Study on Preparation Process of Shanxian Zaopi Granules

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Keywords: Shanxian Zaopi Granules, High-Performance Liquid Chromatography, Excipients, Dry Paste.

Abstract: *Objective*: To establish the preparation method and quality standard of Shanxian Zaopi granules. *Methods*: High-performance liquid chromatography was used to optimize parameters including granule appearance, formation ability, dilution rate, solubility and content of forsythia glycosides, and the type and ratio of auxiliary materials. *Results*: The excipients were synthesized into granules using dextrin and starch at the ratio of 2:3. The optimum ratio of the dry paste and excipients was 1:1.5. Curcuma oil and an equal amount of anhydrous ethanol were evenly sprayed onto the granules. *Conclusion*: The methods for the preparation and quality control of Shanxian Zaopi granules are simple, feasible and reliable. The granules have good properties and conform to the relevant provisions of the Pharmacopoeia of China.

1 INTRODUCTION

Breast cancer is the most prevalent malignancy in women and the second leading cause of cancer-related deaths worldwide (Tong, 2021). Around 2.3 million new cases of breast cancer were reported globally in 2020, with a mortality rate of 6.9% (Sung, 2021). The current treatment modalities including surgery, radiotherapy, chemotherapy, targeted therapy etc. (Cao, 2021) have failed to improve the long-term survival rate substantially (Shen, 2008), and are associated with an increased risk of secondary malignancy (Tong, 2021). In addition, the incidence of postoperative complications is also high at 33% (Nicotera, 2021). The adoption of several unhealthy lifestyle habits in recent years have increased the risk of postmenopausal breast cancer by more than three-fold and the risk of death by 96% (Lofterød, 2020). precancerous breast cancer are breast lesions that may develop into cancer but are not cancerous at present, such as breast enlargement, breast nodules, breast cysts, mastitis, and so on. If left untreated for a long time, they may develop into tumors. Pre-cancerous breast lesions found should be treated actively to prevent development to breast cancer. Only a minority of women progress from hyperplasia to atypical hyperplasia and carcinoma in situ. If a patient develops atypical hyperplasia, there is a risk of developing cancer. The treatment of pre-cancerous breast cancer in Western medicine is still mainly based on surgery, and how to effectively interrupt the development of pre-cancerous disease has not been given enough attention (Deng, 2013). Therefore, the prevention of breast cancer becomes a major clinical challenge to be overcome. Early diagnosis and treatment of breast cancer will greatly improve the survival rate of patients.

Professor Cai Guoliang from our university and Professor Ma Shuanquan, a doctor of Chinese medicine at our affiliated hospital in Shaanxi Province, have developed the "Zaoqiao Xiaopi" prescription based on the "Zhitong Xiaojie" decoction for treating precancerous breast cancer. The formulation consists forsythia, Spina Gleditsiae, Rhizoma Zedoariae, *Fritillaria thunbergii*, Rhizoma Corydalis and bupleurum. Spina Gleditsia can penetrate pus, break firmness, eliminate accumulation and relieve pain, whereas forsythia can clear heat and detoxify, dispel blood stasis and reduce swelling in skin infections. The combination of Spina Gleditsiae and forsythia can clear away heat, detoxify, eliminate accumulation, relieve pain, and alleviate hypochondriasis (Meng, 2018). In addition, both herbs have documented anti-tumor, anti-inflammatory, antibacterial, antioxidant and other pharmacological effects (Zhai, 2021; Xing, 2017). Rhizoma Zedoariae can soothe the liver and promote Qi flow, break blood and expel stasis. Curcumol, a bioactive component of the volatile oil of Rhizoma Zedoariae, promotes tumor cell apoptosis and inhibits

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proliferation and invasion (Nie, 2020). *F. thunbergii* can dissipate phlegm and blood stasis, and augment the action of Rhizoma Zedoariae. Verticine and verticinone, the effective active components of *F. thunbergii*, can reverse chemoresistance of tumor cells and act synergistically with other anti-tumor drugs (Zhao, 2019). Rhizoma Corydalis is used as an adjuvant to promote the flow of Qi, activate blood circulation, and relieve pain. Bupleurum is used to soothe the liver and circulate Qi. According to some researches, Rhizoma Corydalis also has anti-arrhythmic, anti-bacterial, anti-inflammatory and anti-tumor effects, and has protective effects on cardiovascular system, respiratory system, central nervous system, and so on (Wang, 2010). Therefore, the combination of these herbs can remove heat toxins, eliminate blood stasis and phlegm, relieve sclerosis, and target the pathogenesis and cure the root cause (Li, 2016). In this study, the herb formulation was synthesized into a granular form in order to simplify administration. We optimized the preparation process and the number of auxiliary materials, and established a method for quality control.

2 INSTRUMENTS AND MATERIALS

2.1 Instruments

Agilent 1260 high-performance liquid chromatography (HPLC) system, Agilent Zorbax C18 column (4.6 mm × 150 mm, 5 μm), GHX-9270B-1 Thermotank (Fuma, Shanghai, China), DHX-9140A Drying Cabinet (Jinghong, Shanghai, China), HEALFORCENW10UF Water Purifier (Hong Kong), HH-S Digital-displayed Thermostat Water Bath Cauldron (Jiangsu Jintan Medical Instrument Factory), JFSD-100 Crusher (Shanghai Jiading cereals and oils Instrument Co., Ltd.), and Medicine Sifter 3, 4 and 5 were used in this study.

2.2 Materials

Rhizoma Zedoariae (10 g) was suspended in 300 mL water in a flask, and several glass beads were added. The mixture was heated on an electric heating mantle connected to volatile oil detector and reflux condenser. The solution was brought to boil and then simmered for about 5 hours until the amount of oil in the detector no longer increased. The heater was switched off, and the plant matter was stewed. The water was slowly released till the oil rose to the topmost layer and flushed with Scale 0. The amount of volatile oil was calculated to be approximately 2%. Nine batches of the herbs were weighed according to the prescription (15 g forsythia, 12 g Spina Gleditsia, 15 g Rhizoma Zedoariae, 15 g *F. thunbergii*, 12 g Rhizoma Corydalis and 12 g bupleurum). The herbs were soaked in 6 volumes of water for 30 min, and decocted and extracted twice for 30 min each time. Approximately 100 mL of the extract was collected, and an equal amount of 95% ethanol was added. The solution was allowed to rest for 24h, and the sediment was boiled, concentrated and reconstituted to 100 mL. The samples (10μl aliquots) were analyzed by HPLC using Agilent Zorbax C18 column (4.6 mm × 150 mm, 5μm) with acetonitrile-water (25:75) as the mobile phase. The detection wavelength was 277 nm, the detection temperature was 30°C, and the flow rate was 0.1 mL/min. The content of forsythia glycosides in the solution was determined to be 0.0516 mg/mL. The solution was heated and concentrated, and the dry paste was obtained. The paste rate was calculated as dry paste amount (g)/amount of herbal matter (g). Sucrose (batch number: 10012020200802) was provided by Nanning Guizhilong Pharmaceutical raw materials Co., Ltd. Dextrin (batch number: 140310) and soluble starch (batch number: 131209) was obtained from Shaanxi Mr G Pharmaceutical Raw Materials Co., Ltd.

3 METHODS AND RESULTS

3.1 Preparation Method

3.1.1 Selection of Excipients

Sucrose, dextrin and soluble starch are commonly used as excipients for granules (Huang, 2016). However, given the viscous nature of sucrose, it is not suitable for the granulation of water extract containing polysaccharides. Therefore, we used a mixture of dextrin and soluble starch to form the granules, and the appropriate proportion of the excipients was selected according to the formation ability and moisture absorption rate.

3.1.2 Amount of Excipients

The drug solution was extracted as previously described. Briefly, the herbs are soaked in 6 times the amount of water for 30 min, decocted and extracted twice for 30 min each time, and dried into a paste. Different amounts of soluble starch and dextrin were added and mixed evenly to make the granules (Zheng, 2016).

3.2 Results

3.2.1 Ratio of Excipients

The granules were prepared by mixing dextrin and soluble starch at the ratio of 2:3, and showed a high formation rate and low moisture absorption rate (Table 1).

Table 1: Ratio of excipients ratio.

Excipient	Granule formation	Moisture absorption rate
Dextrin	Fragile granules	11.2
Starch	Poor formation and fragile granules	12.4
Dextrin: Starch = 3:2	Different sizes of granules can be synthesized and easily broken into powder form	10.5
Dextrin: Starch = 2:3	Good color and formation	7.8
Dextrin: Starch = 1:1	Size of the granules is not uniform	8.1

3.2.2 The Ratio of Dry Paste and Excipients

Granulation and the characteristics and solubility of the granules were used as indicators to compare the amount of excipient. The optimum ratio of dry paste and excipient was 1:1.5 (Table 2).

Table 2: Ratio of dry paste and excipients.

Ratio of dry paste and excipients	Granulation	Solubility
1:1	Sticky, not easy to pass through the sieve	Good solubility
1:1.3	Easier to pass through the sieve	Good solubility
1:1.5	Easy to pass through the sieve	Good solubility

3.3 Process Validation

The volatile oil of Rhizoma Zedoariae (19 ml from 1,500 g herb) was extracted and mixed with 6,600 g forsythia, Spina Gleditsia, *F. thunbergii*, Rhizoma Corydalis and bupleurum. The herb mixture was soaked in 6 volumes of water for 30 min, heated, and extracted twice for 30 min each time. Around 5,690 mL of the extract was mixed with an equal amount of 95% ethanol and left undisturbed for 24 h. The supernatant was discarded and the sediment was boiled and concentrated into a paste. About 720 g (612 mL) dry paste was obtained, which was then crushed, passed through sieve 5, and mixed with 430 g dextrin and 650 g starch to make the granules. In addition, 19 mL each of Rhizoma Zedoariae volatile oil and anhydrous ethanol were evenly sprayed onto the granules. After drying in an oven at 40℃ (24 h), 1,800 g dried granules were obtained and packed into lots of 9 g. Three such batches were prepared, and 5 samples were randomly selected from each batch. The contents of each were dissolved in warm water to a final volume of 50 ml, and 10μL aliquots were taken for HPLC. The average content of forsythia glycosides was 0.0513 mg/mL. The results were stable.

4 CONCLUSION

Shanxian Zaopi granules are composed of six herbs, namely forsythia, Spina Gleditsiae, Rhizoma Zedoariae, *Fritillaria thunbergii*, Rhizoma Corydalis and bupleurum. The combination of these herbs can remove heat toxins, eliminate blood stasis and phlegm, relieve sclerosis, and target the pathogenesis and cure the root cause. Therefore, the granules can effectively treat precancerous breast cancer for clinical treatment. However, there are fewer studies on other aspects of the effects. The bioavailability is low, and the side effects of the granules are still unclear, so how to apply it more safely and efficiently in clinical treatment is an urgent problem for researchers to solve at present.

In this study, we used high performance liquid chromatography (HPLC) to determine the content of forsythia glycosides, the main active ingredient of forsythia, which can better control the quality of Shanxian Zaopi granules. HPLC has many advantages, such as high separation efficiency, high detection sensitivity, good selectivity, automated operation, and wide range of applications. However, it also has the disadvantage of high analysis cost, expensive liquid chromatograph price and routine maintenance. With the rapid development of Chinese medicine preparation production technology, aim to achieve easier and more sensitive quality control of formulated particles, Some new analytical techniques such as infrared spectroscopy, gas chromatography, high performance liquid chromatography and other qualitative and quantitative detection methods have emerged. Quality control of formulated particles gradually becomes the focus of researchers' attention.The preparation process of granules is complicated. How to improve the preparation technology and perfect the preparation process is a problem that researchers need to consider nowadays. Meanwhile, the extraction process of the granules also needs to be paid attention to, on the basis of which the preparation process will progress smoothly.

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