Determination of ICARIIN in Traditional Chinese Medicine Preparation by HPLC

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Abstract: A method for the determination of ICARIIN in traditional Chinese medicine by High-paerformance liquid chromatography was established, in which octadecyl Silane Bonded Silica Gel was used as Filler, acetonitrile-water (30:70) as mobile phase, the detection wavelength was 270 nm, the flow rate was 1μl /min, the injection volume was 10μl. Column Temperature is 40℃. The average recovery was 99.97%. LINEAR CORRELATION COEFFICIENT r=0.9999, precision and repeatability RSD were 0.35% and 0.70% respectively.

1 INTRODUCTION

The traditional Chinese medicine preparation Wenshen Huazhuo Capsule (formerly known as Jiangzhi Capsule) is a well-known traditional Chinese medicine prescription in Zhangqiu District Traditional Chinese Medicine Hospital. It is based on many years of clinical practice experience and a combination of traditional Chinese medicine theories. The prescription embodies the guiding ideology of holistic concept and differentiation and treatment of traditional Chinese medicine. It has the effects of warming the kidney and resolving turbidity, promoting blood circulation and channeling. It can be used for hyperlipidemia caused by deficiency of kidney yang and blood turbidity and blood stasis. The curative effect is remarkable. The prescription contains medicinal flavors such as roasted Epimedium, Hawthorn, Mistletoe, Polygonatum odoratum, which have the effects of lowering blood fat and nourishing the kidney and removing blood stasis. In order to give full play to the advantages of traditional Chinese medicine in lowering lipids and facilitating the consumption of patients, the preparations for medical institutions have been made, and the preparation of traditional Chinese medicine preparations by medical institutions using traditional techniques has been completed. While exploring and improving its process and formulating process standards, a method for determining the content of icariin is established as the main control index of the internal control agent standard. This article uses HPLC to determine its content.

2 INSTRUMENT AND TEST MEDICINE

Instrument: Shimadzu SPD-20AT high performance liquid chromatograph; Ajilent C18 column 250×4.6mm 5μm

Icariin reference substance: the batch number is 110737-201516, the content is 94.2%, and it is purchased from the China Institute for Food and Drug Control.

Reagents: German Merck chromatographic pure acetonitrile, analytical pure ethanol, Wahaha pure water.

3 METHODS AND RESULTS

3.1 Chromatographic Conditions and System Applicability Test

It can use octadecylsilane-bonded silica gel as filler; use acetonitrile-water (30:70) as mobile phase; SPD-20A ultraviolet detector. Detection wavelength is 270nm, flow rate: 1.0ml/min; injection volume: 10μl;

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Column temperature: 40°C. The number of theoretical plates is calculated based on the peak of icariin as 12143, RSD=0.30%, and tailing factor T=1.030.

3.2 Solution Preparation

Preparation of reference substance solution: accurately weigh an appropriate amount of icariin reference substance, dissolve it with methanol and dilute to the mark, shake it well, and make a solution containing about 35μg/ml of icariin.

Preparation of test solution: take about 0.5g of this product, accurately weigh it, place it in a stoppered conical flask, accurately add 25ml of dilute ethanol, close the stopper, weigh it, and ultrasonically treat it (power 300W, frequency 40kHz) For 30 minutes, it should let it cool, weigh it again, make up the lost weight with dilute ethanol, shake well, filter, and take the additional filtrate to get it.

Preparation of negative control solution: use the same preparation process to prepare a depleted negative control sample, and prepare a negative control solution according to the preparation method of the test solution.

Preparation of sample recovery test solution: take 0.25g of the test product with the measured content, accurately weigh it, place it in a stoppered conical flask, precisely add 15ml of icariin reference substance solution, and add dilute ethanol 10ml, dense plug, weigh it, ultrasonic treatment (power 300W, frequency 40kHz) for 30 minutes, let it cool, then weigh it, use dilute ethanol to make up the lost weight, shake well, filter, and get it. Six copies should be prepared in parallel.

Preparation of linear test solution: weigh accurately 10 mg of icariin reference substance (content: 94.2%), put it in a 50ml measuring flask, add methanol to dissolve and dilute to the mark, shake well, as a reference substance stock solution. Precisely draw the reference substance stock solution to prepare a solution containing about 0.20μg, 0.30μg, 0.40μg, 0.50μg, 0.60μg of icariin.

3.3 Determination of the Wavelength is Detected

By consulting the literature (Li, Chen, Zhang, Wang, Wang 2020) and the 2020 edition of the Chinese Pharmacopoeia (National Pharmacopoeia Commission. 2020) the detection method of icariin in preparations, the wavelength is 270 nm. Therefore, the detection method chooses 270nm as the detection wavelength.

3.4 Methodological Investigation

3.4.1 Specificity

Taking 10μl of icariin reference solution, negative control solution, and test solution respectively, and it can be injected into liquid chromatograph for determination. The chromatograms are shown in figure 1, figure 2, and figure 3.

It can be seen from the chromatogram that at the same position as the reference substance retention time 11.10min, there is no corresponding chromatographic peak in the negative control chromatogram. There is a corresponding chromatographic peak in the chromatogram of the test product. Therefore, the depleted negative control and the solvent do not interfere with the determination.

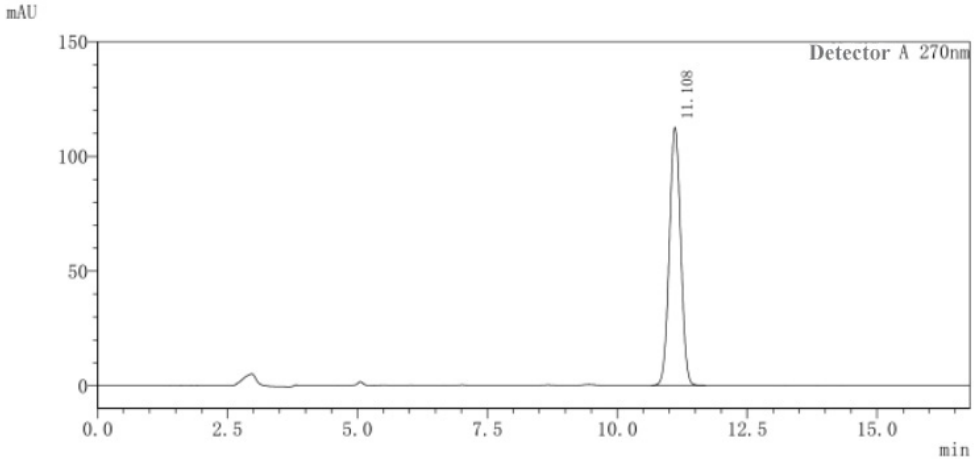

Figure 1: HPLC chromatogram of icariin reference substance.

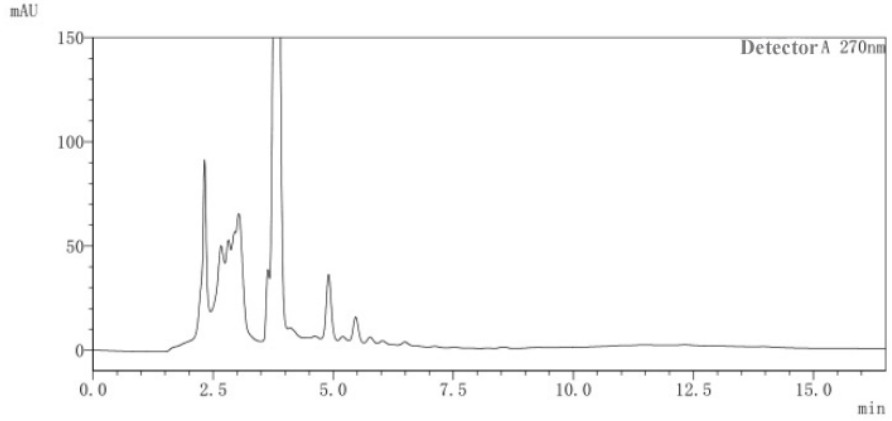

Figure 2: HPLC chromatogram of negative control.

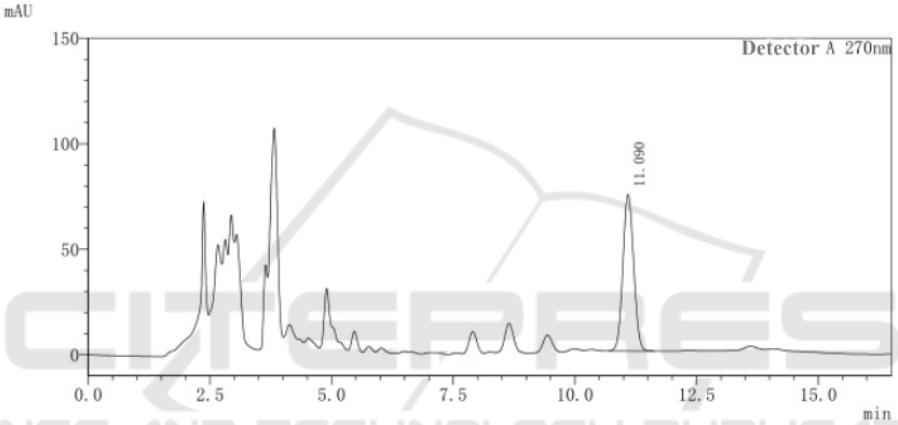

Figure 3: The HPLC chromatogram of the test sample.

3.4.2 Precision

It can take 10μl of the e solution and inject it into the high-performance liquid chromatograph, and inject 5 times continuously to measure the peak area. The results are shown in table 1.

Table 1: Precision test results.

Peak area	1081871	1079740	1079205	1071382	1076269
RSD (%)	0.35				

The test results show that the instrument precision meets the requirements.

3.4.3 Repetitiveness

It should take 10μl of the test solution, inject it into the high performance liquid chromatograph, repeat the determination 6 times, and calculate the content by the external standard method. The results are shown in table 2.

Table 2: Results of the reproducible findings.

Content (%)	0.1847	0.1879	0.1845	0.1852	0.1856	0.1867
Mean content (%)	0.1858					
RSD (%)	0.70					

The test results show that the reproducibility of the measurement method meets the requirements.

3.4.4 Sample Recovery Rate

The next step is to take 10μl of the sample recovery test solution, inject it into the high performance liquid chromatograph, determine, and calculate the content by external standard method. The results are shown in table 3.

Table 3: Results of sample recovery rate.

No.	Sampling amount (g)	Content in the sample (μg)	Control addition (μg)	Measurements (μg)	Rate of recovery (%)	Average recovery rate (%)	RSD (%)
1	0.2780	0.2061	0.2125	0.4184	99.91		
2	0.2762	0.2047	0.2125	0.4170	99.91		
3	0.2747	0.2036	0.2125	0.4161	100.00	99.97	0.06
4	0.2768	0.2052	0.2125	0.4176	99.95		
5	0.2727	0.2021	0.2125	0.4147	100.05		
6	0.2735	0.2027	0.2125	0.4152	100.00		

The test results show that the recovery rate of the measurement method meets the regulations.

3.4.5 Linear and Range

The next is to take 10 μl each of the linear test solution and inject it into the high performance liquid chromatograph, measure the peak area respectively, and calculate the linear correlation. The results are shown in table 4.

Table 4: Results of the linear relationship investigation.

Sample size (μg)	0.1888	0.2832	0.3776	0.4720	0.5664
Peak area	455117	697336	930217	1160026	1393169

Taking the injection volume as the abscissa and the peak area as the ordinate, linear regression is performed to obtain the regression equation, y = 2.4649×106x -2980.2, and the correlation coefficient r=0.9999. It can be seen in figure 5.

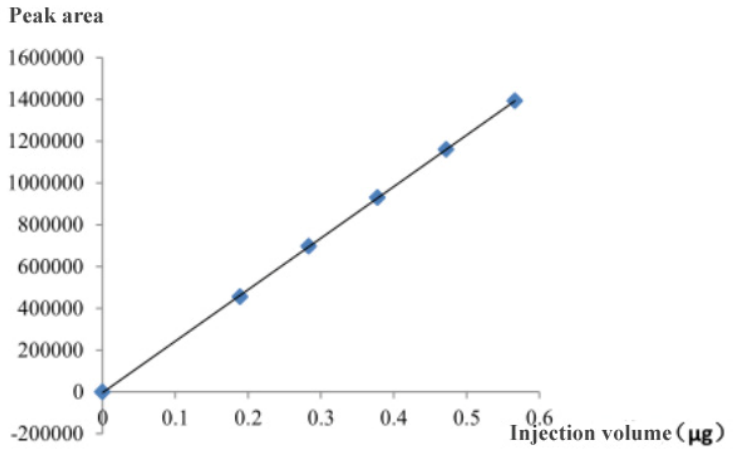

Figure 4: Linear regression curves.

The results show a good linear relationship between sample size and peak area between 0.1888 μg~0.5664 μg.

3.4.6 Sample Stability

At 0 hour, 2 hours, 4 hours, 8 hours, and 24 hours, 10 μl of the test solution was taken and injected into the high performance liquid chromatograph to determine the peak area of icariin. The results are shown in table 5.

Table 5: Results of the stability test.

Time (h)	0	2	4	8	24
Peak area	945015	946455	949716	941619	945068
RSD (%)	0.31				

From the test results, the test product solution is well stable within 24 hours and can meet the determination needs.

3.5 Sample Determination

According to the determination method and conditions, three batches of samples were tested, and the content was calculated by the external standard method. The results are shown in table 6.

Table 6: Results of epicanosin content determination.

Lot.	20200812	20200813	20200813
Content (%)	0.1850	0.1853	0.1863
Content (mg)	0.7900	0.7916	0.7948

4 DISCUSSION

4.1 In this paper, octadecylsilane-bonded silica gel is used as the filler, and acetonitrile-water (30:70) is used as the mobile phase (Wang 2020). The detection wavelength is 270nm, the flow rate is 1.0ml/min, the injection volume is 10μl, and the column temperature is 40°C. The content of icariin in Jiangzhi capsules was determined by external standard method. The method's specificity, accuracy, precision, linearity, stability all meet the requirements of methodology verification.

4.2 Solvent (National Pharmacopoeia Commission. 2020) with dilute ethanol is environmentally friendly and cheap.

4.3 Icariin was extracted by ultrasound for 20 minutes, 30 minutes, 40 minutes, and 60 minutes and the measured results remained unchanged, so 30 minutes of ultrasound was used to extract icariin.

4.4 When the sample dosage is 0.2g, 0.5g, 1.0g, the measured result of icariin remains unchanged, so 0.5g is selected.

4.5 Hyperlipidemia is a disorder of lipid metabolism and an important risk factor for atherosclerosis. It can lead to atherosclerotic diseases such as coronary heart disease, cerebral infarction and peripheral vascular disease, as well as fatty liver and acute pancreatitis other diseases. Hyperlipidemia and atherosclerosis are common in middle-aged and elderly people. Chinese medicine believes that when people are over forty years old, the kidney essence gradually declines, and the qi function is weakened. The clearing will change from turbidity, and lipid cohesion will cause hyperlipidemia, qi deficiency and blood weakness, turbidity, blockage of blood, and blood stasis atherosclerosis. The disease is located in the blood vessels, which is a syndrome of deficiency and deficiency of the underlying condition, deficiency of the liver, spleen, and kidney are the roots, the deficiency of the kidney is the mainstay, and turbid phlegm and blood stasis are the indicators. Clinical treatment often starts with kidney deficiency and blood stasis. The treatment is suitable for warming the kidney and removing turbidity, and promoting blood circulation to clear the pulse. Combination of Chinese medicines in Jiangzhi Capsule, it can warm the kidney and remove turbidity without injuring the yin, promote blood circulation and dredge the collaterals without breaking the blood.

5 CONCLUSION

Hyperlipidemia is a disorder of lipid metabolism and an important risk factor for atherosclerosis. It can lead to atherosclerotic diseases such as coronary heart disease, cerebral infarction and peripheral vascular disease, as well as fatty liver and acute pancreatitis. The combination of all the medicines in this prescription can warm the kidney and dissolve turbidity without injuring the yin. It can also promote blood circulation and dredge the collaterals without breaking the blood. As China enters a well-off society in an all-round way, the improvement of living conditions and the acceleration of the pace of life have brought about changes in the dietary structure, and the population with hyperlipidemia tends to be younger. In order to facilitate the patient's use, the research team explored well-known traditional Chinese medicine prescriptions. According to the Chinese Medicine Law of the People's Republic of China, the relevant requirements of medical institutions apply traditional techniques to configure traditional Chinese medicine preparations. A controllable technical process route was established and the internal control agent standard was formulated with reference to the Chinese Pharmacopoeia. The internal control standard of this preparation, through microscopic identification items, thin-layer identification items, and HPLC determination of the effective ingredient content of the main drugs in the preparation, comprehensively controls the quality of the preparation, and successfully completes the preparation of the preparation work. In this study, the traditional decoction is changed into capsules, the technological parameters are formulated, and the quality control indicators are increased. It has made useful explorations for the protection and discovery of the proven prescriptions and the promotion of the modernization of Chinese medicine.

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