSD† (%)
Intraday reproducibility ($n = 5$)			
20	20.48 ± 0.47	102.43	2.03
40	39.83 ± 0.22	99.57	0.56
80	78.98 ± 0.62	98.72	0.78
160	162.19 ± 1.82	101.37	1.12
Interday reproducibility ($n = 5$)			
20	20.70 ± 0.44	103.51	2.13
40	40.01 ± 0.41	100.02	1.02
80	78.50 ± 0.89	98.12	1.14
160	159.30 ± 2.09	99.56	1.31

* Standard deviation.
† Relative standard deviation.

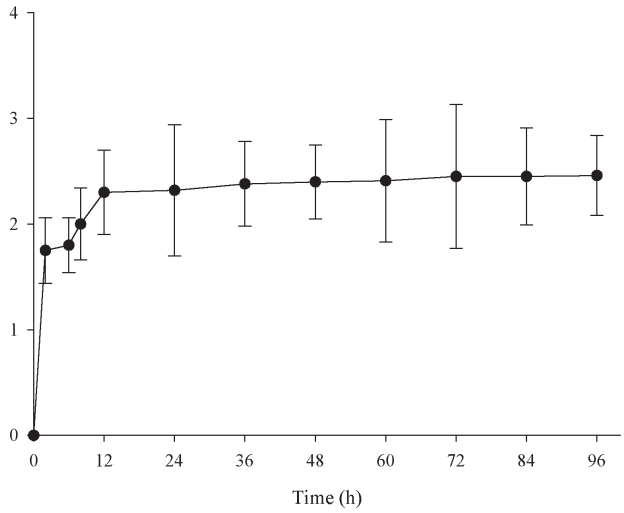

Figure 3. Profile of cumulative excretion of geniposide into urine after oral administration of the YZK decoction.

Determination of geniposide in urine

Urine samples from rats were collected at 2, 6, 8, 12, 24, 36, 48, 60, 72, 84, and 96 h after oral administration of the YZK decoction. Figure 3 shows the cumulative urinary excretion of geniposide. The cumulative urinary excretion of geniposide was 2.46 ± 0.05 of the administered dose. The maximum excretion of geniposide into urine was observed 2 h after dosing. This study indicated that geniposide was quickly excreted into urine. Previous studies showed that geniposide had a low blood distribution and low bioavailability (9). The current results were consistent with this. On the other hand, other mechanisms may have caused this result, such as geniposide may have been poorly absorbed in the stomach and intestines and it may have been quickly and mainly excreted through the kidneys in an intact form. Therefore, further experiments should be done to answer these questions.

Conclusion

Application of an SPE method has permitted the determination of geniposide with relatively high recovery of extraction (> 90%) and elimination of the nonpolar interfering impurities. The designed procedure fulfils the validation requirements and could be applied for in vivo studies.

Acknowledgments

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References

1. Y.Y. Cheng, Y.S. Chan, K.F. Tia, and H.M. Chang. Effect of geniposide on acute jaundice in rats caused by anit poisoning. *Acta Pharm. Sin.* **7(1):** 69–72 (1986).
2. C.M. Harada, N. Tenmyo, M. Aburada, and T. Endo. Pharmacological studies of gardeniae fructus I. Effects of geniposide and genipin on the biloary excretion, the gastric juice secretion, and the gastric contraction, and other pharmacological actions. *Yakugaku Zasshi* **94:** 157–62 (1974).
3. T.H. Tsai, J. Westly, T.F. Lee, and C.F. Chen. Identification and determination of geniposide, genipin, gardenoside, and geniposidic acid from herbs by HPLC/photodiode-array detection. *J. Liq. Chromatogr.* **17(10):** 2199–2205 (1994).
4. M. Masanori, A. Sakae, and O. Yukio. Determination of geniposide, gardenoside, and related iridoid compounds by reversed-phase high-performance liqudi chromatography. *J. Chromatogr.* **357(2):** 293–300 (1986).
5. O. Toshiyuki, S. Kazuhiko, Y. Tsuguchika, Y.Y. Tong, G. Zhang, and Y.H. Chen. Determination of geniposide, gardenoside, geniposidic acid and genipin-1-b-gentiobioside in Gardenia jasminoides by high-performance liqudi chromatography. *J. Chromatog.* **455:** 410–14 (1988).
6. A. Yoshinobu and K. Youichi. Simultaneous determination of geniposide, geniposidic acid and gardenoside in gadenia fruit by high-performance liquid chromatography. *Bunseki Kagaku* **35(3):** 215–18 (1986).
7. H.B. Hu and Q.Y. Ni. TLC-densitometric determination of geniposide in Zhizi and Yueju Wan. *J. Chin. Pharm. Uni.* **20(3):** 179–80 (1989).
8. T.Y. Tseng and T.H. Tsai. Measurement of unbound geniposide in blood, liver, brain and bile of anesthetized rats: an application of pharmacokinetic study and its influence on acupuncture. *Anal. Chim. Acta* **517:** 47–52 (2004).
9. K. Ueno, Y. Takeda, Y. Iwasaki, and F. Yoshizaki. Simultaneous estimation of geniposide and genipin in mouse plasma using high-performance liquid chromatography. *Anal. Sci.* **17(10):** 1237–39 (2001).

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