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Preparation, Solubilization and Content Determination of Silibinin-protamine Complex

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Abstract

With the increase in alcohol production and the drinking population, the incidence of alcoholic liver disease has risen rapidly, and it has become the second largest liver disease after viral hepatitis. This grim situation poses a serious threat to human health. Silibinin has biological functions such as protecting the liver from damage, improving kidney function, inhibiting myocardial damage, and so on. It has strong antioxidant activity and can effectively eliminate free radicals in the human body. However, its poor water solubility leads to low bioavailability, which limits its application in clinical medication to some extent. In this study, the solubility of silibinin in protamine was improved using solid dispersion technology, and its content was determined by high-performance liquid chromatography, in order to provide a new method for the research and development of silibinin drugs to improve bioavailability.

Keywords

Silibinin; Alcoholic liver disease; Protamine; Improve bioavailability.

1. INTRODUCTION

Silibinin is a natural flavonoid, which is extracted and separated from the seeds of milk thistle[1][2][3]. In clinical practice, silibinin is used as a traditional medicine to treat liver diseases. At present, alcoholic liver disease has become the main cause of liver injury[4]. In recent years, with the in-depth exploration of its characteristics and components, its functions of antioxidation, scavenging free radicals, regulating lipid and sugar metabolism, anti-tumor, neurotoxic immunity, anti-photoaging and other pharmacological effects have been gradually confirmed. However, its poor water solubility leads to low bioavailability, which limits its application in clinical medicine to some extent[5,6,7]. The solubility of silibinin in protamine was improved by solid dispersion technology, and the solubility of silibinin in protamine at different concentrations was compared, and the content was determined by HPLC, in order to provide a reference for the research and development of silibinin drugs to improve bioavailability.

2. METHODS

2.1. Solubilization experiment of silibinin

2.1.1 Samples Preparation

Weigh 5.0mg of silibinin and dissolve it in 50μL DMSO, and divide the solution into 10 equal portions, each containing 5μL of DMSO solution with 0.5mg of silibinin.

2.1.2 Solution Preparation

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Weigh protamine powder and prepare solutions of 10.0mg/mL, 11.0mg/mL, 12.0mg/mL, 15.0mg/mL, 20.0mg/mL, 50.0mg/mL, 80.0mg/mL, and 100.0mg/mL by adding an appropriate amount of water to each concentration.

2.1.3 Silibinin-protamine complex

2.0mL of water and 2.0mL of protamine solutions with different concentrations were added to the control group and the test sample respectively. Heat and dissolve, stir the mixture well, and observe the color and sedimentation. In the test group, 1.0mL supernatant was centrifuged to observe the color, and the color gradually became transparent until it was clear.

2.2. Determination of silibinin by HPLC

2.2.1 Silibinin-protamine complex preparation

Accurately weigh 24mg of silibinin, put it in a 10.0mL volumetric flask, add methanol for ultrasonic dissolution and dilute it to mark, and shake it evenly to obtain reference standard. Weigh 0.5mg of silibinin and prepare 10.0mg/mL, 50.0mg/mL and 80.0mg/mL solutions of protamine respectively by adding 2.0mL of water to each. Shake for 24h, centrifuge to obtain the supernatant, and add methanol to mark, shake well, filter, and take the continuous filtrate as the test samples.

2.2.2 Chromatographic conditions and system suitability test

Chromatographic column: XDB-C18(250mm×4.6mm, 5μm); Mobile phase: methanol-1% glacial acetic acid aqueous solution (51：49, V/V), flow rate: 1.0mL/min；Column temperature: 35℃; Detection wavelength: 290 nm; Sample volume: 5.0μL. Accurately suck 5.0μL of blank solvent (methanol), reference solution and test sample for injection. Result: the theoretical plate number is not less than 5000 for silibinin, and the resolution is good.

2.2.3 Dilution linearity

Take an appropriate amount of silibinin and dilute it to prepare a series of standard solutions with concentrations of 0.012, 0.024, 0.048, 0.096, 0.120, 0.240 and 0.480mg/mL respectively, take 20.0μL for injection and record the peak area. See Table 1. With the silibinin concentration (c, mg/mL) as the abscissa and the peak areas sum of silibinin (y) as the ordinate, the working curve is plotted, as shown in Figure 1, and the regression equation y = 32114x+578.49 is obtained. The results show that the linear range of silibinin detection concentration is 0.012 ~ 0.240mg/mL.

Table 1. Results of silibinin standard

Concentration (mg/mL)	0.012	0.024	0.048	0.096	0.120	0.240
Peak area	512.09161	1286.49146	2286.24115	3998.39044	4733.15051	7996.28040

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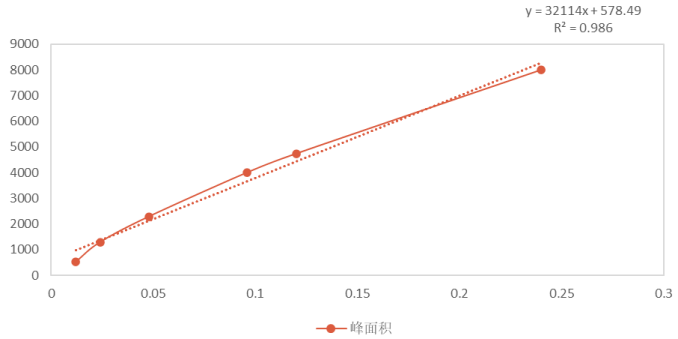

Figure 1. Standard curve of silibinin

3. RESULTS AND DISCUSSION

3.1. Solubilization of silibinin

3.1.1 Test sample

As shown in Figure 2, weigh 5.0mg of silibinin and dissolve it in 50.0μL of DMSO. Once the dissolution is complete, divide the solution into portions containing 0.5mg of silibinin and 5.0μL of DMSO per serving, and use these as the test sample for later testing.

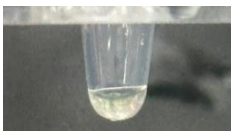

Figure 2. Silibinin is completely dissolved in DMSO

3.1.2 Samples

As shown in Figure 3, each tube contains 0.5mg of silibinin and 5.0μL of DMSO. As water and protamine solutions of 10.0mg/mL, 20.0mg/mL, 50.0mg/mL, 80.0mg/mL and 100.0mg/mL are successively added, the color gradually becomes more transparent and clear, and the precipitation gradually decreases (it becomes transparent after shaking with the 80.0mg/mL solution added).

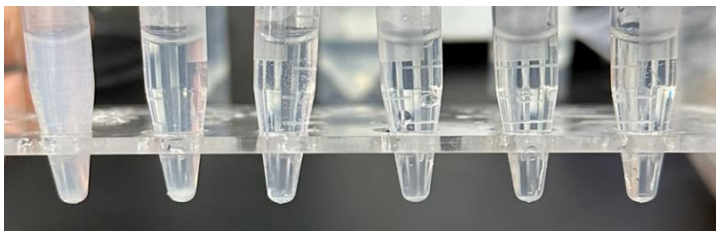

Figure 3. Samples containing different concentrations of protamine solutions

As shown in Figure 4, after being left standing for a period of time, except for the samples with water and 10mg/mL protamine, the powder remained on the wall and dissolved incompletely. The protamine solutions of 20.0mg/mL, 50.0mg/mL, 80.0mg/mL and 100.0mg/mL, however, were clear and transparent.

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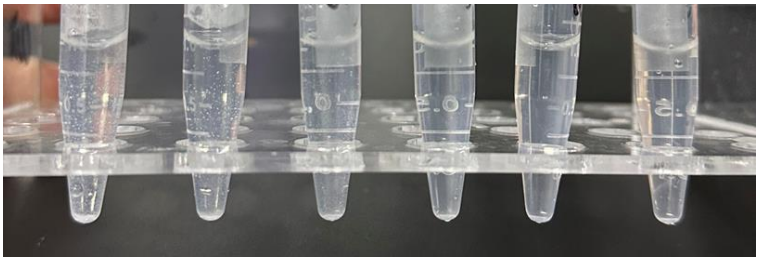

Figure 4. Samples after standing for a while

As shown in Figure 5, the solution with 10.0mg/mL protamine only slightly clung to the wall, so the scope was further narrowed and the best dissolution concentration of protamine suitable for the test sample was screened. We added 10.0mg/mL, 11.0mg/mL, 12.0mg/mL, 15.0mg/mL, 20.0mg/mL protamine solutions into the mixture of 0.5mg silibinin and 5.0μL DMSO in turn, and found that the 12.0mg/mL protamine solution was clear and transparent. Therefore, and the optimal concentration was finally selected.

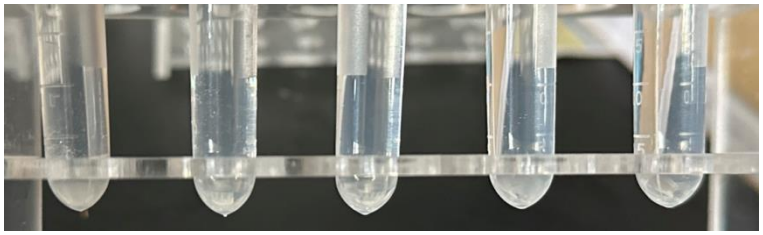

Figure 5. Further screening of suitable protamine concentration

3.2. Determination of the content of the test sample by HPLC

Under the same conditions, content determination was carried out. Test samples prepared with silibinin dissolved in water, 10.0mg/mL, 50.0mg/mL, and 80.0mg/mL protamine solutions were diluted with methanol to prepare stock solutions. The peak areas measured at 290nm were 151.05934, 6629.53564, 3.72E+04 and 8458.13281. Only the sample dissolved in 10.0mg/mL protamine solution met the linear range requirements.

4. CONCLUSION

The solubility of silibinin in protamine solutions with different concentrations showed a gradual increasing trend. From the phenomenon of powder hanging on the wall in the initial water and 10mg/mL protamine solution to the clarity and transparency of 20mg/mL, 50mg/mL, 80mg/mL and 100mg/mL protamine solutions, this change indicates that the increase of protamine concentration significantly improves the solubility of silibinin. The experiment further tested the range from 10mg/mL to 20mg/mL and finally chose 12mg/mL protamine as the better dissolution concentration. In order to accurately determine the content of silibinin at different concentrations, we used HPLC to detect the samples prepared with protamine solutions of 10mg/mL, 50mg/mL and 80mg/mL at the wavelength of 290nm. The results showed that the sample peak area of silibinin was in a linear range only in 10mg/mL protamine solution, which was consistent with the observed clear and transparent solution. It was necessary to further detect the peak area of the sample prepared with 12mg/mL protamine using HPLC in order to confirm that the solubility of silibinin was the best at this concentration.

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