To Optimize and Improve Cang'erzi Rhinitis Capsule that under Multi-standard Parallel Quality Specification

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Abstract: Objective: To optimize and improve Cang'erzi rhinitis capsule that under multi-standard parallel quality specification. Methods: Using TLC to legally identify prescriptions that including Radix Angelicae Dahuricae, Flos Magnoliae and Fructus Xanthii Using the high performance liquid chromatography to measure the baicalin's content in Xanthium rhinitis capsules. Results: Radix Angelicae Dahuricae, Flos Magnoliae and Fructus Xanthii could be detected by TLC,and the spots were clear, specific and negative control no interference; The correlation coefficient 0.083 μg ~ 0.834 μg was r ≥ 0.999.The average recovery was 98.5% and RSD (the relative standard deviation) was 0.9%. Conclusion: The research data show that the amount of baicalin in the samples of three different manufacturers is obviously different, and it is necessary and feasible to unify the national standard, which is more beneficial to control the quality of Xanthium sibiricum rhinitis capsule.

1. Introduction

The prescription of Cangerzi Biyan capsule mainly includes Cangerzi extract powder, gypsum extract powder, angelica extract powder, borneol, volatile oil of Magnolia, menthol, Magnolia extract powder and scutellaria extract powder. At present, there are 6 standards for Cangerzi Biyan capsule, including ws3-b-2701-97 (drug standard of the Ministry of Health), ybz09362005 (standard of the State Food and Drug Administration), ws3-b-2701-97-1, ws3-b-2701-97-2, ws3-b-2701-97-3 and ws3-b-2701-97-4. The current situation of multi-standard products brings many inconveniences to drug inspection and drug supervision departments, and also brings different degrees of wrong judgment and unnecessary medication errors to drug patients. In order to strengthen the market supervision of this kind of drugs and ensure the safety of drug use for patients, the author specially establishes the "most rigorous standard" to relieve the market pressure on the quality control of this product, make its quality controllable, and ensure the safety and effectiveness of clinical drug use. The current situation of multi-standard products brings many inconveniences to drug inspection and drug supervision departments, and also brings different degrees of wrong judgment and unnecessary medication errors to drug users. In order to strengthen the supervision of this kind of drug market and ensure the safety of patients' medication, the author specially establishes "the most rigorous standard" to relieve the pressure of the market on the quality control of this product, to make its quality controllable and to ensure the safety and effectiveness of clinical medication.

2. Instruments and Drug Testing

Agilent Technologies 1260 hplc, 1260DAD VL detector; Electronic balance (model FA224, manufacturer, Shun Yu Heng Ping instrument); Column of electronic analytical balance (BP211D model SaiDolis, d=0.01mg): ODS2, 5μm, specification ID4.6mm×200mm; Solvent filtration system (sand core filtration device), Shanghai Lichen Bonsai Instrument Technology Co., Ltd.; Ge Neng brand ultrasonic cleaner, model G-040S; Silica gel G thin layer plate, Qingdao Ocean Chemical Co., Ltd., batch number 20180920, specification 25×75mm; Reflux device; Third-use uv analyzer,

Shanghai Gu Cun Electro-optic Instrument Factory. Chromatographic methanol, Xanthium sibiricum rhinitis capsule (3 manufacturers), baicalin reference substance (Beijing century aoke biotechnology co., ltd, batch number 21967-41-9, content 98.0%).

3. Methods and Results

3.1 Identification by TLC

(1) TLC Identification of Angelica dahurica

Take 3g of the content of this product, add 50ml dilute hydrochloric acid into a round bottom flask, heat and reflux in water bath for 60min, filter, extract the filtrate with ether twice, 30ml each time, and set aside the water layer. Combine ether extracts, wash with water to neutrality, volatilize naturally, and dissolve the residue with 1ml of ethanol as sample solution. Another negative sample of Angelica dahurica was taken and a negative control solution was prepared by the same method. Take 1g of radix Angelicae dahuricae as control, add 20ml dilute hydrochloric acid, heat and reflux in water bath for 60min, filter, extract the filtrate with ether twice, 10ml each time. Combine ether extracts, wash with water to neutrality, volatilize naturally, add 1ml of ethanol to the residue to dissolve it, and prepare a control medicinal solution. According to the test of thin-layer chromatography (general principles 0502, Part IV, Chinese Pharmacopoeia, 2015 Edition), take 10 μ l of each of the above-mentioned, control drug solution, sample solution and negative control solution, and place them on the same silica gel G thin-layer plate. Using glacial acetic acid ethyl acetate toluene (1:2:8) as developing agent, develop, take out, air dry and inspect under ultraviolet light (365nm). In the thin-layer chromatography of the test article, there are fluorescent spots of the same color on the corresponding position of the chromatogram of the reference medicinal materials, and there are no spots on the corresponding position of the negative control solution of Angelica dahurica.

(2) Identification of Flos Magnoliae by TLC

Take the aqueous layer solution in item 2.1.1, add ethyl acetate to extract twice, 30ml each time, combine the extract, wash it with water to neutral, and concentrate to 1ml as the sample solution. In addition, negative samples of Magnolia were taken and negative control solution was prepared by the same method. Take 1g of control herbs of Magnolia, add 20ml of dilute hydrochloric acid, heat in water bath and reflow for 60min, filter, extract 10ml of filtrate twice with ether, combine the extract, wash with water to neutral, and concentrate to 1ml as the control herbs solution. According to the test of thin layer chromatography (general rule 0502 of the fourth part of Chinese pharmacopoeia, 2015 edition), 10μL each of the above-mentioned control medicinal material solution, sample solution and negative control solution were absorbed. Respectively spot on the same silica gel g thin layer plate, use glacial acetic acid-ethyl acetate-toluene (1: 2: 8) as developing agent, unfold, take out, air dry, and place under ultraviolet lamp (365nm) for inspection. In the chromatogram of the test sample, fluorescent spots of the same color are displayed at the corresponding positions with the chromatogram of the control medicinal materials, while the negative control solution has no spots at the corresponding positions.

(3) Identification of Xanthium sibiricum by TLC

Take 4g of the content of this product, add 50ml of 10%NaOH solution, heat in water bath for 60min, and filter. The filtrate was extracted with ethyl acetate twice with 30ml each time, the extracts were combined, washed with water to neutrality, and concentrated to 1ml as sample solution. Another negative sample of Xanthium sibiricum was taken and a negative control solution was prepared by the same method. Take 1g of Xanthium sibiricum control drug, add 20ml of 10%NaOH solution, heat in water bath for 60min, filter, extract the filtrate with ethyl acetate twice, 10ml each time, and prepare the control drug solution by the same method. According to the test of thin layer chromatography (general principles 0502 in the fourth part of Chinese Pharmacopoeia, 2015 Edition), suck 10 μ l of the above-mentioned, control drug solution, sample solution and negative control solution respectively. Spot them on the same silica gel G thin layer plate, take glacial acetic acid ethyl acetate toluene (1:4:16) as the developing agent, develop, take out and dry them, put them in

iodine steam for 10 minutes, inspect. In the chromatogram of the test sample, there are spots of the same color in the corresponding position of the chromatogram of the reference medicinal materials, and there are no spots in the corresponding position of the negative control solution.

3.2 Determination of Baicalin by HPLC

(1) Chromatographic conditions: chromatographic column: Diamonsil C 18 (ID 4. 6mm ×200mm, ODS2 5μm); Mobile phase: methanol-water-phosphoric acid (47: 53: 0.2); The flow rate was 1. 0mL/min. Detected at 278nm wavelength; The temperature of chromatographic column is 35℃; The injection volume of each sample is 20 μ l.

(2) Test Method and Solution Preparation: When preparing the test sample solution, weigh about 0.1g of Xanthium sibiricum rhinitis capsule and pour it into a volumetric flask with a scale of 50ml. Pour 40ml 50% 50% methanol into the volumetric flask, then perform ultrasonic operation for 30min. After seeing the drug dissolved in the volumetric flask, take it out from the ultrasonic, cool it to room temperature, dilute it to scale with 50% methanol, swing it evenly, filter it with the filter system, and take the filtrate as the test solution. Weigh 20.86mg of baicalin reference substance, place it in a 100mL volumetric flask, dissolve and dilute it with 50% methanol, fix the volume to the calibration line, shake it evenly, and use it as reference substance reserve solution. Accurately measure 3mL into a 50mL volumetric flask and dilute to scale with 50% methanol as the reference solution. The chromatogram is shown in fig. 4. In order to better observe the linear concentration range of baicalin under the chromatographic conditions mentioned above, a pipette gun is used to suck 1mL, 2mL, 3mL, 5mL and 10mL of baicalin reference substance stock solution respectively, and then the sample is poured into a 50mL volumetric flask, diluted to scale with 50% methanol respectively, and injected according to the same chromatographic conditions, taking the peak area as the y axis and the concentration as the x axis to draw a standard curve. The linear regression equation of baicalin can be obtained: A=20432.13C-91.15, r=0.9999. it can be seen that the injection amount of baicalin has a good linear relationship with peak area in the range of 0.083~0.834μg g. Therefore, the content of baicalin can be determined by this method. For the precision inspection of the instrument needed for the test, the same reference solution should be used for 5 consecutive injections, and the relative standard deviation (RSD) of the peak area is 0.82%, indicating that the performance of the instrument is in line with the requirements. In the stability test of sample detection, the randomly selected sample solution was detected every two hours for 8 consecutive times, and the RSD of baicalin peak area in the chromatogram was 1.2%, indicating that there was no significant change in baicalin in the sample solution within 16 hours. After repeated tests, 5 samples of Xanthium sibiricum Biyan capsule of the same batch were detected, and the test solution was prepared according to the sample solution method. The peak area of baicalin could be obtained by running the liquid phase, and the RSD was 0.9%. The method for the determination of baicalin has high reproducibility and durability. In order to verify the accuracy of the experimental method, this experiment also designed the sample adding recovery test, using the sample adding recovery method. The total content of baicalin (n = 9) in the samples of Xanthium sibiricum Biyan capsule was determined. Calculate their recoveries as shown in Table 1.

Table 1 Investigation test results of baicalin recovery rate

Sample size /g	Sample content /mg	Addition /mg	Real measurement /mg	Recovery rate/%	Average recovery rate/%	RSD/%
0.1058	7.3214	0.0417	7.2453	98.4		
0.1032	7.3222	0.0417	7.3344	99.6		
0.1101	7.2154	0.0417	7.0031	96.5		
0.1084	7.3321	0.0834	7.3339	98.9		
0.1055	7.1226	0.0834	7.0403	97.7	98.5	0.9
0.1069	7.2247	0.0834	7.1692	98.1		
0.1059	7.2832	0.01668	7.4053	99.4		
0.1048	7.2162	0.01668	7.2796	98.6		
0.1104	7.2152	0.01668	7.3599	99.7		

(3) In order to confirm that the sample chromatogram has no interference from other impurity peaks at the retention time position of baicalin peak in the chromatogram of the reference substance, a negative sample is specially prepared. The negative sample does not contain Scutellaria baicalensis Georgi. The solution of the negative sample is prepared according to the method used in 2.2.2. The same method is used for determination under chromatographic conditions. There is no peak of negative sample in the retention time position.

of baicalin peak in the chromatogram of sample solution. Samples of Xanthium sibiricum rhinitis capsules from three manufacturing enterprises purchased from pharmacies were tested by the same method under item 2.2.2, and baicalin reference substance solution was additionally taken for chromatographic determination by the same method. See Table 2 for data and Figures 1, 2 and 3 for chromatogram.

Table 2 Determination of baicalin content in Cangziziyanyan capsules from three manufacturers

Sample lot number	Baicalin content /mg/ granule			Average content /mg/ granule	Relative deviation/%
171206	4.5236	4.7865	4.4527	4.5876	0.4
180206	7.7226	7.2688	7.3157	7.4357	0.2
20181101	6.1235	6.1346	6.1168	6.1250	0.3

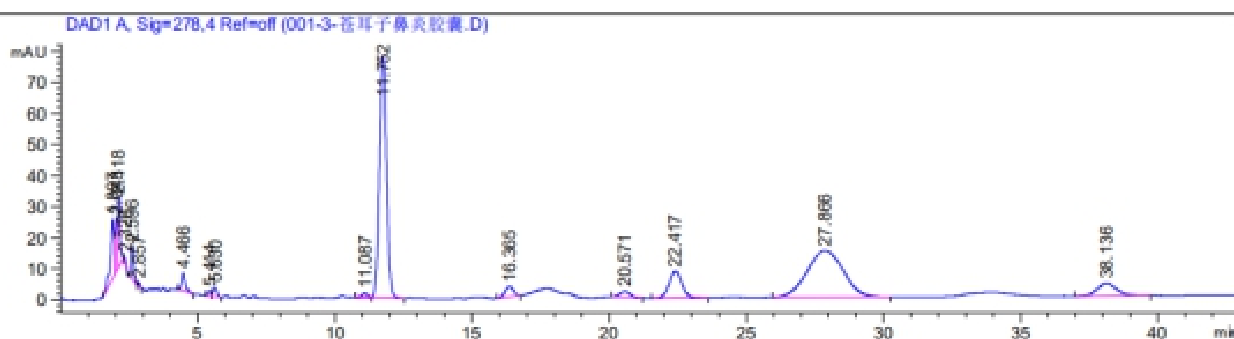

Figure 1 Sample 1 HPLC

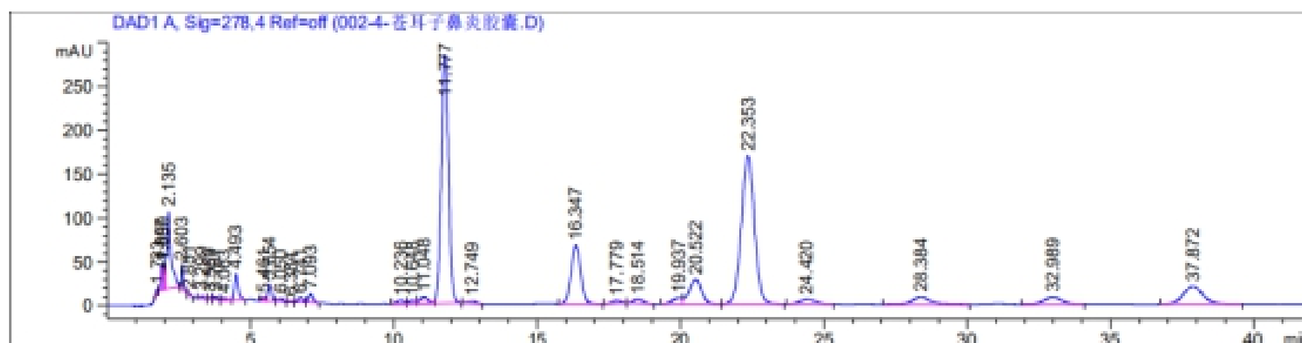

Figure 2 Sample 2 HPLC

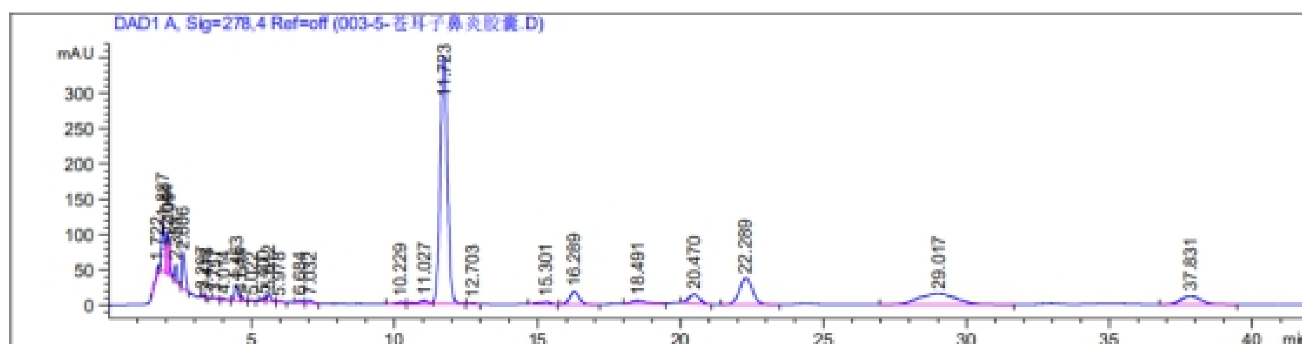

Figure 3 Sample 3 HPLC

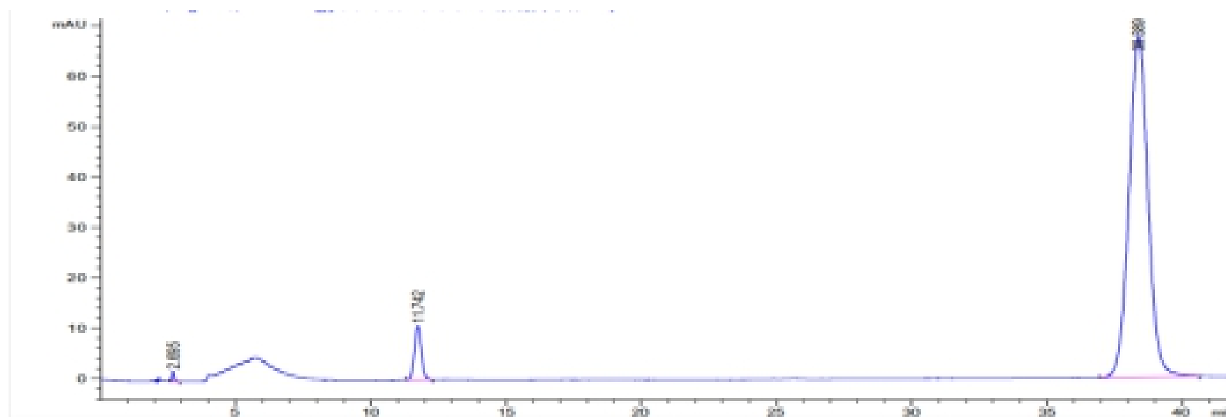

Fig. 4 High performance liquid chromatography of baicalin reference solution

4. Summary

The detection method of "Xanthium sibiricum rhinitis capsule" is studied from samples of three commercial production enterprises, and the detection results of various manufacturers are compared, and a more scientific, more advanced, more practical, more standardized, more accurate and more accurate quality control standard is preferred. At present, our country is in a crucial and important stage from a big pharmaceutical country to a powerful pharmaceutical country. Therefore, the smooth implementation of this project will lead to the implementation of drug standards improvement actions, so that more clinically widely used drug standards with definite curative effects will be included in the Chinese Pharmacopoeia. This is of great significance to ensure that public medication is fast, convenient, safe and effective, and to promote the upgrading of the pharmaceutical industry and product quality.

Under the above experimental chromatographic conditions, a plurality of well separated component peaks can be detected in the chromatographic system of the content determination chromatogram of scutellaria baicalensis Georgi in the prescription. In this subject, only baicalin, an effective chemical component in the prescription, has been determined, and the study of other component peaks in chromatography needs further discussion.

There are obvious differences in product quality under different quality standards. The reason for this result is probably due to the difference in the quality of Scutellaria baicalensis Georgi. According to literature reports, the extraction rate and the content of effective chemical components of Scutellaria baicalensis Georgi from different habitats and different harvest seasons are different, and there is currently no drug standard for content determination or different control standards. Under this kind of product quality control background, it will bring favorable opportunities to illegal or illegal producers. Therefore, the current product standard of Xanthium sibiricum rhinitis capsule needs to be further optimized and perfected, and a national unified quality standard with controllable quality should be gradually established.

According to the above experimental results, the TLC identification and baicalin content determination method of Xanthium sibiricum rhinitis capsule are feasible. The thin-layer identification of samples from three commercial production enterprises can pass the test of this method. According to the determination results of baicalin content and referring to the theoretical content of baicalin stipulated in the national standard of scutellaria baicalensis, it is relatively objective and feasible to initially determine the content limit of baicalin in Xanthi rhinitis capsule to be not less than 5mg per capsule. This index of not less than 5mg per granule can be used as the national drug standard for this preparation.

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