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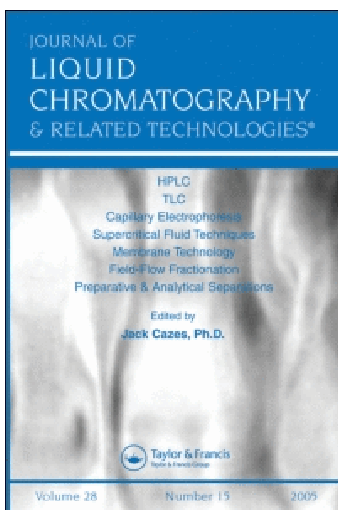

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MEASUREMENT AND PHARMACOKINETIC STUDY OF GASTRODIN IN RAT BLOOD BY CAPILLARY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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ABSTRACT

A simple, accurate, and rapid capillary micellar electrokinetic chromatography method was developed for the determination of gastrodin in rat blood. All experiments were performed in a 47 cm (40 cm effective length) $\times$ 75 μm i.d. uncoated fused-silica capillary with UV detection at 200 nm. A running buffer composed of 50 mM sodium tetraborate, 15 mM sodium dodecyl sulfate (SDS) (pH = 9.50) was found to be suitable. The method was linear in the range of 2.50 ~ 200.0 μg/mL (R = 0.999), and the detection limit was 0.50 μg/mL. Only a small amount of blood (about 50 μL) was required for each analysis.

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The recovery of gastrodin in rat blood was 91.51 ~ 96.39%. This method was suitable for the determination of gastrodin after femoral vein injection and could be used for pharmacokinetic studies of this drug in rat blood.

INTRODUCTION

Gastrodin, one of the main active principles isolated from the traditional medicinal herb "tianma," is considered to have very beneficial properties. It is said to have the functions of improving the memory and preventing the aging process. Tiama is often prescribed as a sedative, as well as for rheumatism, paralysis, headaches, and hemiplegia (1,2). It has also been used in China for the treatment of cardiovascular and cerebrovascular diseases for many years (3). In view of these important effects, an accurate assay method for the determination of gastrodin in a biological sample is very important. As a first step, we are concerned with measuring drug concentrations of gastrodin in rat blood considering the fact that most of the drugs attained the intended part of the body by blood circulation.

Several studies have been reported for the determination of gastrodin by sepectrophotometry (4), high-performance liquid chromatography (HPLC) (5–7), and capillary electrophoresis (CE) (8,9). However, the above method focused on the components analysis in the Chinese traditional herb, its preparations and liquid injections, and not on the measurement of the gastrodin concentration in biological samples or plasma. This paper describes a simple and rapid capillary micellar electrokinetic chromatographic method with ultraviolet (UV) detection to determine the concentration of gastrodin in rat blood and its related pharmacokinetic profile.

EXPERIMENTAL

Materials

Gastrodin hydrochloride (the structure is shown in Figure 1) was purchased from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China), coumarin (internal standard and also shown in Figure 1) from the Third Chemical Factory of Shenyang, sodium tetraborate and sodium hydroxide were purchased from Beijing Fine Chemical Company, and phosphor acid from Beijing Hongxing Chemical Factory. SDS was purchased from Sigma (USA). Gastrodin injection liquid was purchased from the local drug store. All other chemicals and reagents were analytical grade or better and used as received. Water was purified by a Milli-Q water system (Millipore, Bedford, MA).

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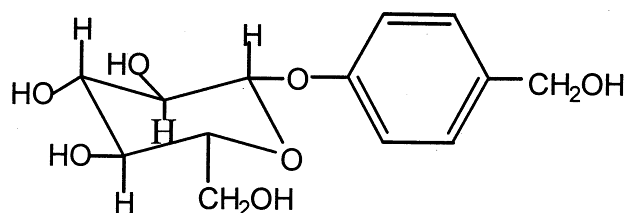
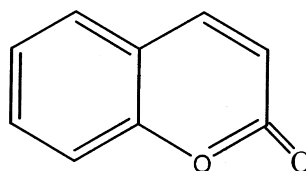


Gastrodin



Coumarin

Figure 1. The chemical structure of gastrodin and coumarin.

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Apparatus

All experiments were performed on a Beckman P/ACE 5000 capillary electrophoresis system (Fullerton CA) with an UV detector. An uncoated fused-silica capillary of 47 cm length (40 cm effective length from the anode to the detector) × 75 μm i.d. × 360 μm o.d. (Yongnian Optical Fiber Factory, Heibei, China) was used as a separation tube. The capillary was assembled in a cartridge format and the temperature was maintained at 20°C by a thermostatic system. Samples were pressure injected at 0.5 p.s.i. for 2 s. A personal computer was used to control the P/ACE instrument and to perform data analysis using the P/ACE software (Beckman, USA). A pH-HJ90B pH meter (Aerospace Computer Company, Beijing, China) was used for the pH measurements.

Blood Sample Preparation

Male Sprague-Dawley rats (about 200 g) were obtained from the Zoological Institute, Chinese Academy of Sciences (Beijing, China), and were forbidden food overnight but allowed free access to water. The rats were anesthetized with amino ethyl formate. The drug was injected in a single bolus through the femoral

vein of the rat. Blood samples (50 μL) were collected from another femoral vein at a different time following gastrodin (50 mg/kg i.v.) injection. Each sample was transferred to a heparinized microfuge tube (1.0 mL) and centrifuged at 4000 g for 10 min. The resulting plasma sample was mixed with 12 μL internal standard (coumarin, 0.1 mg/mL) solution. The above solution was mixed with 1.5 volume acetone and centrifuged at 4000 g for 30 min, and then the supernatant was collected in a new centrifuge and was stored in a refrigerator until analysis. These samples were used for making the pharmacokinetic profile.

Calibration Curves and Recovery Test

Calibration of the method was carried out by addition of known amounts of gastrodin and internal standard to drug-free blood samples that were treated as described above. The calibration curves were established in the range of 2.50 ~ 200.0 μg/mL and the internal standard concentrations were fixed at 25 μg/mL. The actual sample concentrations were calculated by the determination of the peak-height ratios of gastrodin and the coumarin, and the value was compared to the calibration curve.

Certain known concentration standard gastrodin solutions were added to the blood sample and were treated with the same method. The peak area ratio (gastrodin/coumarin) was calculated according to the calibration curve, the calculated value was compared to the value added to the plasma and was used for calculating the recoveries.

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RESULTS AND DISCUSSION

The effects of separation voltage, buffer pH, and SDS concentration on separation were studied. With increasing voltage, the migration time of gastrodin and coumarin decreased, and the efficiency of gastrodin obtained the highest value at 24 kV; the mobilities of gastrodin and internal standard changed as did the electroosmosis at the pH range of 8.50 ~ 10.50, which showed that the effective mobilities of them did not change; the resolution between gastrodin and acetone (adding to the blood to eliminate the protein) became large with the increasing of SDS when its concentration was lower than 15 mM, but varied little after that. Although the resolution between gastrodin and internal standard improved continuously with the increasing SDS concentration, the migration time was prolonged at high SDS concentration, so 15 mM SDS was selected. The most suitable conditions selected were: 50 mM sodium tetraborate, 15 mM SDS at pH = 9.50, the separation voltage was 24 kV.

At the optimal conditions, the typical electropherograms of drug-free plasma obtained at different times after femoral vein injections were shown in Figure 2. There were no apparent interfering peaks from the plasma components, either on gastrodin or the internal standard. The migration time was about 3.66 min and 4.67 min for gastrodin and coumarin, respectively.

The peak area ratios (gastrodin to coumarin) of eight concentrations of gastrodin were linearly related to the concentrations of the drug, and the equation of the regression line was $y = 0.0106x + 0.339$ ($r = 0.999$), as shown in Figure 3.

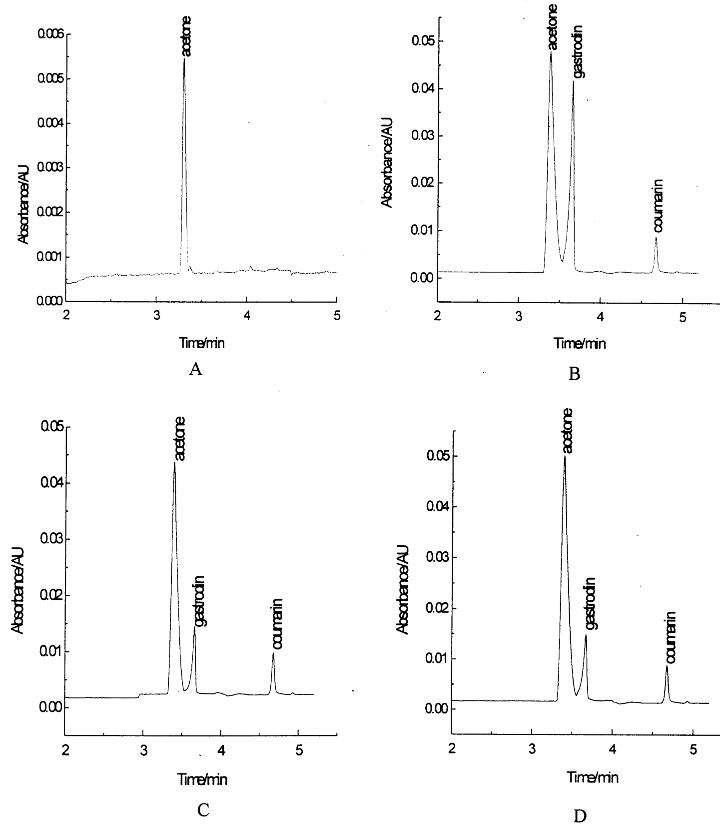

Figure 2. The typical electropherograms of drug-free rat plasma (A), plasma sample containing gastrodin collected from a rat at 2.33 min (B), 9.30 min (C) and 14.00 min (D) after gastrodin injection (50 mg/kg i.v.). Peaks: 1, gastrodin, 2, coumarin (internal standard). Capillary: 47 cm (40 cm effective length) × 75 μm i.d., 360 μm o.d. Conditions: buffer, 50 mM sodium tetraborate at pH = 9.50, 15 mM SDS, Detection, UV at 200 nm, injection, pressure at 0.5 p.s.i. for 2 s, separation voltage: 24 kV.

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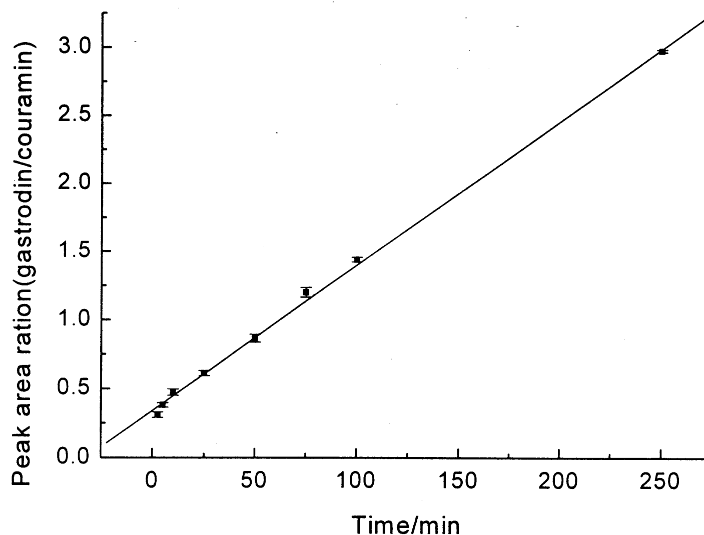

Figure 3. The calibration curve of gastrodin in rat blood plasma. Data are expressed as means of 3 determinations ± S.D. All the conditions are the same as in Figure 2.

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The recoveries of gastrodin from rat plasma were between 91.51 ~ 96.39% (Table 1). The detection limit of gastrodin, at the signal-to-noise of 3 : 1 was 0.50 μg/mL in rat plasma.

The method was validated for reproducibility of the migration time and the peak area ratios (gastrodin to coumarin). The relative standard deviation (RSD) values of the migration time and peak area ratios for five injections was 1.01 ~ 2.28% and 2.68 ~ 4.99% (inter day), and 1.63 ~ 2.32% and 2.72 ~ 6.49% (intra day), respectively.

Drug concentrations in plasma were plotted against time after femoral vein injections of gastrodin (shown in Figure 4). The profile was described by

Table 1. Recoveries of Gastrodin in Rat Blood at Different Concentration

Amount Added (μg)	Amount Determined (μg/mL)	Recoveries	RSD (n = 3)
5	4.625	92.50	4.85
10	9.151	91.51	2.61
30	28.918	96.39	2.97

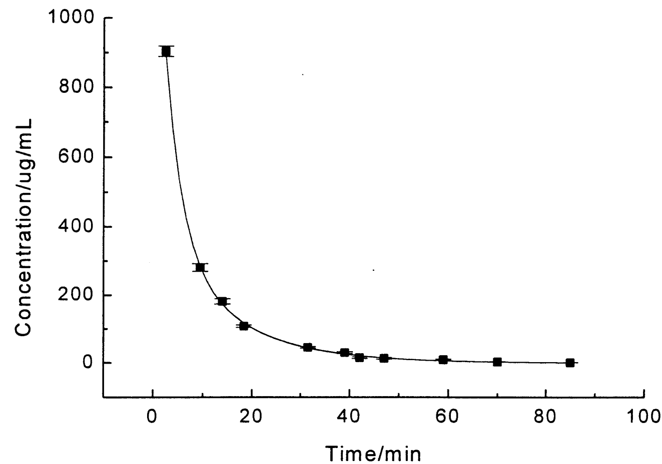

Figure 4. Plasma concentration-time curve after gastrodin injection. All the conditions are the same as in Figure 2.

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compartmental models using the computer program 3P87 and proven according to one-compartment open pharmacokinetic model:

$$C = e^{-kt}$$

The pharmacokinetic parameters, as derived from the data and calculated by 3P87, are shown in Table 2.

Table 2. Pharmacokinetic Parameters of Gastrodin in Rat Plasma After Injection (50 mg/kg, Data are Expressed as Means ± S.D, n = 5)

Parameter	Unit	Values
C0	μg/mL	438.250 ± 28.46
Ke	1/min	0.070 ± 0.005
V(c)	(mg/kg)/(μg/mL)	0.137 ± 0.011
T1/2 (ke)	Min	9.832 ± 0.36
AUC	(μg/mL) min	6216.622 ± 112.0
CL (s)	Mg/kg/min/(μg/mL)	0.0096 ± 0.00052

C0: concentration at time zero; Ke: terminal elimination rate constant; V: volume of apparent distribution; T1/2: terminal elimination half-life; AUC: the area under the concentration curves; CL: clearance.

CONCLUSION

A capillary micellar electrokinetic chromatography (MECC) method with UV detection was developed for determining the gastrodin. It provided the advantages of short analysis time, low operation cost, and simple instruments. This method could be used to analysis the gastrodin in rat plasma after femoral vein injection and to obtain the pharmacokinetic profiles of it.

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