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Research Article

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Preliminary study on serum pharmacochemistry of *Magnolia officinalis*

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ABSTRACT

To establish a method research the parameters of serum pharmacochemistry of Magnolia Officinalis. Based on the established HPLC fingerprint of Magnolia Officinalis, analysis and comparison were made between the HPLC fingerprints of rat serum samples obtained after orally taking extract fractions of Magnolia Officinalis and those of control rat serum samples. Three compounds were detected after oral administration of Magnolia Officinalis, two of which are Magnolol and Honokiol. These three compounds absorbed into blood are capable of being the effective constituents of Magnolia Officinalis. Further studies on them will help clarify the bioactive constituents and mechanisms of Magnolia Officinalis.

Key words: *Magnolia Officinalis*, serum pharmacochemistry, high performance liquid chromatography

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INTRODUCTION

Magnoliae Officinalis is a Chinese traditional medicine. It originated from the dried cortex of trunk, branch and root of *Magnolia officinalis* Rehd.et Wils.and *Magnolia officinalis* Rehd.et Wils.var.biloba Rehd.et Wils.[1].The major active constituents of it are Magnolol, Honokiol, volatile oil and minor alkaloids, etc. Traditional Chinese Medicine consider that *Magnoliae Officinalis* have the ability of promoting qi circulation to remove dampness and relieving asthma. So it often be used in Abdomen distending pain, indigestion, Vomiting, diarrhea and Asthma which Caused by Severe cough, etc[2] . At present, there are a large number of studies on chemical constituents and pharmacology of *Magnoliae Officinalis*, but there is no Serum Pharmacochemistry study on it. For this reason, the author referred the relevant literature[3-5] , Based on the established HPLC fingerprint of *Magnolia Officinalis*, analysis and comparison were made between the HPLC fingerprints of rat serum samples obtained after orally taking extract fractions of *Magnolia officinalis* and those of control rat serum samples. Identify the compounds which were absorbed into blood, the active constituents were identified by using the serum pharmacochemistry method.

1.1 Instrument

Agilent1260 HPLC gradient system equipped with a photodiode array detector(Agilent Company, USA); Sartorius BS224S electronic analytical balance(Sartorius Company, PR China); ALLEGRA X-15 Rrefrigerated centrifuge(Backman Company, USA); ZH-2 Automatic vortex mixer (Pharmacopoeia Standard Instrument Factory of Tianjin, PR China); MTN-2800W bath-typed nitrogen blowing instrument(Autosciense Company, PR China); Finnpipette colour transferpettor (Thermo Company, USA).

1.2 Materials

Acetonitrile, Methanol(CP FISHER Company, USA), Phosphoric acid and ethyl acetate(AR Chengdu Kelong Chemical Co., Ltd. ,PR China), Wahaha purified water(Hangzhou Wahaha Group Co., Ltd. ,PR China).

1.3 Reference substance and sample

Magnolol(110729-200310)and Honokiol(110730-200609) were all obtained from The National Institute For The Control of Pharmaceutical and Biological Products; The stock solution was prepared by dissolving the appropriate amounts of ~2.0 mg each standards Magnolol and Honokiol in 3ml of diluent (Methanol) with sonication for 25 min. The final volume of each solution was then diluted to 5ml with the diluent at room temperature. *Magnoliae Officinalis* were Purchased from New Lotus Chinese Herbal Medicine Co., Ltd. Chengdu, china. The medicinal herbs were identified by Professor Song LK, School of Life Science and Engineering, Southwest Jiaotong University , Chengdu, china.

1.4 Experiment Animals

SD rats(female, weighing about 200±20g, 6 weeks old) were purchased from Laboratory Animal Center of Chengdu University of Traditional Chinese, certificate number: SCXK（2008-11）. They were pre-housed for 3d and were fasted for about one week before the experiment.

EXPERMENTAL SECTION

2.1 HPLC condition

The HPLC condition was as following: column, Ultimate XB-C18 (5 μm, 46mm×250mm, Welch company, USA); Guard column, Guard-Pak C-1(Simadzu company, Japan); column temperature, 30 °C; mobile phases, linear gradient system of 0.05% aqueous phosphoric acid (A)and acetonitril (B), A/B 80/20 (0 min), 70/30 (7 min), 40/60 (25 min), 40/60 (35 min), 20/80 (45 min), 0/100 (60 min), 0/100 (65 min); flow rate, 1 ml/min; detecting wavelength, 200nm; injection volume,20m l.

2.2 Preparation of the Test Samples

2.2.1 Preparation of *Magnoliae Officinalis* Fed samples

The dried *Magnoliae Officinalis*(50g) were extracted with water (1000ml) under reflux for 20 min. The solution was filtered with paper, and the residue was further extracted as the same way. The solutions were mixed and evaporated to turbid liquor which equivalent to 3g Crude drug /ml in vacuo at 60 °C.

2.2.2 Preparation of *Magnoliae Officinalis* test solution

The turbid liquor of *Magnoliae Officinalis*(1/3ml) was diluted with methanol into the suitable concentration 1g Crude drug/10ml, ultrasonicated for 30 min and filtrated through 0.45mm membrane filter, and the aliquot (20ml) was applied into HPLC.

2.3 Preparation of Blood Sample

2.3.1 Preparation of the Control serum

The SD rats were fasted for 12 hours (free water), weighed, orally administered distilled water at the dosage of 1ml/100g, and 10min later, 5.0 ml of the blood was collected from femoral artery of the rat. The blood was centrifuged at 3500 rpm for 15 min at room temperature, One milliliter of the serum obtained was mixed with 2ml of ethyl acetate and ultrasonicated for 3 min. The mixture was centrifuged at 3000 rpm for 15 min at room temperature, the ethyl acetate layer was separated, and the residue was further extracted as the same way. The ethyl acetate layers were mixed and concentrated by nitrogen. The condensate was mixed with 100ul of methanol, ultrasonicated for 5 min and centrifuged at 12000 rpm for 10 min, and the supernatant was collected for analysis, then ,the aliquot (20ul) was applied into HPLC.

2.3.2 Preparation of Drug-containing Serum

Fifteen SD rats were randomly divided into five groups(3min group, 6min group, 10min group, 20min group, 30min group), fasted for 12 hours (free water), weighed, orally administered at the dosage of 1ml the turbid liquor of *Magnoliae Officinalis* /100g, 3min, 6min, 10min, 20min or 30 min later, 5.0 ml of the blood was collected from femoral artery of the rat. The blood was centrifuged at 3500 rpm for 15 min at room temperature, One milliliter of the serum obtained was mixed with 2ml of ethyl acetate and ultrasonicated for 3 min. The mixture was centrifuged at 3000 rpm for 15 min at room temperature, the ethyl acetate layer was separated, and the residue was further extracted as the same way. The ethyl acetate layers were mixed and obtained was concentrated by nitrogen. The condensate was mixed with 100ul of methanol, ultrasonicated for 5 min and centrifuged at 12000 rpm for 10 min. The supernatant was collected for analysis, then, the aliquot (20ul) was applied into HPLC.

RESULTS AND DISCUSSION

3.1 the selection of preparation of drug-containing serum

Experiments were used acetonitrile precipitation, ethanol precipitation, methanol precipitation, ethyl acetate

extraction and solid phase extraction method for processing of serum samples. Components from the measured impact of treatment, interference, and the conditions of chromatographic analysis of the impact of aspects to consider, determined to use the ethyl acetate extraction.

3.2 the selection of Blood collection time point

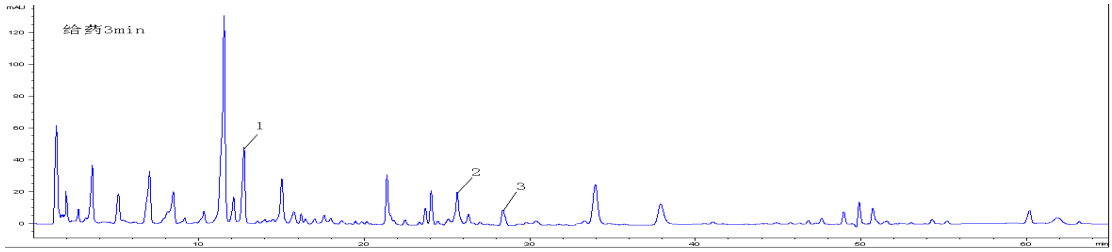

Fig. 1.Serum after oral administration (3min)

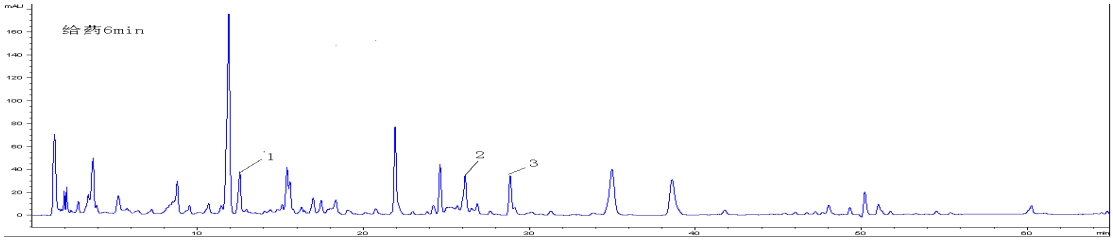

Fig. 2.Serum after oral administration (6min)

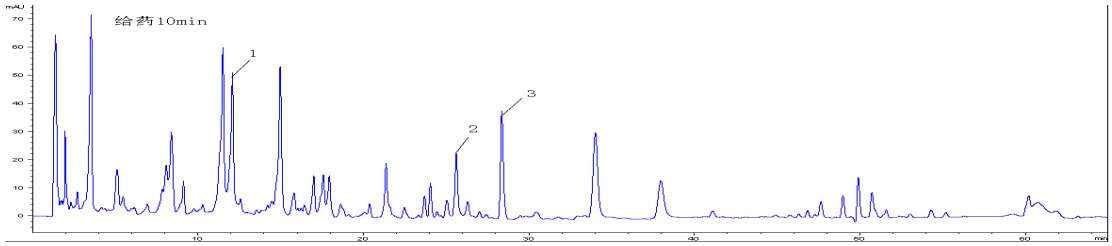

Fig. 3.Serum after oral administration (10min)

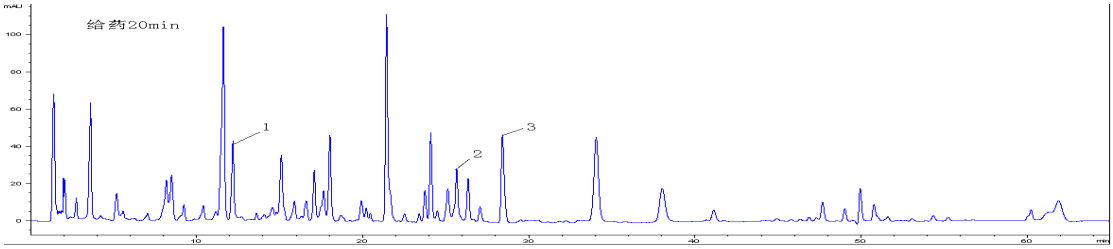

Fig. 4.Serum after oral administration (20min)

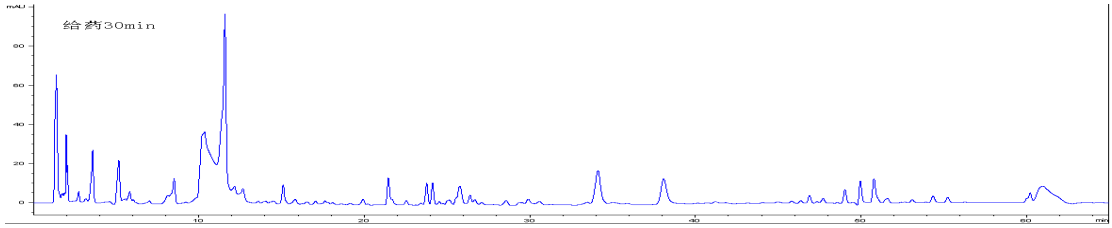

Fig. 5.Serum after oral administration (30min)

Figure 1to5 shows the detection wavelength of 220nm, and other chromatographic conditions fixed, collected blood after orally administered 3 minutes, Peaks 1, 2, 3 have been detected in the blood, achieved to a higher response

value, and response to the higher value at about tenth minutes. As times go on, both contents of them become more and more low. And was not detected in serum after oral administration (30min). The result is consistent with literature[6-7], Therefore it would be concluded that in vivo *Magnoliae Officinalis*' distribution of active ingredients with quick, rapid decline in plasma concentration, etc.

The serum samples which collected after fed with medicinal decoction *Magnoliae Officinalis* 10 minutes, with a larger number of peaks, and a higher response value，Therefore, choice 10min after fed with medicinal decoction *Magnoliae Officinalis* as the best the blood collection time point.

3.3 HPLC Profiles of drug-containing serum

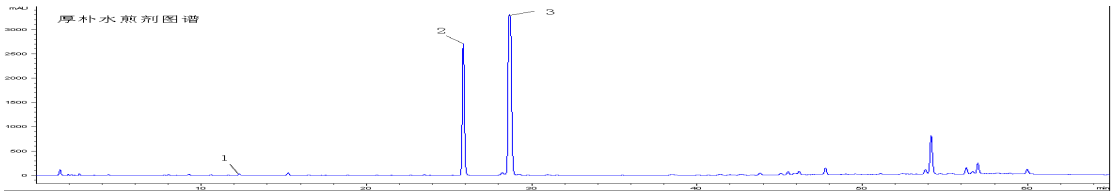

Fig.6.The turbid liquor of *Magnoliae Officinalis*

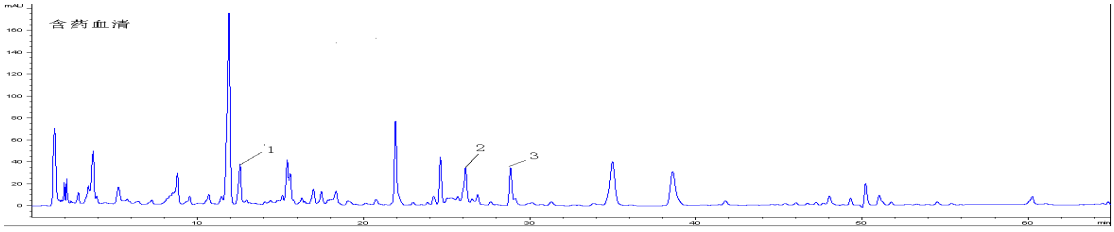

Fig.7.Drug-containing Serum

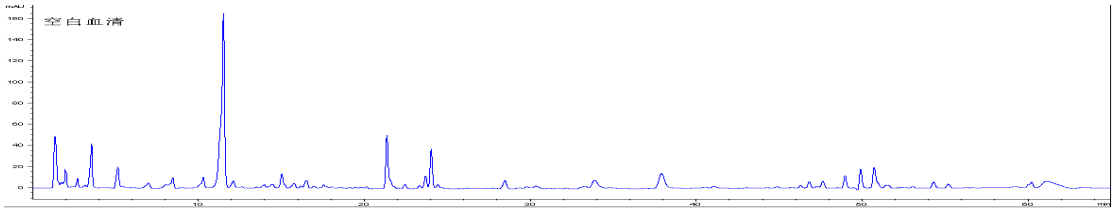

Fig.8.Control serum

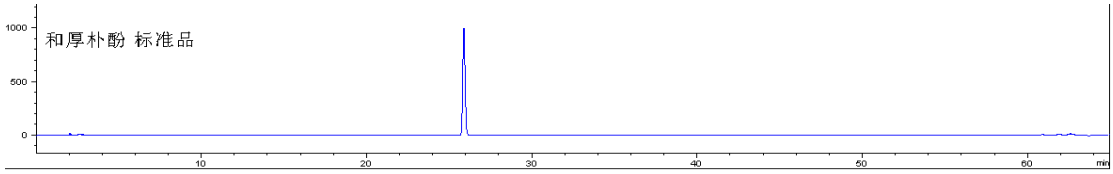

Fig.9.Magnolol

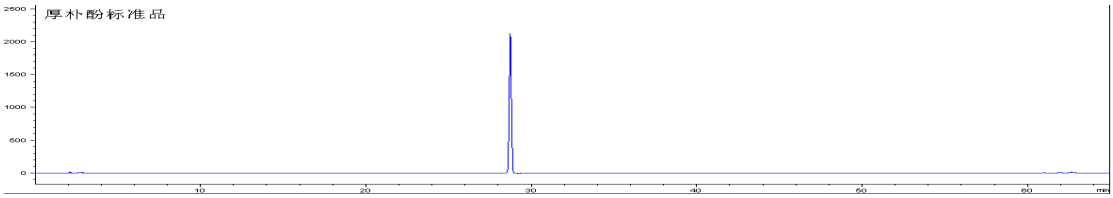

Fig.10.Honokiol

Fig.6 to10 shows the representative HPLC profiles for control serum, the turbid liquor of *Magnoliae Officinalis* and the serum of rat after the oral administration of *Magnoliae Officinalis*, respectively. Three distinct peaks were detected in the serum of the rat treated with *Magnoliae Officinalis*, Two of them were identified as Magnolol (Fig. 9) and Honokiol (Fig. 10) by comparison with the retention times and UV spectrums of the standard compounds. The third peak wait to be identified by other means.

3.4 Method validation

The precision： The analysis was repeated using the same sample for 6 times in the same day and additionally on 3 consecutive days to determine intra- and inter-day precision, Relative standard deviations (RSD) of retention time and peak area were all within ±2% and no significant difference was observed, indicating good precision of the method.

Stability: The RSD for the serum samples reanalyzed after 4, 8, 16 and 20h of storage was not more than 2.0%, indicating that the sample solutions were stable for at least 20h when stored at 2–8 ◦C or room temperature.

Repeatability of the method: The repeatability was determined by analyzing of five test serums which prepared by the same method. The RSD of relative to the total peak- peak area was less than 5%, the RSD of relative retention time was less than 2%, indicating good repeatability of the method.

Above experimental data can be established to ensure that the Serum Pharmacochemistry of *Magnolia Officinalis* is operability and effectiveness.

CONCLUSION

In this study, an accurate and reliable analytical method for the simultaneous determination of *Magnolia Officinalis* and the serum of rat after the oral administration of *Magnoliae Officinalis* was developed by HPLC method. The proposed method is promising to be the routine analysis for the serum pharmacochemistry of *Magnolia Officinalis* with simplicity, accuracy and reliability.

In our study, three compounds were detected after oral administration of *Magnolia Officinalis*, two of which are Magnolol and Honokiol, those compounds which being absorbed into blood can show the bioactivity. The results clarified that these two compounds Magnolol and Honokiol have the related pharmacological effects to the folk usage.

Acknowledgments

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