 DOI: 10.19600/j.cnki.issn2152 - 3924.2024.01.007

Determination of Salvianolic Acid B in Yiqi Huayu Prescription by HPLC

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Abstract [**Objectives**] To determine the content of salvianolic acid B in Yiqi Huayu Prescription by HPLC. [**Methods**] The chromatographic column was ZORBAX Eclipse Plus C_{18} (4.6 nm × 250 nm, 5 μm); the mobile phase was acetonitrile-0.1% phosphoric acid (21∶79), the detection wavelength was 286 nm, the column temperature was 30 ℃, and the flow rate was 1.0 mL/min. A method for determination of salvianolic acid B in Yiqi Huayu Prescription was established. [**Results**] The linear relationship of salvianolic acid B was good in the range of 0.021 4 - 0.406 4 mg/mL. The regression equation was $Y = 5\,995.989\,84\,X - 0.073\,32$, $r = 0.999\,9$. The average recovery rate was 98.88% (*RSD* = 1.6%). [**Conclusions**] The method is reliable, accurate and specific, and can be used for the determination of salvianolic acid B in Yiqi Huayu Prescription.

Key words Yiqi Huayu Prescription, Salvianolic acid B, High performance liquid chromatography, Content determination

1 Introduction

Yiqi Huayu Prescription consists of *Salvia miltiorrhiza*, *Panax notoginseng*, *Rhodiola rosea*, *Agrimonia pilosa* and *Ficus hirta* Vahl. It is recorded in the *Internal Canon of Medicine* that blood and qi are synonymous but of the same kind[1]. The blood and qi are inseparable, and early disease lies in qi while long disease lies in blood. Replenishing qi and blood can strengthen the body, activating blood circulation and removing blood stasis can eliminate symptoms and cure diseases. Treatment and tonifying go hand in hand, and the combination of deficiency and excess can help to realize the simultaneous treatment of principal and subordinate symptoms. Yiqi Huayu Prescription has been developed on this, and the clinical effect is significant. It has the functions of invigorating qi, promoting blood circulation, dredging collaterals and relieving pain. *S. miltiorrhiza* is the monarch drug in the prescription. Exploring the content determination method of salvianolic acid B, an effective component of *S. miltiorrhiza* in Yiqi Huayu Prescription[2-3], can lay a foundation for developing this prescription into hospital preparation in the later period, so as to better serve patients.

2 Materials

2.1 Instruments Agilent 1100 high performance liquid chromatograph; BS224S electronic balance (Beijing Sartorius Instrument System Co., Ltd., d = 0.000 1 g); KQ3200E medical ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); Dongfang-A directly-heated electrothermal constant-temperature drying oven (Guangzhou Dongfang Electric Heating Drying Equipment Factory); HH-S4 digital display thermostat water bath (Jintan Medical Instrument Factory).

2.2 Reagents and medicinal materials Salvianolic acid B reference substance (batch number: 121521-90-2); *S. miltiorrhiza*, *P. notoginseng* and *R. rosea* were all purchased from Guangzhou Zhixin Chinese Herbal Pieces Co., Ltd.; *A. pilosa* and *F. hirta* Vahl were purchased from Yulin Bencaotang Chinese Herbal Pieces Co., Ltd.; acetonitrile (chromatographically pure), other reagents were analytically pure, and water was ultrapure water.

3 Methods and results

3.1 Preparation of reference solution 10.16 mg of salvianolic acid B was weighed accurately, placed in a 25 mL flask, mixed with methanol to volume, and shaken well to obtain reference stock solution (0.406 4 mg/mL). 2 mL of solution was put into a 10 mL flask with precision, mixed with methanol to volume, and shaken well as the reference solution.

3.2 Preparation of test solution *S. miltiorrhiza* (20 g), *P. notoginseng* (10 g), *A. pilosa* (20 g), *R. rosea* (10 g) and *F. hirta* Vahl (25 g) were weighed according to the prescription, and put in a 1 000 mL flask. 500 mL of water was added, and it was soaked for 15 min, decocted for 45 min, filtered, and the filtrate was concentrated to 100 mL to obtain the stock solution of the test product. 2 mL of stock solution was taken for test, put in a 50 mL flask, mixed with methanol to volume, and shaken well as the test solution.

3.3 Preparation of negative control solution According to the prescription amount, the rest of the medicinal materials except *S. miltiorrhiza* were weighed and prepared according to the method under Section **3.2**, and the negative control stock solution was obtained. 2 mL of negative control solution was precisely measured, placed in a 50 mL flask, mixed with methanol to the desired volume, and shaken well as the negative control solution.

3.4 Chromatographic conditions and system adaptability test The chromatographic column was ZORBAX Eclipse Plus C_{18} column (4.6 nm × 250 nm, 5 μm); the mobile phase was acetonitrile-0.1% phosphoric acid (21∶79), the detection wavelength was 286 nm, the column temperature was 30 ℃, and the flow rate was 1.0 mL/min. 10 μL of three solutions under Section **3.1**, **3.2**

Received: December 13, 2023 Accepted: January 18, 2024
Supported by Zhongshan Medical Research Project (2021A020487).
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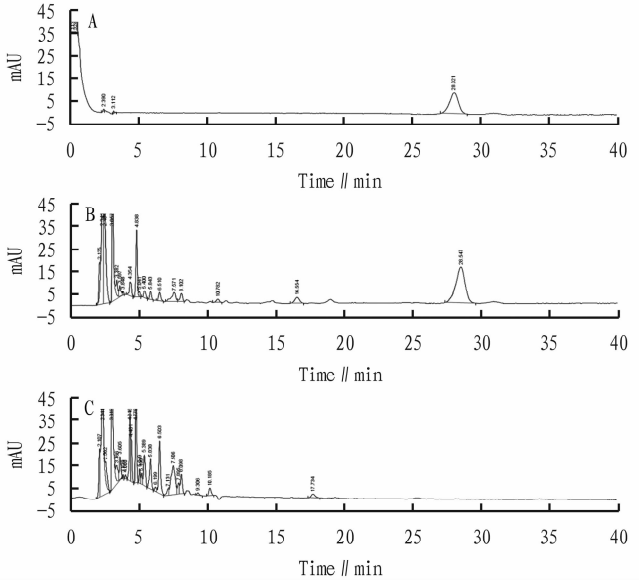
and **3.3** were taken, injected and analyzed respectively, and the chromatography was recorded. The number of theoretical plates calculated according to salvianolic acid B should not be less than 1 000. Comparing with the retention time, it can be seen that the negative control solution had no interference at the position of salvianolic acid B. See Fig. 1 for details of chromatography.



Note: A. reference substance; B. test substance; C. negative control.

Fig. 1 High performance liquid chromatogram of salvianolic acid B

3.5 Investigation of linear relationship 0.8, 2, 3, 5 and 10 mL of reference stock solutions under Section **3.1** were accurately suctioned and placed in a 10 mL flask separately, and mixed with methanol to volume. The above five samples of reference solution were injected, and linear regression was carried out with reference solution concentration C (mg/mL) as abscissa and peak area A as ordinate.

The regression equation is: $Y = 5\,995.989\,84\,X - 0.073\,32$, $r = 0.999\,9$, indicating that the concentration of salvianolic acid B showed a good linear relationship with the peak area in the range of 0.021 4 – 0.406 4 mg/mL (Fig. 2).

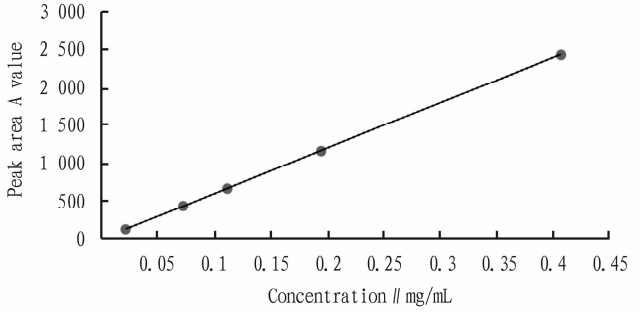


Fig. 2 Standard curve of salvianolic acid B reference substance

3.6 Precision Test The reference solution was injected continuously for 5 times, and the chromatographic peak area was recorded. The relative standard deviation of peak area *RSD* = 1.6% (Table 1).

Table 1 Precision test results

Group	A value	*RSD*//%
1	647.3	1.60
2	650.0	
3	673.2	
4	661.5	
5	647.1	
6	655.5	

3.7 Stability test The test solution was injected at 4, 8, 12, 16, 20 and 24 h, respectively, and the chromatographic peak area was recorded, *RSD* = 1.2%, which indicated good stability (Table 2).

Table 2 Stability test results

Time//h	A value	*RSD*//%
0	599.5	1.2
4	585.3	
8	602.7	
12	600.5	
16	598.5	

3.8 Repeatability test 6 groups were weighed according to the prescription amount, and 6 test solutions were prepared according to the test solution preparation method under Section **3.2**, and the content of salvianolic acid B was determined and calculated respectively. The average content was 0.101 2 mg/mL, *RSD* = 1.0%, indicating that the method had good repeatability (Table 3).

Table 3 Repeatability test results

Group	A value	Content // mg/mL	*RSD*//%
1	599.5	0.100 0	1.0
2	598.5	0.099 8	
3	606.5	0.101 2	
4	596.1	0.099 4	
5	600.4	0.100 2	
6	612.2	0.102 1	

3.9 Recovery rate test 6 groups of samples for test (2.843 83 mg/mL) with known content were measured accurately and placed in a 50 mL flask, each with 1 mL. The same amount of salvianolic acid B reference substance (about 2.84 mg) as 1 mL of test stock solution was accurately added respectively, and proper amount of methanol was added for ultrasonic treatment until the reference substance was fully dissolved, and methanol was added to the desired volume and shaken well. The determination was carried out according to the chromatographic conditions under Section **3.4**, and the recovery rate was calculated. The average recovery rate was 98.88%, *RSD* = 1.6% ($n = 6$) (Table 4).

3.10 Determination of sample content 3 groups were weighed according to the prescription amount, and 3 test solutions were prepared according to the test solution preparation method under Section **3.2** for determination. The content of salvianolic acid B in sample was 0.101 3, 0.126 3 and 0.110 5 mg/mL, respectively.

Table 4 Test results of sample recovery rate

Group	Mass concentration // mg/mL	Addition // mg	Measured amount // mg/mL	Recovery rate	Average recovery rate	*RSD* // %
1	2.843 8	2.75	5.522 5	0.974 1	0.988 8	1.6
2	2.843 8	2.95	5.726 9	0.977 3		
3	2.843 8	3.02	5.903 7	1.013 2		
4	2.843 8	2.84	5.651 0	0.988 4		
5	2.843 8	2.89	5.708 5	0.991 2		
6	2.843 8	2.79	5.588 4	0.983 7		

4 Discussion

In this experiment, the content of salvianolic acid B was determined by HPLC and the methodological investigation was carried out. Salvianolic acid B had a specific absorption peak at 286 nm, and the concentration of salvianolic acid B was proportional to the peak area. When screening the chromatographic conditions, we tried to use acetonitrile: 0.1% phosphoric acid and acetonitrile: 0.02% phosphoric acid for gradient elution[4], and use acetonitrile: 0.1% phosphoric acid for isocratic elution[5]. By comparing the chromatogram, the chromatogram under isocratic elution with acetonitrile: 0.1% phosphoric acid (21 ∶ 79) showed better peak shape, better degree of separation and more stable baseline than the chromatogram eluted with gradient.

This method is reliable, accurate and specific, and can be used for the determination of salvianolic acid B in Yiqi Huayu Prescription.

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