Study on the Quality Standard of Fuzheng Wangan Granules

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Abstract [**Objectives**] This study aims to establish the quality standard of Fuzheng Wangan granules. [**Methods**] The qualitative identification of Radix Bupleuri and Radix Paeoniae Alba was carried out by the thin layer chromatography (TLC). The content of ginsenosides Rg1, Re and Rb was determined by the high performance liquid chromatography (HPLC). [**Results**] The spots of Radix Bupleuri and Radix Paeoniae Alba were clear, and the negative control had no interference. The total content of ginsenoside Rg1, Re and Rb ranged from 0.076 2 to 0.073 9 mg/g. [**Conclusions**] The method for the quality control of Fuzheng Wangan granules established in this study is stable and feasible.

Key words Fuzheng Wangan granules, Quality standard

1 Introduction

Fuzheng Wangan Prescription is an experienced prescription that has been applied in the Department of Hepatology of the First Affiliated Hospital of Guangxi University of Chinese Medicine for many years. It is a new recipe formed based on the new understanding of "deficiency and loss of liver" in the malignant progression of chronic liver disease. It can not only tonify liver deficiency but also remove liver stasis, improve the intrahepatic/external microenvironment of the liver and thus alleviate or even block the state of persistent liver injury[1-2]. In this paper, in order to better exert the efficacy of Fuzheng Wangan granules and control the safety of its clinical use, the quality standard of Fuzheng Wangan granules was established to provide better pure Chinese medicine granule preparation for patients with liver disease under the premise of ensuring stable quality.

2 Materials

2.1 Instruments Main instruments include UV reflection transmission imager (model: YOKO-CS, Wuhan Pharmaceutical New Technology Development Co., Ltd.), electric blast drying box (model: DHG-9240A, Shanghai Yiheng Scientific Instrument Co., Ltd.), electronic balance (model: GH-252, Japan AND Electronic Balance Co., Ltd.), Chinese medicine extractor (model: 100836, Hunan Hengyang Jinyifan Pharmaceutical Equipment Industrial Co., Ltd.), 500L single effect vacuum concentrator (model: WZ-500, Wuhan Huarong Dingchang Light Industry Equipment Co., Ltd.), YK-80 swing granulator (Nanjing Tengyong Dry Heat Equipment Co., Ltd.), and automatic particle packaging machine (Tianjin Sanqiao Packaging Machinery Co., Ltd.).

2.2 Reagents Radix Bupleuri control drug (batch No.: 120992-201509), paeoniflorin reference substance (batch No.: 110736-202044), ginsenoside Rg1 reference substance (batch No.: 110703-202034), Re reference substance (batch No.: 110754-202028), and Rb1 reference substance (batch No.: 110704-201122) were all purchased from the National Institute of Food and Drug Control. Chromatographic-grade acetonitrile was purchased from Fisher, USA. Silicone G plate (10 mm × 10 mm) was brought from Qingdao Marine Chemical Plant. The water was ultra-pure water, and the reagents such as trichloromethane, n-butanol and concentrated ammonia water were analytically pure. Fuzheng Wangan granules (batch No.: 20220708, 20220710, and 20220803) were provided by the First Affiliated Hospital of Guangxi University of Chinese Medicine.

3 Methods and results

3.1 Qualitative identification[3-4]

3.1.1 Qualitative identification of Radix Bupleuri. Firstly, 20 g of this drug was treated by ultrasound in 50 mL of ethanol for 30 min, and then the solution was filtered. The filtrate was dried in a water bath, to which 20 mL of pure water was added. After it was put in a separating funnel, to which 20 mL of chloroform was added twice for extraction. After the chloroform was discarded, 20 mL of water saturated n-butanol was added twice for extraction, and then the upper n-butanol solution was collected. Afterwards, 20 mL of ammonia test solution was added to wash it twice, and then the ammonia test solution was discarded. 50 mL of saturated aqueous solution of n-butanol was added to wash it, and then the water solution was discarded. The n-butanol solution was dried in a water bath, and 1 mL of methanol was added to it to obtain the test solution. The negative sample solution was prepared by the same method. Meanwhile, 1 g of Radix Bupleuri control drug was taken to prepare the control drug solution by the same method. According to the thin layer chromatography (general rule 0502), 3 μL of the test product solution and 5 μL of the control drug solution were put on the same silica gel G thin layer plate, and the lower layer solution of trichloromethane-methanol-water (13∶6∶2) placed below 10 ℃ was used as the development agent. The solution was unfolded, taken out, dried, and sprayed with 40% sulfuric acid solution of 2% p-dimethylaminobenzaldehyde. It was blown with hot air until the color of the spots was clear. The spots were examined under daylight and an ultraviolet lamp (365 nm)

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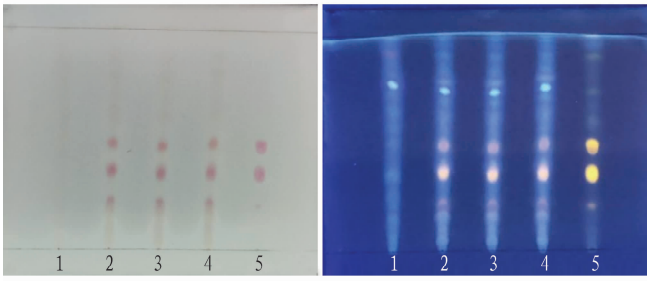
respectively. In the chromatogram of the test products, the same color spots or fluorescent spots are shown at the corresponding position of the chromatogram of the control drug (Fig. 1).



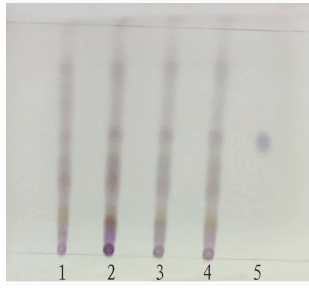
NOTE 1. Negative sample lacking Radix Bupleuri; 2. Fuzheng Wangan granules (batch No.: 20220708); 3. Fuzheng Wangan granules (batch No.: 20220710); 4. Fuzheng Wangan granules (batch No.: 20220803); 5. Radix Bupleuri control drug (batch No.: 120992-201509).

Fig. 1 Chromatograms for the identification of Radix Bupleuri by the thin layer chromatography

3.1.2 Qualitative identification of Radix Paeoniae Alba[5]. At first, 10 g of this drug was treated by ultrasound in 50 mL of ethanol for 30 min, and then the solution was filtered. The filtrate was dried in a water bath, to which 20 mL of pure water was added. After it was put in a separating funnel, to which 20 mL of trichloromethane was added three times for extraction. After the trichloromethane was discarded, 20 mL of water saturated n-butanol was added three times for extraction, and then the upper n-butanol solution was collected. Afterwards, 20 mL of ammonia test solution was added to wash it three times, and then the ammonia test solution was discarded. 50 mL of saturated aqueous solution of n-butanol was added to wash it, and then the water solution was discarded. The n-butanol solution was dried in a water bath, and 1 mL of methanol was added to it to obtain the test solution. Paeoniflorin reference substance was taken, to which methanol was added to prepare a solution containing 1 mg/mL paeoniflorin as the reference product solution. The negative sample lacking Radix Paeoniae Alba was prepared by the method of the test solution. According to the thin layer chromatography (general rule 0502), 10 μL of the test product solution and the negative solution lacking Radix Paeoniae Alba as well as 2 μL of the paeoniflorin control solution were put on the same silica gel G thin layer plate, and the mixture of trichloromethane, ethyl acetate, methanol and formic acid (40∶5∶12∶0.2) was used as the development agent. The solution was unfolded, taken out, dried, and sprayed with 5% vanillin sulfuric acid solution. It was heated at 105 ℃ until the spots were clear, and observed under sunlight (Fig. 2).

3.2 Content determination

3.2.1 Chromatographic conditions. Chromatographic column: Inertsil ODS-3 column (4.6 mm × 250 mm, 5 μm). Flow rate: 1 mL/min; column temperature: 30 ℃; detection wavelength: 203 nm; sampling volume: 10 μL; acetonitrile was as mobile phase A, while water was as mobile phase B, and gradient elution



NOTE 1. Negative sample lacking Radix Paeoniae Alba; 2. Fuzheng Wangan granules (batch No.: 20220708); 3. Fuzheng Wangan granules (batch No.: 20220710); 4. Fuzheng Wangan granules (batch No.: 20220803); 5. Paeoniflorin control drug (batch No.: 110736-202044).

Fig. 2 Chromatogram for the identification of Radix Paeoniae Alba by the thin layer chromatography

was conducted (0 – 15 min, 20% A; 15 – 40 min, 20% – 25% A; 40 – 70 min, 25% – 40%).

3.2.2 Preparation of the test solution. Firstly, 50 g of Fuzheng Wangan granules were weighed accurately, and dissolved with appropriate amount of water. 40 mL of trichloromethane was added twice for extraction. After the trichloromethane layer was discarded, 30 mL of water saturated n-butanol was added three times for extraction, and then the upper n-butanol solution was collected. Hereafter, 40 mL of ammonia test solution was added to wash it twice, and then the ammonia test solution was discarded. 40 mL of saturated aqueous solution of n-butanol was added to wash it, and then the water solution was discarded. After the n-butanol solution was dried in a water bath, the residue was dissolved with methanol, and transferred to a 5 mL volumetric bottle, to which methanol was added until reaching the scale. After shaking well, it was filtered through 0.45 μm microporous filter membrane, and the filtrate was filtered with 0.45 μm microporous filter membrane to obtain the test product solution.

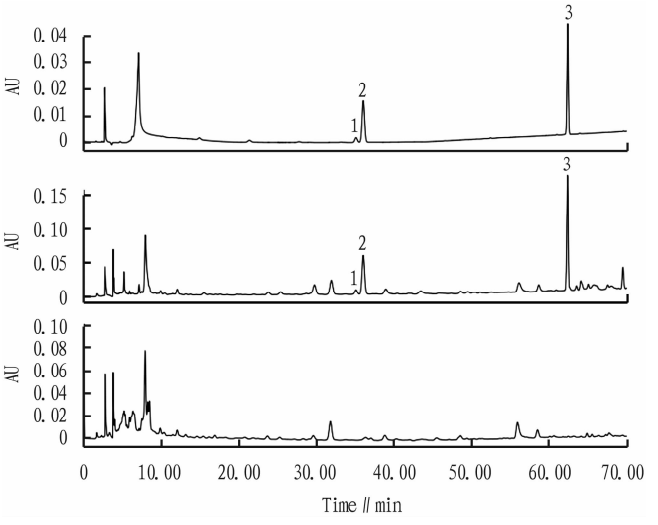
3.2.3 Preparation of the control solutions. Appropriate amounts of ginsenoside Rg1, Re and Rb1 control products were weighed accurately, to which methanol was added to prepare mixed solutions with concentration of 0.055 5, 0.619 8, and 1.209 6 mg/mL. After being shaken well, they were filtered with 0.45 μm microporous filter membrane to obtain the control solutions.

3.2.4 Preparation of the negative control solution. The negative sample lacking ginseng was taken to prepare the negative control solution by the method of "preparing the test product solution".

3.2.5 Methodological investigation. The content of ginsenoside Rg1, Re and Rb1 in Fuzheng Wangan granules was determined by the external standard method and HPLC, and the linearity, range, precision, stability, repeatability and recovery of samples were investigated.

(i) Specificity test. At first, 10 μL of the test solution, control solution and negative control solution obtained in Sections **3.2.2**, **3.2.3** and **3.2.4** were determined according to the chromatographic conditions in section **3.2.1**. In the chromatogram of the test solution, there were corresponding absorption peaks at the same retention time as that of the control solution, and the separation and tailing factors of ginsenoside Rg1, Re and Rb1 met the

requirements. The negative control had no interference, indicating that the specificity of the method was good. The chromatograms are shown in Fig. 3.



NOTE 1. Ginsenoside Rg1; 2. Ginsenoside Re; 3. Ginsenoside Rb1. A. Control solution; B. Test solution; C. Negative control solution.

Fig. 3 High performance liquid chromatograms

(ii) Investigation of linear relationship. The mixed control solution of ginsenoside Rg1 (Re or Rb1) was taken and diluted to 0.046 25, 0.037 00, 0.027 75, 0.018 50 and 0.009 25 mg/mL (0.516 5, 0.413 2, 0.309 9, 0.206 6, and 0.103 3 mg/mL or 1.008 0, 0.806 4, 0.604 8, 0.403 2, and 0.201 6 mg/mL), respectively. 10 μL of each sample was injected into high performance liquid chromatograph, and the peak area was determined according to the chromatographic conditions in Section **3.2.1**. The content of the mixed control product was as the horizontal coordinate, and the peak area was as the vertical coordinate to draw the standard curve and obtain regression equations and linear ranges. The results of the regression equations are shown in Table 1.

Table 1 Verification results

Component	Regression equation	*R* value	Linear range // μg/L
Ginsenoside Rg1	$y = 395\,299x + 2\,848.7$	0.999 9	0.092 5 – 0.555 5
Ginsenoside Re	$y = 323\,767x + 21\,196.0$	0.999 7	1.033 0 – 6.198 0
Ginsenoside Rb1	$y = 240\,818x + 2\,622.1$	0.999 5	2.016 0 – 12.096 0

(iii) Precision test. Fuzheng Wangan granules (batch No.: 20220708) were taken to prepare the test solution according to the method in Section **3.2.2**. Samples were injected continuously for 6 times according to the chromatographic conditions in Section **3.2.1**, and the *RSD* value of each component was investigated and calculated respectively. The results show that the *RSD* value of the peak area of ginsenoside Rg1, Re and Rb1 was 0.73%, 0.50% and 1.92%, respectively, indicating that the precision of the instrument was good.

(iv) Repeatability test. Fuzheng Wangan granules (batch No.: 20220708) were taken to prepare the test solution according to the method in Section **3.2.2**. Samples were injected and determined according to the chromatographic conditions in Section **3.2.1** at 0, 2, 4, 8, 12 and 24 h after the preparation of the test solution, respectively. The results reveal that the *RSD* value of the peak area of ginsenoside Rg1, Re and Rb1 was 0.59%, 0.38% and 2.85%, respectively, indicating that the test solution was stable within 24 h.

(v) Stability test. Fuzheng Wangan granules (batch No.: 20220708) were taken to prepare 6 test solutions according to the method in Section **3.2.2**. Samples were injected and determined according to the chromatographic conditions in Section **3.2.1**. The results show that the *RSD* value of the peak area of ginsenoside Rg1, Re and Rb1 was 1.52%, 1.29% and 2.78%, respectively, indicating that the method had good repeatability.

(vi) Sampling recovery test. Three samples of Fuzheng Wangan granules (batch No.: 20220708) were taken and added to 0.5, 1.0 and 1.5 times of ginsenoside Rg1, Re and Rb1 control products, respectively. The test solution was prepared according to the method in Section **3.2.2**, and samples were injected and determined according to the chromatographic conditions in Section **3.2.1**. The sampling recovery rate was calculated. The average recovery rate of ginsenoside Rg1, Re and Rb1 was 99.20%, 99.19% and 99.37%, respectively. The *RSD* value of recovery rate was 1.90%, 1.79% and 1.87%, respectively, indicating that the recovery rate of this method was good.

3.2.6 Sample determination and results. Different batches of Fuzheng Wangan granules were respectively weighed to prepare the test solution according to the method in Section **3.2.2**. Samples were injected and determined according to the chromatographic conditions in Section **3.2.1**. The peak area of ginsenoside Rg1, Re and Rb1 was determined. The results are shown in Table 2.

Table 2 Content of ginsenoside Rg1, Re and Rb1 in Fuzheng Wangan granules mg/g

Batch number	Ginsenoside Rg1	Ginsenoside Re	Ginsenoside Rb1	Total content
20220708	0.001 9	0.021 6	0.052 6	0.076 2
20220710	0.001 9	0.021 6	0.053 7	0.077 2
20220803	0.001 9	0.021 6	0.050 3	0.073 9

4 Discussion

This recipe mainly consists of more than ten medicinal materials such as Radix Paeoniae Alba, Radix Bupleuri, Carapax Trionycis, and Herba Artemisiae Scopariaeporia. Among them, Radix Paeoniae Alba is neutral in nature and can nourish the lungs for assisting metal and calming wood. It is sweet and can enter the spleen. It can not only strengthen the spleen and suppress the liver, but also treat the pain in the liver. Herba Artemisiae Scopariaeporia is bitter in taste and can dilate bile duct, promote bile secretion, assist liver detoxification, promote liver cell regeneration and prevent liver necrosis[6]. Radix Bupleuri is bitter in taste, and can enter the gall bladder meridian, and help the stomach digest food to eliminate gastrointestinal food accumulation. Studies

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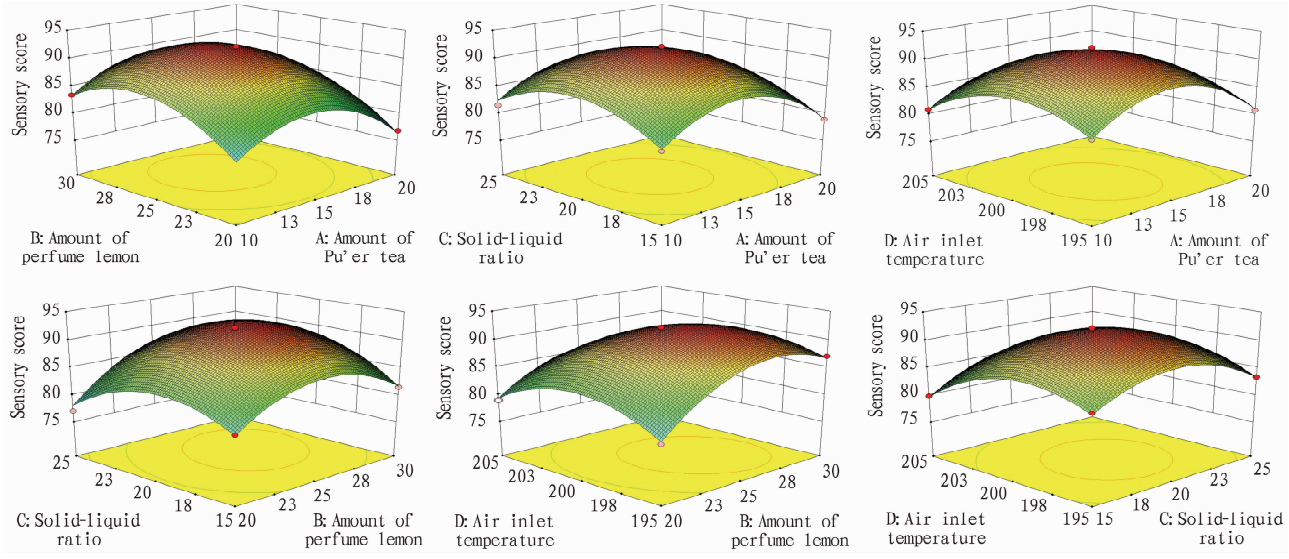

Fig. 2 Optimization results of response surface test

4 Conclusions

In this study, large-leaf Pu'er tea and perfume lemon were utilized as the primary raw materials, and the sensory score served as the benchmark for the development of lemon-flavored solid instant tea. The results indicated that the impact of each factor on the sensory score was ranked as follows: the amount of lemon added > the air inlet temperature > the amount of Pu'er tea > the solid-liquid ratio. Through the validation of the regression model and taking into account practical considerations, the optimal formulation was determined to consist of 15 g of Pu'er tea, 25 g of perfume lemon, a solid-liquid ratio of 1 ∶ 20 (g/mL), and an air inlet temperature of 200 ℃. The lemon-flavored solid instant tea produced under the specified conditions exhibited the most favorable flavor, a stable texture, and the highest sensory evaluation score. The physicochemical properties of the product formulated under these conditions were as follows: moisture content (5.82 ± 0.24) %, total sugar content (38.10 ± 1.47) %, dissolution time (9 ± 3) sec, and powder yield (19.50 ± 0.34) %. These results aligned with the relevant standards.

References

[1] SU J, LI CW, LIANG HZ, *et al*. Study on technology of fresh leaves solid fermentation of Pu'erh tea[J]. Food Research and Development, 2017, 38(20): 114 – 118. (in Chinese).

[2] WEN LH, CAO FR. Research progress on the regulation of obesity by Pu'er tea[J]. Guangdong Tea Industry, 2021(5): 2 – 7. (in Chinese).

[3] LEE LK, FOO KY. Recent advances on the beneficial use and health implications of Pu-Erh tea[J]. Food Research International, 2013, 53(2): 619 – 628.

[4] DAI JH, WANG XY, JIANG HF, *et al*. Optimization of formula of flavored Pu'er tea beverage by response surface method[J]. Farm Products Processing, 2024(17): 51 – 56. (in Chinese).

[5] GAO W, LIU JB, ZHU MY, *et al*. Distribution of phenolics in different tissues of four kinds of lemon and their antioxidant activity[J]. Journal of Chinese Institute of Food Science and Technology, 2019, 19(2): 281 – 290. (in Chinese).

[6] LIU ZT, ZHOU Y. Green planting techniques for fragrant lemon[J]. Practical Techniques and Information for Fruit Trees, 2022(12): 34 – 38. (in Chinese).

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have found that small molecules of Radix Bupleuri can bind to multiple targets of hepatitis B virus to inhibit the replication of hepatitis B virus in the liver[7]. Carapax Trionycis is salt in taste, and has the function of tonifying the kidney. Studies have shown that it can inhibit angiogenesis, thus preventing and treating tumors[8].

References

[1] WANG TS, ZHANG RZ, WANG MG, *et al*. Theoretical connotations of liver failure "Toxic Pathogen-Toxic Turbidity" theory from the angle of "Gut-liver Axis"[J]. Western Journal of Traditional Chinese Medicine, 2022, 35(6): 67 – 70. (in Chinese).

[2] GUAN ZJ, WEI AL, MAO DW. Theory of "three passages and two channels" in Zhuang medicine with the pathogenesis of chronic serious hepatitis[J]. Chinese Journal of Clinical Research, 2022, 35(8): 1156 – 1158. (in Chinese).

[3] TAN LY, ZHAI KX, ZHANG AH, *et al*. Study on quality standard of Yinchai tuire granules[J]. Lishizhen Medicine and Materia Medica Research, 2021, 32(6): 1386 – 1390. (in Chinese).

[4] National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (Voume 1)[M]. Beijing: China Pharmaceutical Science and Technology Press, 2020: 293. (in Chinese).

[5] National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (Voume 1)[M]. Beijing: China Medical Science and Technology Press, 2020: 108. (in Chinese).

[6] YU L, ZHAO X, LI T, *et al*. Study on the therapeutic effect of several main formulas of Yin Chen on jaundice hepatitis[J]. Asia-Pacific Traditional Medicine, 2015, 11(5): 37 – 39. (in Chinese).

[7] SHEN X, ZHANG G, CHEN Y. A preliminary study on the network molecular mechanism of Chaihu against hepatitis B[J]. Shaanxi Journal of Traditional Chinese Medicine, 2015, 36(1): 113 – 114. (in Chinese).

[8] ZENG Z. The study of turtle shell on the inhibition of angiogenesis in zebrafish model by down-regulating ENO3 and PFKM in glycolysis pathway [D]. Chengdu: Chengdu University of Traditional Chinese Medicine, 2019. (in Chinese).