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Determination of the Content of Hydroxyl Safflor Yellow A in Mongolian Medicine Yihahari-12

Wei Li[1], Chao Yu[1], Jing Yan[2], Zhenjie Fei[1]
[1]Xilin Gol League Inspection and Testing Center, Xilinhot 026000, Inner Mongolia, China
[2]Baotou Inspection and Testing Center, Baotou 014000, Inner Mongolia, China

Abstract: *Objective:* To establish a method for determining the content of Mongolian medicine Yihahari-12 by establishing a high-performance liquid chromatography analysis method. *Method:* High-performance liquid chromatography was used to determine the content of hydroxysafflor yellow pigment A in Ihrhari-12. The specific experimental conditions were as follows: a chromatographic column with an inner diameter of 4.6 mm, a length of 250 mm, and a particle size of 5 μm was used as a filler, and octadecylsilane bonded silica gel was used; The column temperature is 35℃; The mobile phase is methanol acetonitrile (ratio of 26:74); The detection wavelength is set to 403 nm, the flow rate is 1 mL/min, and the injection volume is 20 μL. The linear relationship investigation shows that hydroxysaffron yellow pigment A exhibits a good linear relationship within the concentration range of 0.02 mg/mL to 0.10 mg/mL. The regression equation is Y=-0.000631+0.0x, with an R value of 0.9999; The average recovery rate is 93.66%. As a result, this content determination method for hydroxysaffron yellow pigment A has the characteristics of good stability, good repeatability, strong specificity, and convenience and speed.

Keywords: Mongolian medicine Yihahari-12; High-performance liquid chromatography method; Quality standard

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1. Preface

Mongolian medicine Yihahari-12 is a powdered medicine composed of 12 ingredients, including black borneol, Gansong, Tumuoxiang, *Gardenia jasminoides*, safflower, and *Terminalia chebula*. It is derived from the book "The Joy of the Observer." This formula is cool in nature and primarily treats "Xier" diseases, symptoms such as yellow eyes and skin, plague, plague affecting the stomach, thirst and irritability, indigestion, etc. [1]. It has the effect of clearing "Xier" heat and is effective for various heat syndromes in the stomach, liver, and gallbladder [2].

"Xier" is a Mongolian term that literally translates to "yellow. "In Mongolian medicine, it refers to Xier, which is also commonly translated as Shila, Dan, or Bile. The basic theory of Mongolian medicine compares the human body structure to nature. The Five Elements theory is based on air, fire, earth, water, and space, which are established on the foundation of ancient naive materialism. In this theory, "Xier" is classified as fire, with the function of providing thermal energy to the body, promoting digestion, and enhancing appetite; Xier enables vision and moistens the skin; it imparts energy and wisdom, making people decisive, courageous, and resourceful. There is no disagreement about the location of Xier's residence, which is located between the navel and the heart [3]. The residences of the various Xiers are in the liver, gallbladder, sweat glands,

lymphatic system, blood, stomach, etc. The residence of Xier disease factors is in the liver and gallbladder.

Mongolian medicine believes that the causes of diseases can be summarized into six types, with "Xie Ri" (heat) being the cause of all heat-related diseases. However, under normal circumstances, the three roots are only potential factors. When the body's self-regulatory function is exceeded by external influences, it leads to dysfunction and disorder, resulting in symptoms. External factors can generally be divided into four categories: lifestyle, diet, climate, and others, including injuries, microbial invasion, etc. The treatment of diseases in Mongolian medicine is based on the principle of syndrome differentiation and treatment, utilizing the 17 types of efficacy of Mongolian medicine to balance and adjust the internal three roots, restoring them to equilibrium, thereby achieving the goal of preventing and treating diseases. The description of yellowing of the eyes and skin mainly manifests as jaundice, characterized by yellowing of the body, eyes, and urine, with yellowing of the eyes being a significant feature. Jaundice is a common disease of the hepatobiliary system. Internal injury from diet, external damp-heat, and blood stasis in the body are the main causes of jaundice, which leads to the overflow of bile from the liver and gallbladder, obstruction of qi movement, and abnormal dispersion.

Safflower (*Carthamus tinctorius L.*), a herbaceous plant belonging to the genus Carthamus in the family Asteraceae, is typically annual. It is also known as Cao Honghua, Honglanhua, or Cihonghua. The flowers are harvested in summer when they turn from yellow to red and are processed by drying in the shade or sun. Medicinal safflower is listed in the Chinese Pharmacopoeia, with functions and indications including promoting blood circulation and removing blood stasis, alleviating pain, and being used for amenorrhea, dysmenorrhea, incomplete lochia, abdominal mass and pain, chest obstruction and heart pain, abdominal pain due to blood stasis, sharp pain in the chest and ribs, injuries from falls, and sore and swollen sores [4]. It is an important active ingredient in this preparation.

From the perspective of pharmacological effects, safflower can dilate blood vessels, improve microcirculation; resist arrhythmia, lower blood pressure; reduce inflammation, enhance immune function; resist allergies; have anti-aging and anti-tumor effects; be used clinically for the prevention and treatment of neurological diseases, promoting blood circulation, improving blood circulation, improving sleep, etc. [5]; improve liver function, and is widely used in the treatment of angina pectoris, stroke, anemia, coronary heart disease, etc. [6].

Safflower mainly contains various active ingredients such as flavonoids, fatty acids, pigments, phenolic acids, and volatile oils [7]. The pharmacological effect of safflower is attributed to its flavonoid component, safflower yellow (SY).

The component with a higher content of safflower yellow pigment is hydroxysafflor yellow A. From the perspective of pharmacological effects, hydroxysafflor yellow A can reduce neuronal cell apoptosis and exert antioxidant effects. Jin Ming et al. found through experimental research that hydroxysafflor yellow A can dose-dependently scavenge hydroxyl radicals and has various antioxidant effects, such as inhibiting erythrocyte membrane rupture and suppressing lipid peroxidation in mouse liver homogenates [8]. Hydroxysafflor yellow A can intervene in atherosclerosis by lowering blood lipids and exerting antioxidant effects, and has vasoconstrictive effects [9].

Currently, reports on the determination of the content of Yihazhi preparations mainly focus on components such as gardenia, with few reports on safflower [10–11]. This situation may lead to insufficient comprehensive quality control in terms of reliability and pharmacodynamic stability of Yihahari-12. High-precision detection methods can provide detailed chromatographic data, revealing the absorption characteristics of hydroxysafflor yellow A at specific wavelengths. The use of high-performance liquid chromatography (HPLC) to detect hydroxysafflor yellow A is currently a key step in studying its content. Experimental results show that hydroxysafflor yellow A exhibits a significant maximum absorption peak near 403 nm, indicating that its light absorption capacity reaches its peak at this specific wavelength [12]. This unique absorption characteristic can be used as an effective means to determine its content.

2. Materials, methods, and results

2.1. Materials and methods

2.1.1. Instrument

Digital ultrasonic cleaner: Kunshan Ultrasound KQ-500DE

High-performance liquid chromatograph: Shimadzu LC-2030C

Electronic balance: Sartorius SQP

Electronic balance: Precisa XT220A

Digital display thermostatic water bath: Changzhou Guohua HH-8

2.1.2. Reagents and chemicals

Hydroxysafflor yellow A: Batch No. 111637-202111, National Institute for Food and Drug Control, China

Safflower reference material: Batch No. 120907-200006 National Institute for Food and Drug Control, China

Yihahari-12: Batch No.2301052, 2207251, and 2412081, Xilin Gol League Mongolian Medicine Hospital

Methanol and acetonitrile (chromatographically pure): Chengdu Xijiarui Technology Co., Ltd.

Methanol (analytical grade): Tianjin Pharmaceutical Company

2.2. Method: Assay

2.2.1. Chromatographic conditions

Chromatographic column: packed with octadecylsilane chemically bonded silica gel;

Mobile phase: methanol-acetonitrile (26:74) as the mobile phase; flow rate at 1.0 mL/min; detection wavelength at 403 nm; column temperature at 35℃; injection volume at 20 μL.

2.2.2. Preparation of control solution

Accurately weigh approximately 12 mg of hydroxysafflor yellow A reference substance, place it into a 250 ml volumetric flask, and add 25% methanol to dilute to volume, preparing a solution of 0.045 mg/mL as the reference solution.

2.2.3. Preparation of test solution

Accurately weigh approximately 1 g of Yihahari-12 sample and place it into a 100 mL conical flask with a stopper. Add 25 mL of 25% methanol solution and record the initial weight. Seal the conical flask tightly and place it in an ultrasonic extractor for 40 minutes. After the ultrasonic extraction is complete, remove the conical flask and allow it to cool to room temperature. Make up the weight of the solvent lost due to evaporation. Mix the extract thoroughly and filter it through filter paper. Collect the filtrate as the test solution.

2.2.4. Preparation of negative samples

Take 11 types of medicinal materials other than safflower from Yihahari-12, grind them into powder, weigh them, and mix them thoroughly according to the proportions specified in the formula to prepare the negative sample of Yihahari-12.

2.3. Results

2.3.1. Specificity

Weigh 1 g of the negative sample prepared according to “2.2.4” and place it into a conical flask. Prepare a negative sample solution according to “2.2.3.” After analysis under “2.2.1.”, chromatography analysis shows that a chromatographic peak is detected at the characteristic retention time of the hydroxysafflor yellow A reference substance in the test solution, while no interfering peak is observed in the negative control within this retention time interval. This phenomenon can fully demonstrate that the sample exhibits good specificity under this analytical method.

2.3.2. Investigation of linear relationship

Using the method described under "2.2.2." to prepare the control solution, standard solutions with concentrations of 0.02, 0.04, 0.06, 0.08, and 0. 10 mg/mL were injected for determination. The chromatograms corresponding to each concentration based on the peak area were recorded, and a linear regression analysis was performed on the peak area (X) versus mass concentration (Y, mg/mL). The results showed peak areas of 1532396, 3022403, 4531785, 6019570, and 7486033, respectively. The regression line equation was Y=-0.000631+0.0X, with an R value of 0.9999. It is evident that hydroxysafflor yellow A exhibits a good linear relationship within the concentration range of 0.02 mg/mL to 0.10 mg/mL.

2.3.3. Stability test

Take the same batch of test samples with known content, prepare the test solution, and conduct measurements every 4 hours starting from 0 hours, with a total of 7 measurements until the end of 24 hours. The results show that the peak areas of hydroxysafflor yellow A are 1649151, 1646763, 1648859, 1700623, 1650172, 1646963, and 1646038, respectively, with an RSD of 1. 20%. There is no significant change in the peak areas, indicating that the test solution is stable within 24 hours.

2.3.4. Repeatability test

Accurately weigh 6 samples of 1 g each from the same batch with known content, and prepare the test solution for each sample according to the standard method. Analyze under the chromatographic conditions specified in "2.2.1.", and record the chromatographic peak area data of hydroxysafflor yellow A. The experimental results indicate that the average content of hydroxysafflor yellow A is 0.022 mg/g, with a relative standard deviation (RSD) of 3.53%, indicating that the method has good repeatability and reliability (**Table 1**).

Table 1. Repetitive test

Serial number	Weight (g)	Peak area	Content (mg/g)	Average content (mg/g)	RSD (%)
1	1.0057	1781175	0.023	0.022	3.53
2	1.0047	1690570	0.022		
3	1.0046	1750032	0.023		
4	1.0022	1677399	0.023		
5	1.0001	1623453	0.021		
6	1.0020	1649706	0.021		

2.3.5. Sample recovery rate test

To evaluate the accuracy of the analytical method, approximately 1 g of samples with known content from the same batch were precisely weighed and divided into three portions. These samples were placed in conical flasks with stoppers, and the test solution was prepared according to the method described in "2.2.2." Additionally, 1mL of the reference solution (with a concentration of 0.016 mg/mL, equivalent to 80% of the target component content in the test sample) was added. The determination was conducted under the chromatographic conditions specified in "2.2.1." The results indicated that the average recovery rate of hydroxysafflor yellow A was 93. 66%, with a relative standard deviation (RSD) of 0.95%. These findings demonstrate that the accuracy of this method meets the required standards (**Table 2**).

Table 2. Sample addition recovery test

Serial number	Weight (g)	Standard addition amount (mg)	Content (mg/g)	Recovery rate (%)	Average (%)	RSD (%)
1	1. 0041	0. 016	0. 01739	92		
2	1. 0028	0. 016	0. 01723	93	93. 66	0. 95
3	1. 0007	0. 016	0. 01667	96		

2.3.6. Durability test

After replacing the chromatographic column, three samples of Yihahari-12 with known content from the same batch were taken, and approximately 1 g of each was precisely weighed and used to prepare the test solution. The peak areas of hydroxyl safflor yellow A determined under the chromatographic conditions of "2.2.1." were 1725557, 1688207, and1753349, respectively, with an RSD of 1.89%. This indicates that the chromatographic columns of the same type from different manufacturers do not significantly affect the determination of hydroxyl safflor yellow A content.

3. Discussion and outlook

Mongolian medicine Yihahari-12 is a commonly used medication in Mongolian medicine. However, there is currently no established quality control method for the safflower component in Yihahari-12 preparations in the current drug standards and related literature. The determination results using high-performance liquid chromatography (HPLC) show that hydroxysafflor yellow A has a good linear relationship, which can be detected within the range of 0. 02 μg/ml to 0. 10 μg/ml; the average recovery rate is 93.66%; the relative standard deviation (RSD) in stability tests is 1.20%; the RSD in repeatability tests is 3.53%, indicating good robustness. This method has good reproducibility, high sensitivity, and is simple and rapid. In summary, a testing method for the content of Mongolian medicine Yihahari-12 has been successfully established, providing a basis for product quality control. Although this study has established a testing method for the content of Mongolian medicine Yihahari-12, it still needs to be continuously optimized and validated in subsequent research, in order to provide a more comprehensive solution for the quality control of ethnic medicine. Mongolian medicine has minimal side effects and great development potential. The increasing research on Mongolian medicine in recent years also indicates its broad prospects. Therefore, the quality standards for Mongolian medicine also need to be emphasized and developed, so that the development and production of Mongolian medicine can keep pace with modernization.

Disclosure statement

The authors declare no conflict of interest.

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