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# A Potential Method for Recycling of Gastrodin Separated from Urine

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*Gastrodia rhizome* is a famous Chinese medicinal plant and has been widely used in China for centuries. In this paper, we have, preparatively seperated gastrodin from the rats' urine after metabolism. Male Wistar rats were orally given crude gastrodin water solution and their urine was collected for continuously 6 h. The collected urine was absorbed with Macroporous resin D101 and desorbed with ethanol water solution (5:95, v/v) to give crude gastrodin. The crude gastrodin was then purified by silica gel column chromatography to get 262 mg gastrodin over 98 % in purity. The total recovery yield of gastrodin was *ca.* 70.4 %. Though low recovery, our results provided a potential way to recycle gastrodin because of huge amounts need of *Gastrodia rhizome* in China every year.

**Key Words: Gastrodin, *Gastrodia rhizome*, Rat metabolism, Urine, Recycling.**

## INTRODUCTION

Recycling is very important for natural resources, it helps conserve energy, reduces pollution and save raw materials, The recycling of water, glass, paper, cardboard or plastic is common, but there is few attempts about the recycling of the bio-active components of Chinese herbal medicines, especially for valuable medicines.

*Gastrodia rhizome*, 'Tian-Ma' in Chinese, is the dried rhizome of Gastrodia elata Blume, which is a notable and commonly used Chinese herb and a family member of Orchidaceae[1]. For centuries, *Gastrodia rhizome* has been used, as a traditional medicine in China, to cure facial paralysis, rheumatism, infantile convulsion, lumbago, neuralgia, headache and other neuralgic and nervous disorders[2]. Gastrodin (*p*-hydroxymethyl phenyl β-D-glucopyranoside, its structure shown in Fig. 1A), separated from *Gastrodia rhizome*, has proved to be its most bio-active component. In recent years, gastrodin draws lots of attentions not only for its effectively used for neuro-protection, treatment of some disorders of central nervous system and cardiac problems[3-5], but also for its low toxicity[6].

Chinese Pharmacopoeia prescribes that gastrodin is the only reference substance of *Gastrodia rhizome* and its content should be no less than 0.2 % in dried *Gastrodia rhizome*. As such, the separation of gastrodin is crucial for properly use of *Gastrodia rhizome*. Lots of efforts have been made: *i.e.*, Ong *et al.*[7] used the pressurized liquid extraction at room temperature to extract gastrodin from *Gastrodia rhizome* and claimed that such an extraction process could minimize the use of organic solvents. Li and Chen[2] separated gastrodin *via* high-speed counter-current chromatography and could obtain gastrodin in a one-step separation.

Some references reported that when rats was feed *Gastrodia rhizome*, after rats metabolism, most of its components were decomposed while gastrodin basically remained unchanged and its original form was expelled from the body with its urine[8]. As such, we proposed, in this paper, a new route to separate gastrodin, which was, in brief, to obtain it from the urine of rat metabolism of *Gastrodia rhizome*. As far as the huge amount daily use of *Gastrodia rhizome* in China is concerned, our method provides potential route for recycling use of gastrodin. And to the best of our knowledge, it is the first report of the separation of gastrodin from the urine of rat metabolism.

## EXPERIMENTAL

**Animal, reagents and plant materials:** Male wistar rats (200-220 g) were obtained from Vital River Laboratories (Beijing, China). Animals were housed in an environmentally controlled room with a 12 h light/dark cycle and free access to commercial ration and deionized water. The study protocol was approved by the Beijing University of Traditional Chinese Medicine Animal Care and use commit.

Macroporous resin was purchased from Tianjin Haiguang chemical corporation; ethanol were AR grade purchased from

Beijing Chemical reagent factory; deionized water was purchased from Wahaha Corporation. The medicinal plant materials were obtained from An-guo medical hall in Hebei province, China; the standard gastrodin was purchased from the National Agency for Food and Drug Administration and Control.

**Preparation of crude gastrodin water solution from the *Gastrodia rhizome*:** The dried *Gastrodia rhizome* was first ground to powder. The powder (150.0 g) was then immersed with 420 mL ethanol and sonicated for 1 h. The mixture was filtered and then the residue was repeatedly extracted twice (420 mL ethanol each time). The filtrate was combined and evaporated by rotary evaporation at 45 ºC. The dried products (10.8 g) were then re-dissolved in 40 mL water.

**Rat metabolism and gas purification:** After overnight fasting, each rat was given 2 mL crude gastrodin water solution by oral administration; the rats were then placed in metabolism cages to collect their urine for continuous 6 h. During this period, the rats were fasted but permitted free access to water.

The collected urine was separated by macro-porous resin D101 as reported procedure[9]. In brief, Macro-porous resin D101 was first washed several times with deionized water until the pH of liquid phase was closed to 6.0. The rats' urine was condensed to *ca.* 15 mL by rotary evaporation, added to the above resin and held for 8 h under agitation at 25 ºC. After reaching adsorption equilibrium, the resin were washed with 3 BV deionized water and then desorbed with ethanol-water (5:95, v/v) solution. The desorbed solution was monitored by HPLC, merged, then evaporated by rotary evaporation at 45 ºC to get the dried crude gastrodin.

The dried crude gastrodin was purified by silica gel column chromatography eluting successively with ethyl acetate/methanol (v/v: 8:1) and to yield gastrodin.

**HPLC analysis:** Both the crude sample and the purified gastrodin were analyzed by HPLC. The HPLC system is consisted of Waters 1525 pump, a manual sample injector and a Waters 2489 UV detector (Waters, Milford, MA, USA). The column used was a reversed-phase symmetry $C_{18}$ column (250 mm × 4.6 mm i.d., 5 μm; cosmosil). The mobile phase was acetonitrile (solvent A)-water (solvent B) in the gradient mode as follows: 0-2 min, 5 % A; 2-12 min, 5-44 % A; 12-15 min, 44-56 % A; 15-16 min, 56-5 % A. The flow rate was 1.0 mL/min and the effluent was monitored at 272 nm. Routine sample calculations were made by comparing the peak area with that of the standard.

## RESULTS AND DISCUSSION

Fig. 1A presented the HPLC profile of crude gastrodin from *Gastrodia rhizome* and the chemical structure of gastrodin; peak A corresponded to gastrodin. It could be seen

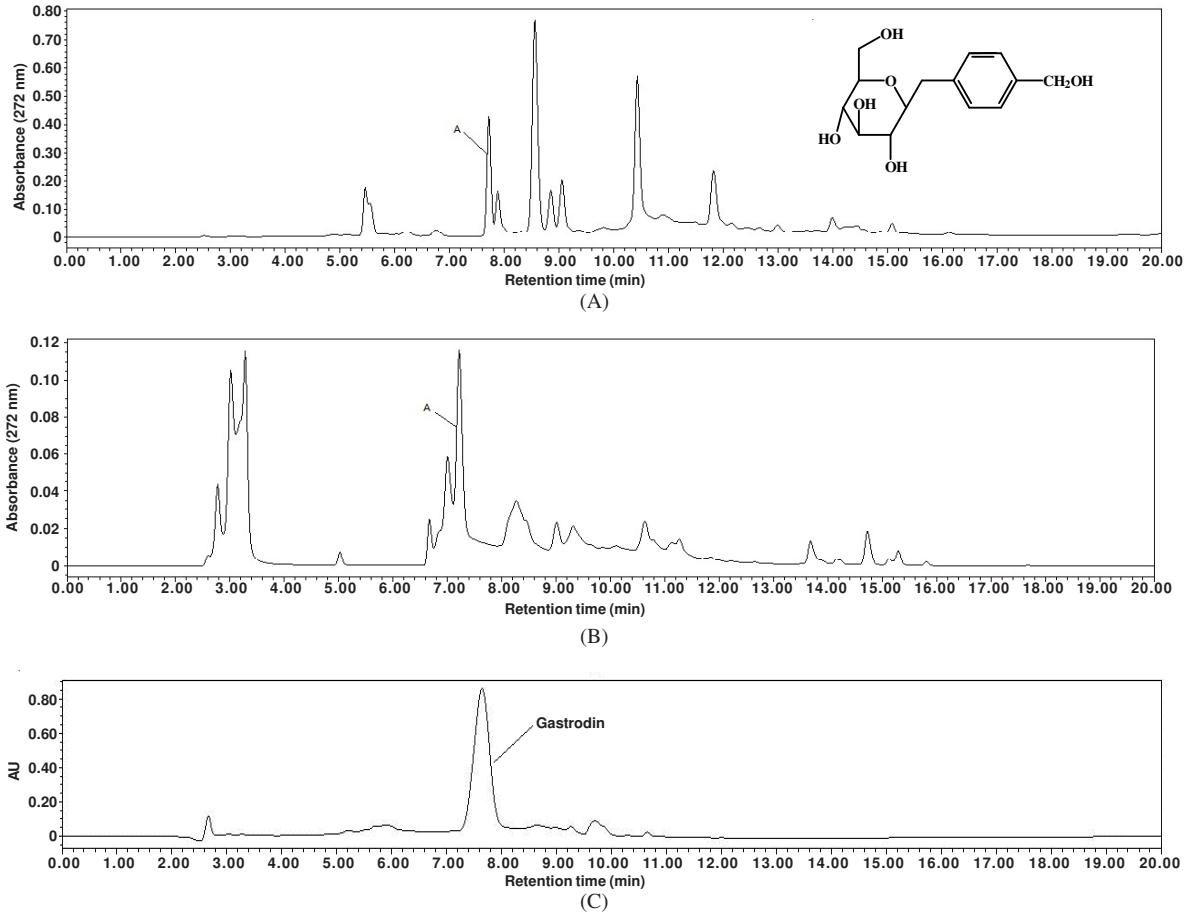


Fig. 1. (A) Chromatogram of crude gastrodin from *Gastrodia rhizome* by HPLC analysis; A = gastrodin. Conditions: column, reversed-phase symmetry $C_{18}$ column (250 mm × 4.6 mm i.d., 5 μm); mobile phase, acetonitrile (solvent A)-water (solvent B) in the gradient mode as follows: 0-2 min, 5 % A; 2-12 min, 5-44 % A; 12-15 min, 44-56 % A; 15-16 min, 56-5 % A. The flow rate was 1.0 mL/min and the effluent was monitored at 272 nm. (B) Chromatogram of rats' urine in the first 2 h after being given crude gastrodin water solution. (C) Chromatogram of crude gastrodin isolated by macroporous resin D101

from this figure that the content of gastrodin was 6.92 %. There were also many other compounds with a broad range of polarity, from which gastrodin is going to be separated.

Fig. 1B listed the chromatogram of rats' urine within the first 2 h after being given crude gastrodin water solution. In the experiment, each rat was given 2 mL crude gastrodin water solution orally each time, totally 372 mg gastrodin was given. It could be seen that, within initial 2 h, 46.9 % of the total gastrodin was expelled into urine, while for the first 6 h, 75.0 % was expelled (the data was not shown and it was calculated by regression equation of gastrodin). The first 6 h' urine was isolated with macroporous resin D101 to get 400 mg crude gastrodin (Fig. 1C). The crude gastrodin was purified by column chromatography and washed with ethyl acetate/ methanol (v/v, 8:1) to give 262 mg of gastrodin with a purity of over 98 %.

Based on the above mentioned, we could see that recovery from rats' urine was over 70.4 % in 6 h. It was not very high, though, but considering the huge amount use in China every year (the *gastrodia rhizome* output is over 600 tons in 2007), our method provided a potential way for recycling of gastrodin.

**Concluding remarks:** In this paper, we had, for the first time, reported the separation of gastrodin from the urine of rats after metabolism. In this purification process, large amount of organic solvents was avoided to get the aimed compound. Although its recovery yield was not high, considering its the huge amounts of consumption in China, our research provided a potential way to recycle gastrodin.

This method is also suitable for the recycling of salidroside and lithospermoside, the former stimulates DNA repair[10], protects hippocampal neurogenesis[11] enhances the immune response of aged rats[12] and the latter shows good anticancer activities[13]. In brief, our method provides a potential way for recycling of bioactive components of Chinese herbal medicines, especial the valuable ones.

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