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Study on the Content Determination of Shenbei Beigua Ointment

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Abstract: *Objective:* A TLCS method was established for the determination of the content of Shenbei Beigua ointment, and the product quality of six samples from two formulations was evaluated. *Methods:* The determination method was thin-layer chromatography scanning (TLCS), using a developing solvent composed of ethyl acetate–methanol–strong ammonia water (17: 2: 1). The plates were heated at 105 °C for 5 minutes, then sprayed with a mixture of dilute bismuth potassium iodide and 1% ferric chloride in ethanol (10: 1), and scanned at a wavelength of 500 nm. *Results:* Peimine showed good linearity in the concentration range of 0.21–2.1 μg with a correlation coefficient of r = 0.9997, and Peiminine also exhibited good linearity in the same range with r = 0.9995. The accuracy was ≥ 95.0%, and the relative standard deviation (RSD) was ≤ 5.0% (n = 6). *Conclusion:* This method allows for the simultaneous determination of peimine and peiminine, providing a reliable reference for the quality control of the product.

Keywords: Shenbei Beiguai ointment; Peimine; Peiminine; Content determination; TLCS; Quality control

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1. Introduction

Shenbei Beiguai ointment is a traditional Chinese medicine preparation produced by Shanghai Jing ‘an Pharmaceutical Co., LTD. It contains 5 herbs, such as Beigua ointment, Codonopsis, Zhejiang fritillaria (*Fritillaria thunbergii Miq*), Nansha ginseng, and dried ginger. In the Chinese Pharmacopoeia (2025 edition), the content of Fritillaria is determined by high-performance liquid chromatography coupled with evaporative light scattering detection [1, 2]. Considering that this product is a decoction, it also contains four other herbs. If the high-performance liquid chromatography with evaporative light scattering detection method is used, significant interference occurs; therefore, thin-layer chromatography scanning (TLCS) was ultimately chosen for determination. After research and exploration, the TLC scanning method was established to determine the content of Fritillarine A and Fritillarine B in Zhejiang fritillaria [3].

2. Experimental materials

2.1. Instruments and reagents

(1) Fritillary A reference substance (China Institute for Food and Drug Control, batch number:110750-202414) [4].

(2) Fritilbetin B reference substance (China Institute for Food and Drug Control, batch number:110751-202414).

(3) Using high-efficiency Silica gel prefabricated thin layer plate (TLC Silica gel 60 e.mark).

(4) All reagents were analytical pure [China Pharmaceutical (Group) Shanghai Chemical Reagent Co., LTD. [5].

2.2. Information of test products

Table 1 below shows the information of the test products used in this study.

Table 1. Information of test products

Variety	Number 1	Number 2	Number 3
Sample (available in sugar form)	250510-1	250511-1	250512-1
Sample (low sugar type)	250426-1	250426-2	250427-1

3. Methods and results

3.1. Preparation of the test solution

Take 40g (sugar form) or 20g (no sugar form) of this product, weigh it carefully, add water to make 100mL, dissolve, shake well, adjust the pH value to 3–4 with 6mol/L hydrochloric acid solution, extract with dichloromethane twice, 60mL each time, discard the dichloromethane solution, The acid solution was adjusted to pH 11 with 40% sodium hydroxide solution, extracted with dichloromethane 4 times, 100mL each time, combined with the extract, concentrated to dry under reduced pressure, the residue was dissolved with dichloromethane: methanol (1:1) mixed solution, transferred to a 2mL volumetric flask, and diluted to the scale, shaken well, centrifuged, and the supernatant was used as the test solution [6].

3.2. Preparation of the reference solution

Accurately weigh approximately 10 mg each of Fritillarine A and Fritillarine B, and transfer both into the same 50 mL volumetric flask. Add methanol to volume, and mix thoroughly to ensure complete dissolution. The resulting solution contains 0.2 mg/mL of Fritillarine A and 0.2 mg/mL of Fritillarine [7].

3.3. Preparation of negative control solution

The negative blank samples of *Fritillaria thunbergii Miq* without Zhejiang-Fritillary were prepared according to the prescribed proportion, and the negative control solution was made according to the preparation method under Section 3.1. above [8].

3.4. Thin layer chromatography and scanning conditions

According to the thin-layer chromatography test described in the Chinese Pharmacopoeia (2025 edition), General Rules (0502), 15 μL of the test solution and 3 μL and 10 μL of the control solutions were spotted on the same silica gel G thin-layer plate. Ethyl acetate–methanol–concentrated ammonia (17:2:1) was used as the developing solvent. The plate was developed at low temperature (2–8 °C), then removed and dried. Heat at 105°C for 10 minutes, let cool,

spray with dilute potassium bismuth iodide test solut-1% ferric chloride ethanol solution (10: 1). Mix the solution until the spot color is clear, then dry the plate. Cover the thin-layer plate with a glass plate of the same size and fix it with tape around the edges. Perform thin-layer chromatography scanning within 2 hours at a wavelength of 500 nm. Measure the integral absorbance values of the test and control substances, then calculate the content accordingly [9].

3.5. Recovery test

A total of 20 g of the sugar-type test substance (Sample no.: 250510-1) and 10 g of the low-sugar-type test substance (Sample no.: 250426-1) were accurately weighed and prepared according to the method described in Section 3.1 for the preparation of test substance solutions. Control solutions containing Fritillarine A (1.0832 mg/mL) and Fritillarine B (1.1786 mg/mL) were added to the samples in volumes of 50 μL, 75 μL, and 100 μL, corresponding to Fritillarine A amounts of 54.16 μg, 81.24 μg, and 108.32 μg, and Fritillarine B amounts of 58.93 μg, 88.40 μg, and 117.86 μg, respectively.

The recovery results showed: (1) Fritillarine A: Average recovery of 98.5% with an RSD of 1.9%; (2) Fritillarine B: Average recovery of 99.6% with an RSD of 2.3%. For a separate batch, the average recovery of Fritillarine A and Fritillarine B was 100.3% (RSD: 3.8%) and 98.9% (RSD: 2.3%), respectively [10].

3.6. Repeatability test

This product was prepared and determined according to the preparation method of the test product solution in the text. A total of 6 test product solutions were prepared and determined. The results showed that the RSD of Fritillitin A in the sugar test product was 2.3%, the RSD of Fritillitin B was 3.4%, and the RSD of the total was 1.9%. The results showed that the RSD of Fritillitin A, Fritillitin B, and total amount were 2.4%, 2.9% and 3.9%, respectively, in the low glucose type test [11].

3.7. Parallel test

3.7.1. In-board parallelism test

The sample solution of 10 microliters (μL) was accurately taken by precision pipetting equipment and added to the silica gel G thin layer chromatography plate with the same number (n = 6), expanded, and scanned. The results showed that the RSD of Fritilletin A and Fritilletin B in the sugar form was 0.5% and 0.7%, respectively.

3.7.2. Inter-plate parallelism test

Ten microliters (μL) of the sample solution was accurately taken by precision pipetting equipment, and then added to the silica gel G thin layer chromatography plates with different numbers (n = 6), expanded, and scanned. The results showed that the RSD of Fritillitin A and Fritillitin B in the sugar form samples was 0.8% and 1.2%, respectively [12].

3.8. Linear range

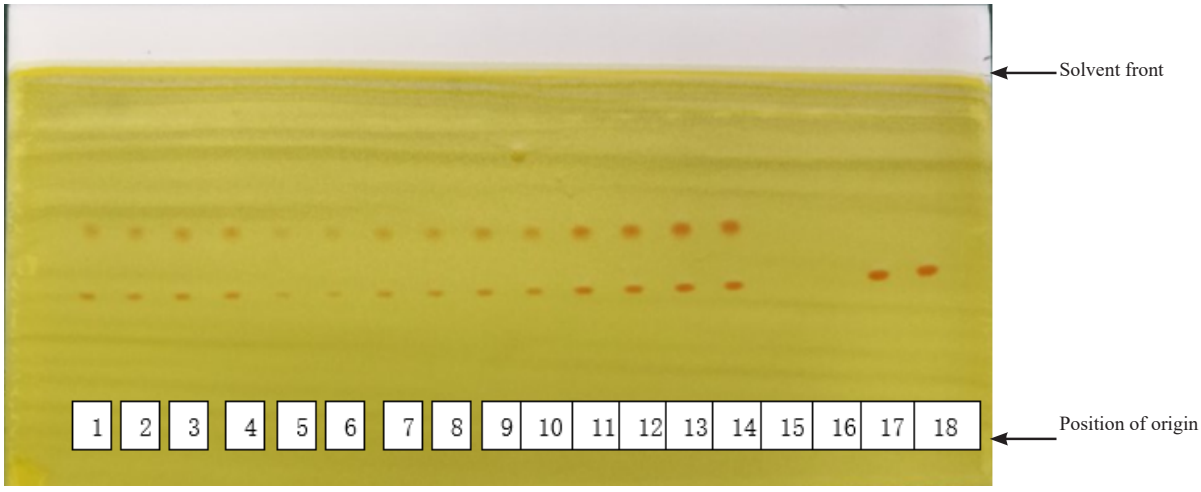
Accurately pipette the Fritillarine A reference solution (0.1028 mg/mL) in volumes of 2 μL, 4 μL, 6 μL, 10 μL, 15 μL, and 20 μL, and the Fritillarine B reference solution (0.1036 mg/mL) in the same volumes. Spot each onto the same silica gel G thin-layer plate, and perform scanning according to the specified procedure. The spot sample volume (μg) was taken as the abscission, and the absorbance integral value was taken as the ordinate. The standard curve was drawn, and the regression equation of pantilbetin A was obtained: $Y = 6651.9\rho + 622.2$, $r = 0.9997$, in the range of 0.21–2.1 μg [13]. The regression equation was: $Y = 6456.6\rho + 3076.1$, $r = 0.9995$, in the range of 0.21–2.1μg; There was a good linear relationship between the amount of sample spots and the absorbance integral value of chromatographic spots [14].

3.9. Stability test

A control solution containing 0.2056 mg/mL of Fritillarine A and 0.2072 mg/mL of Fritillarine B was prepared. From this, 10 μL of the control solution and 15 μL of the test solution were taken and analyzed under the chromatographic conditions described above. The concentration of the test solution was determined within 3 hours for a total of 6 times. The results showed that the solution was basically stable during 0–180 min (RSD = 0.9%).

3.10. Specific test

Precisely measured volumes of 15 μL of the test solution, 15 μL of the negative control solution, and 3 μL and 10 μL of the control solution were spotted onto the same thin-layer chromatography (TLC) plate. The plate was developed and analyzed under the chromatographic conditions described above. The resulting content is shown in **Figure 1** [15].



1–2 There is a sugar test solution
3–4 Low sugar test solution
5–6 Reference solution (3μl)
7–8 Reference solution (10μl)
9–10 solution of sugar test substance
11–12 Low sugar test solution
13–14 Test solution + control solution
15–16 blank sample solution
17–18 Fritilbetin A control solution

Figure 1. Chromatographic diagram of the content determination of Shenbei Beigua ointment

3.11. Sample determination

Six batches of samples were taken to determine the content according to law, and the results are shown in **Table 2**.

Table 2. Sample determination results

No.	Fritillary A (μg/g)	Fritillarine B (μg/g)	Total amount (μg/g)
Sugar form (250510-1)	7.2649	5.2675	12.5324
Sugar type (250511-1)	7.3603	5.7549	13.1152
Sugar type (250512-1)	7.1895	6.0237	13.2132
Low sugar type (250426-1)	10.3098	8.7549	19.0647
Low glucose type (250426-2)	10.2798	8.6512	18.9310
Low glucose type (250427-1)	10.5759	8.6439	19.2198

4. Discuss

4.1. Selection of test solution preparation methods

This product is a decoction, containing a large amount of sugar. To identify a more effective method for extracting Fritillaria components and eliminating sugar interference, various extraction methods were investigated. The extraction efficiency of each method for Fritillaria alkaloids was evaluated to determine the most suitable extraction method for this product. Four kinds of unfolding agents were used for the test. The sample volume was 20μl, the inspection conditions were spraying dilute potassium bismuth iodide test solution, the temperature control was 19–21℃, and the humidity control was 35–45%. Results: The extraction method was better than the reflux extraction method. The pH value had a great influence on the extraction efficiency of alkaloid, and the extraction efficiency of chloroform was higher than that of mixed solvent. This method is preliminarily planned to be used, and then detailed research will be carried out on this basis.

4.2. Selection of unfolding agent

To determine the appropriate unfolding agent, the six commonly used unfolding agents for the differentiation of Zhezheimbolium alkaloids are: ethyl acetate-methanol-concentrated ammonia (20: 1: 1), cyclohexan-ethyl acetate-diethylamine (6: 4: 1), petroleum ether (60–90 ° C) -ethyl acetate-methanol-concentrated ammonia (10: 10: 2: 1), ethyl acetate-trichloromethane methanol (30: 20: 3), ethyl acetate-methanol-concentrated ammonia water (17: 2: 1), trichloromethane-ethyl acetate-methanol-concentrated ammonia water (30: 40: 15: 10) placed below 10℃ in the lower layer of the solution were investigated. The results showed that ethyl acetate-methanol-concentrated ammonia water (17: 2: 1) had the best separation and moderate retention time, and was finally selected as the unfolding agent in this method.

4.3. Selection of constant volume solvent

After dissolving the residue obtained after extraction, it was found that better solubility could be obtained by dissolving the mixed solution of dichloromethane and methanol (1: 1).

4.4. Selection of detection wavelength

After the spectral scanning of the control solution of Fritillarine A and Fritillarine B and the spots of the test solution after expansion, the spectrogram of the control solution of Fritillarine A and Fritillarine B was basically the same as that of Fritillarine A and Fritillarine B in the test. According to the test results, the maximum wavelength was 500nm, so the scanning wavelength of this method was set as 500nm.

5. Conclusion

The developed method enables the simultaneous determination of peimine and peiminine, offering a reliable approach for quality control of the product. This provides a valuable reference for ensuring consistency and standardization in future applications.

Disclosure statement

The author declares no conflict of interest.

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