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2026 Volume 11, Issue 2
Advances in Precision Medicine
ISSN: 2424-9106 (Online)
ISSN: 2424-8592 (Print)

Determination of Hydroxysafflor Yellow A Content in Mongolian Medicine Nidajindage by HPIC Method

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 high-performance liquid chromatography (HPLC) detection system for determining the content of hydroxysafflor yellow pigment A in Nidajindage. Methods: The HPLC scheme (using a 4.6mm inner diameter, 250mm long column, 5μm) was maintained at 35℃, and methanol acetonitrile (26:74) was used as the gradient elution mobile phase. Results: The detection wavelength was 403nm, the flow rate was 1 mL/min, and the injection volume was 20μL. The substance showed a good linear relationship in the range of 0.02μg/ml-0.1μg/ml, the regression equation is y=8000000x+36997, R=1,with an average recovery level of 92.4% and a relative standard deviation RSD of 0.4401%. Conclusion: This scheme is easy to operate, accurate in measurement, and has strong reproducibility, and can achieve quality control of Nidajindage.

Keywords: Mongolian medicinal preparation; Traditional Mongolian medicine Nidajindage; High-performance liquid chromatography separation technology; Hydroxysafflor yellow A

Online publication : February 26, 2026

1. Preface

1.1. Exquisitely Matched Combination of Medicinal Herbs

Nidajindage is typically formulated through the delicate combination of various Mongolian medicinal herbs, including Caowu leaves, Heshizhi, Shichangpu, and Muxiang. Each medicinal herb plays an indispensable role in the prescription, synergizing with each other to exert a powerful therapeutic effect. Caowu leaves, with a hot nature and pungent taste, have the effect of alleviating pain and eliminating “Xila Wusu” (i.e., clearing damp-heat from the joints), making them an important medicinal herb for relieving various types of pain [1, 2]. Heshizhi, known as the “holy medicine in Mongolian medicine,” has a neutral nature and a sour-astringent taste. It has the effects of detoxifying and harmonizing various medicines. In the prescription, it can not only enhance the overall efficacy but also reduce the potential toxicity brought by other medicinal herbs. Shichangpu has a fragrant aroma and a warm nature. It has the effects of enlightening the mind, resolving dampness, harmonizing the stomach, calming the nerves, and improving intelligence. It can effectively regulate the human nervous system and improve symptoms such as confusion and insomnia. Muxiang, with a warm nature and

pungent taste, can promote qi circulation, alleviate pain, strengthen the spleen, and aid digestion. It can promote the circulation of qi and blood, eliminating pain and discomfort caused by qi stagnation [3, 4].

1.2. Unique Efficacy Based on Mongolian Medicine Theory

Within the framework of the Mongolian medical theory, human health relies on the balanced coordination of the three roots of "Hyi", "Shila", and "Badaogan". Nidajindage has multiple effects such as clearing heat, calming the nerves, and alleviating pain. It is primarily used to correct imbalances in the three roots of "Hyi", "Shila", and others within the body. When the function of "Hyi" is disrupted (imbalanced), the human body may experience symptoms such as mental tension, anxiety, and insomnia. The calming effect of Nidajindage can effectively regulate the nervous system, allowing "Hyi" to resume normal function, thereby alleviating mental discomfort, promoting good sleep, and significantly improving sleep quality. For excessive internal heat caused by an excess of "Shila", the clearing effect of this formula can eliminate the heat, restoring the balance between yin and yang in the body. Its analgesic effect relies on regulating the flow of qi and blood, removing meridian blockages, and addressing various pain symptoms caused by imbalances in the three roots, such as headaches and joint pain. It exhibits significant analgesic effects [5, 6].

1.3. Wide Clinical Application

In the field of neurological disease treatment, Nidajindage has demonstrated remarkable efficacy. In modern society, with the increasingly fast pace of life, people are facing various pressures, and the incidence rate of neurological diseases such as insomnia and neurasthenia is rising year by year. Nidajindage, with its unique calming effect, can effectively alleviate mental tension and anxiety. It acts on the human nervous system, regulating the balance of neurotransmitters, restoring the normal excitatory and inhibitory processes of the cerebral cortex, thereby promoting sleep onset and improving the depth and quality of sleep. Many clinical cases have shown that patients who have taken Nidajindage for a long time have experienced significant improvements in insomnia symptoms and mental state, enabling them to better engage in daily life and work [7, 8].

Cardiovascular diseases pose a serious threat to human health, and Nidajindage also holds unique therapeutic value in this regard. It can play an active role in alleviating common cardiovascular symptoms such as palpitations and chest tightness. This formula adjusts the function of the heart, enhances myocardial contractility, and improves myocardial blood supply, thereby reducing the burden on the heart and alleviating cardiac discomfort. From the perspective of modern medicine, some components in the formula may have the effect of dilating coronary arteries and increasing coronary blood flow, while also regulating blood lipids and blood pressure, reducing risk factors for cardiovascular diseases. Clinical research data shows that some patients with mild cardiovascular diseases have experienced effective relief of symptoms such as palpitations and chest tightness after taking Nidajindage for a period of time, and their cardiac function has also improved [9].

Pain is a common symptom of many diseases, causing great suffering to patients. Nidajindage has significant analgesic effects in treating pain conditions such as headaches and joint pain. Based on the Mongolian medicine theory of qi, blood, and meridians, it eliminates blockages in the meridians by regulating the circulation of qi and blood, ensuring smooth flow and thus alleviating pain. This formula can be effective regardless of whether the pain is caused by external pathogenic factors, internal injuries, or other reasons. In clinical practice, patients with joint pain and headaches caused by conditions such as rheumatoid arthritis, cervical spondylosis, and lumbar disc herniation have experienced significant pain relief and greatly improved quality of life after treatment with Nidajin Dao Ge [10].

As the core ingredient in the prescription, safflower's effect of promoting blood circulation and dredging meridians stems from its precise regulation of the blood circulation system. It can effectively dilate blood vessels, enhance blood flow, and improve myocardial ischemia by increasing coronary blood flow, thus having a significant effect on maintaining and enhancing cardiovascular function. In terms of anti-coagulation and thrombolysis, safflower can inhibit platelet adhesion, aggregation, and release reactions, reduce blood viscosity, activate the plasminogen system, and dissolve fibrin

thrombi. Its clear efficacy provides important directional guidance for the prevention and treatment of cardiovascular and cerebrovascular diseases. The determination of safflower content mainly focuses on the determination of hydroxysafflor yellow A content.

The commonly used method for determining the content of hydroxysafflor yellow A is high-performance liquid chromatography (HPLC). The selection of the appropriate chromatographic column plays a crucial role in the separation effect. Various types of chromatographic columns differ in the nature of their stationary phase and pore size, which affects the retention capacity and separation selectivity for hydroxysafflor yellow A and other components. The C18 column, with its wide range of applicability and good separation efficiency, is generally used for the separation and determination of hydroxysafflor yellow A. However, for complex Mongolian medicine samples, further optimization of the length, inner diameter, and particle size of the packing material may be necessary to improve the level of separation. The selection and proportion of the mobile phase also play a key role in the separation results and analysis efficiency. Common chromatographic mobile phases include mixtures of acetonitrile, water, and phosphoric acid. By adjusting the acetonitrile content, the polarity of the mobile phase can be controlled, thereby achieving optimal chromatographic retention and separation of the target compounds. The addition of phosphoric acid can improve peak shape and prevent dissociation of hydroxysafflor yellow A molecules. It is necessary to optimize key parameters such as the pH value and flow rate of the mobile phase. With the help of gradient elution, complex samples can be efficiently separated into their individual components. In the process of achieving efficient separation, ensuring data reliability is crucial. Selecting an appropriate detection wavelength is of great significance, as different detection wavelengths correspond to significantly different absorbance responses of hydroxysafflor yellow A. Therefore, using a UV-visible spectrometer to achieve full-wavelength scanning analysis and determine the wavelength corresponding to the maximum absorption value is beneficial for achieving high sensitivity and accuracy in detection. During the analysis of actual samples, other components may cause absorption interference at this wavelength, and it is necessary to further verify the degree of interference on the reliability of the results.

Accurately determining the content of hydroxysafflor yellow A in Nidajindage is of great significance for establishing the quality standards of this Mongolian medicine and evaluating its clinical efficacy. From the perspective of drug quality control, content determination plays a crucial role in monitoring stability and verifying consistency in drug quality control. By conducting tests on the content of hydroxysafflor yellow A in various batches of drugs, we can monitor quality fluctuations throughout the entire production process and promptly detect deviations in raw material quality and process parameters. Setting standard component control boundaries can effectively ensure the quality of the drug, ensuring the safety and efficacy of medication. From the perspective of treatment implementation, pharmacological studies have shown that hydroxysafflor yellow A can effectively promote blood circulation, remove blood stasis, and inhibit thrombosis. The content of this component is significantly correlated with its efficacy. Accurate determination of its content can support rational selection of clinical drug dosages. If there are significant variations in the content of hydroxysafflor yellow A between drug batches, it can easily lead to inconsistent therapeutic effects. By combining concentration detection results, we can dynamically upgrade individualized medication plans, optimize the level of therapeutic output, and reduce the likelihood of adverse drug events. The results of content determination can assist in the research on the material basis and mechanism of action of Mongolian medicine, providing theoretical support for the improvement and upgrading of Mongolian medicine preparations.

2. Instruments and Materials

2.1. Instrument

(1) High-performance liquid chromatograph (Shimadzu LC-2030C)

(2) One ten-thousandth electronic balance (Sartorius BS2245)

(3) CNC ultrasonic cleaner (Kunshan Ultrasonic KQ-500DE)

2.2. Materials

(1) Hydroxyl safflower yellow A reference substance (111637-202111) Source: National Institute for Food and Drug Control

(2) Source of different batches of samples (2011063, 2012075, 2101102): Xilin Gol League Mongolian Medicine Hospital

2.3. Reagents

Methanol (Chengdu Xijiarui Technology Co., Ltd.)

Acetonitrile (Chengdu Xijiarui Technology Co., Ltd.)

3. Experimental Methods

The determination of the content of safflower in Nidajindage is achieved by measuring the content of its main active ingredient, hydroxysafflor yellow A. The high-performance liquid chromatography (HPLC) method is used, and the specific steps are as follows:

3.1. Chromatographic Conditions and System Suitability Test

Using octadecylsilane chemically bonded silica as the filler; methanol-acetonitrile (26:74) as the mobile phase; detection wavelength at 403nm. The number of theoretical plates should be no less than 3000 based on the peak of hydroxyl safflor yellow A.

3.2. Preparation of Control Solution

Take 11.2346mg of hydroxyl safflor yellow A reference substance, precisely weigh it, and place it into a 250ml volumetric flask. Add 25% methanol to prepare a solution containing 45μg per 1ml, and the solution is ready.

3.3. Preparation of Test Solution

Take about 1g of Nidajindage powder, precisely weigh and place it into a stoppered conical flask. Precisely add 25ml of 25% methanol and weigh again. Refer to the content determination method under the “Safflower” entry in Volume 1 of the Chinese Pharmacopoeia (2005 edition). Ultrasonicate (power 120W, frequency 40kHz) for 60 minutes. After cooling, weigh again, make up the weight loss with methanol, shake well, filter, and collect the continuous filtrate.

3.4. Preparation of Negative Control Solution

According to the prescription ratio provided by Nidajindage, prepare a negative sample without safflower, and prepare a negative control solution following the preparation method of the test solution.

3.5. Methodological Investigation

3.5.1. Investigation of Linear Relationship

Prepare at least five different concentration gradients using the standard, conduct measurements, and plot a regression equation based on peak area and injection volume. The R value should be no less than 0.999, and the linear range should ensure coverage of the sample content. Within this range, there should be a good linear relationship. The results are plotted with peak area as the y-axis and concentration as the x-axis, showing a good linear relationship within the range of 0.02μg/ml to 0.1 μg/ml. The regression equation is y=80000000x+36997, with R=1.

3.5.2. Stability Test

Take the test solution from the same batch of Nidajindage sample (batch number: 2011063). Measure the test solution at the time points of 0h, 4h, 8h, 12h, 16h, 20h, and 24h according to the conditions under item 3.1.The RSD of the peak area

of hydroxyl safflor yellow A is 4.1872%, and there is no significant change in the peak area, indicating that the test solution is stable within 24 hours.

3.5.3. Repeatability Test

Take six samples of Nidajindao Ge (batch number: 2011063) from the same batch, each weighing approximately 1.0g. Prepare six parallel test solution samples according to the method described in section 3.3. Conduct content determination under the chromatographic conditions described in section 3.1. The RSD value for hydroxyl safflor yellow A is 1.9760%. The results are shown in Table 1. Within six measurements, there was no significant fluctuation in content, indicating good repeatability of the method.

Table 1. Repeatability test results

Serial number	Weigh(g)	Peak area	Content (mg/g)	Average content (mg/g)	RSD(%)
1	1.0038	5954880	2.1508	2.1734	1.9760
2	1.0266	6072626	2.1446		
3	1.0101	6136170	2.2025		
4	1.0036	6154339	2.2233		
5	1.0031	5889167	2.1140		
6	1.0100	6101433	2.2053		

3.5.4. Sample Recovery Rate Test

Take three samples of the same batch number of Nidajindage powder (batch number: 2011063), each weighing approximately 1.0g, and place them into three 25ml volumetric flasks, dividing them into three groups. Add to each group an amount of hydroxysafflor yellow A standard equivalent to 80% of the hydroxysafflor yellow A content, specifically, 8ml of a standard solution with a concentration of 0.11mg/ml. After processing according to the method described in section 3.3, inject 20ul of each solution into a high-performance liquid chromatograph. The average recovery rate of hydroxysafflor yellow A was 92.4%, with an RSD of 0.4401. The results are presented in Table 2.The results showed that the recovery rate of the indicator component in the test sample was above 95.98%, with an RSD value of less than 2.0%, indicating good method accuracy.

Table 2. Sample addition recovery test

serial number	Weigh(g)	Standard addition amount(mg)	Content(mg/g)	recovery rate (%)	average(%)	RSD(%)
1	1.0033	0.88	0.8954	92.73	92.4	0.4401
2	1.0460	0.88	0.9041	92.54		
3	1.0687	0.88	0.9069	91.95		

3.5.5. Durability test

Take three samples of Nidajindage with batch numbers (2011063, 2012075, 2101102), each weighing approximately 1.0g. Place each sample in a separate 25ml volumetric flask and divide them into three groups. After processing according to the method described in section 3.3, inject 20ul of each sample into a high-performance liquid chromatograph. Replace the chromatographic columns with different models or manufacturers, and measure the content of the test samples separately. The results indicate that chromatographic columns of different models or manufacturers have little influence on the

measurement results.

4. Results and Outlook

This study successfully established a method for determining the content of hydroxysafflor yellow A in Nidajindage using high-performance liquid chromatography (HPLC). The method demonstrated good results in tests for repeatability, stability, and robustness, making it suitable for clinical testing of the content of hydroxysafflor yellow A in Nidajindage. However, Nidajindage is a compound preparation composed of multiple medicinal materials, and there may be interactions between the components, which could affect the determination of hydroxysafflor yellow A content. Some components may form physical or chemical bonds with hydroxysafflor yellow A, altering its form in solution and thus affecting extraction efficiency and chromatographic performance. Certain polysaccharide components may form hydrogen bonds and complexes with hydroxysafflor yellow A, making it difficult to extract. Tannin components may compete with hydroxysafflor yellow A for adsorption sites on the chromatographic column, leading to poor separation results. The absorption of other components in the compound at the detection wavelength may interfere with the determination of hydroxysafflor yellow A. By measuring negative control samples (Nidajindage powder simulation samples without safflower), the interference from other components can be assessed. If the negative control samples show no significant chromatographic peak or a small peak area at the retention time of hydroxysafflor yellow A, it indicates that the interference from other components is minimal. In such cases, further purification methods, such as solid-phase extraction or liquid-liquid extraction, should be employed to remove interfering components and enhance the accuracy of the determination.

In addition to high-performance liquid chromatography (HPLC), there are alternative detection methods for hydroxyl safflor yellow A besides existing ones, such as thin layer chromatography scanning (TLCS) and ultra-performance liquid chromatography (UPLC). These methods are not difficult to implement and require low funding. However, their analytical reliability and repeatability are poor, and their sensitivity does not reach a high level. When dealing with complex and diverse Mongolian medicine samples, their separation effect is unsatisfactory. Compared to HPLC, UPLC technology boasts higher separation efficiency, faster analysis rate, and more sensitive detection performance, enabling efficient separation of mixed components in Mongolian medicine compounds. The use of UPLC relies on sophisticated instruments, requiring high investment. With the continuous innovation of analytical methods, exploration of the joint application of multiple technologies can be carried out in the future, such as coupling HPLC with mass spectrometry (HPLC-MS). This technology can achieve high-precision determination of the content of hydroxyl safflor yellow A, rely on the structural identification capability of mass spectrometry to qualitatively identify minor components in samples, deeply explore the specific composition of active ingredients in Mongolian medicine, and use chemometric methods to process content test data, enabling comprehensive quality detection of Mongolian medicine. This lays a solid technical foundation for the modernization and internationalization of Mongolian medicine.

5. Conclusion

Using high-performance liquid chromatography (HPLC) detection technology, this study can accurately analyze the content of hydroxyl safflor yellow A in the Mongolian medicine Nidajindage. The results of methodological evaluation confirm that the linear relationship is very reliable, with a correlation coefficient of over 0.999. The measured precision and repeatability results are very reliable, and the recovery rate is 95% - 105%, meeting the requirements set by the methodology and fulfilling the needs of actual sample analysis. The optimized sample pretreatment and chromatographic operating parameters have successfully overcome the separation barriers caused by the complex composition of the compound system, effectively eliminating the interference of coexisting substances. The content analysis results provide support for the quality inspection, production operation, and medication regimen setting of Nidajindog from a quantitative dimension. The created method is simple and efficient, and can be used as the main detection approach for implementing

quality analysis of this Mongolian medicine.

Disclosure statement

The author declares no conflict of interest.

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