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Determination of Isomatrine in Traditional Mongolian Medicine Agaru-15 Powder by HPLC

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Abstract: A qualitative and quantitative method for the determination of flavonoids in traditional Mongolian medicine agaru-15 powder was established. C18.6 mm chromatographic column is selected × 250 mm, particle size 5 μm. The mobile phase system is acetonitrile water, gradient elution. The detection wavelength was 295 nm. The range of isomatrine was 0.284–2.84 μg. The regression equation is y = 25.811x - 0.1969, r = 0.99990. The recovery was 91.39% and RSD was 1.42%. All the methods mentioned in this paper have the characteristics of simplicity, accuracy, high sensitivity, good reproducibility and strong durability, which provide a reference for the quality control of agaru-15 powder.
Keywords: Agaru-15 powder; Isomatrine; High performance liquid chromatography; Content determination

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1. Introduction

Agaru-15 powder is a traditional Mongolian medicine preparation widely used in Inner Mongolia. Its efficacy is to calm Qi, heat and viscosity [1]. It is mainly used for Qi and blood asthma, cough, late stage of fever, heart heat, heart Qi and blood headache. It is now included in the specification for Mongolian medicine preparations in Inner Mongolia (2007 Edition) (Volume I) [1]. The prescription of agaru-15 powder is composed of fifteen herbs, based on *Sophora flavescens* function in clearing heat, drying and dampness, supplemented by fourteen other herbs. There are two main types of chemical components in Sophora flavescens, namely alkaloids and flavonoids, which are also the main pharmacologically active components of Sophora flavescens. In the current standard of agaru-15 powder, only the alkaloid components of Sophora flavescens are identified, and the test items of flavonoids contained in Sophora flavescens are not involved at all, which cannot scientifically and objectively reflect the effectiveness of the drug. Therefore, the relevant test items of Sophora flavescens in agaru-15 powder are supplemented [2].

2. Material

Thermofisher Ultimate 3000 high-performance liquid chromatograph, Waters e2695 high-performance liquid chromatograph, Satoris BSA224S electronic balance (d = 0.1mg), Satoris BP211D electronic balance (d = 0.01 mg), Kunshan ultrasonic instrument KQ500DE numerical control ultrasonic cleaning instrument.

Anthocyanin reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 190214, purity: 98.06%), matrine reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 190511, purity: 96.49%), isomatrine reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 190409, purity: 98.95%) Matrinol I reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 190513, purity: 99.74%), Gaolihuaisu reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 19070502, purity: 99.50%) and Clover bean red sandalwood glycoside reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 19080508, purity: 99.75%), five batches of agaru-15 powder samples were from Hohhot Mongolian medicine hospital, Alashan League Mongolian medicine hospital, Xianghuang Banner Mongolian medicine hospital, Xilin Gol League Mongolian medicine hospital and Inner Mongolia International Mongolian medicine hospital [3].

3. Experimental methods and results

3.1. Chromatographic conditions and system suitability test

Chromatographic column: the filler is octadecyl silane-bonded silica gel. Omni Hubble C18 column (SN: 132235) (specification: 250) is used in this experiment × 4.6mm, particle size 5 μm) And thermofisher BDS hypersil C18 column (SN: 12185170jr4) (specification: 250 × 4.6 mm, particle size 5 μm); Gradient elution with acetonitrile water as mobile phase, 0–25min, 40–50% a, 25–30 min, 50–70 A, 30–40 min, 70% a, 40–50min, 70–40% a; Detection wavelength: 295 nm; Flow rate: 1.0 mL/min; The injection volume of standard sample and test sample is the same as 10 μL. Under the above chromatographic conditions, the theoretical plates calculated by the isomatrine peak is not less than 4000, the resolution with adjacent component peaks is greater than 1.5, and the symmetry factor of chromatographic peaks is 0.595–1.05. Typical chromatograms are shown in **Figure 1–Figure 3**.

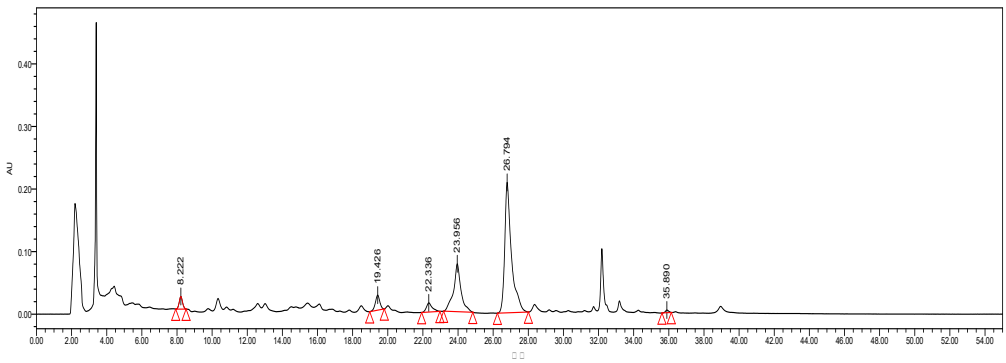

Figure 1. Chromatogram of mixed standard separation effect (the peak sequence is as follows: Sandalwood glycoside of clover bean, anthocyanin of prickly awn stalk, gaolihuaisu, matriol, matrine and isomatrine).

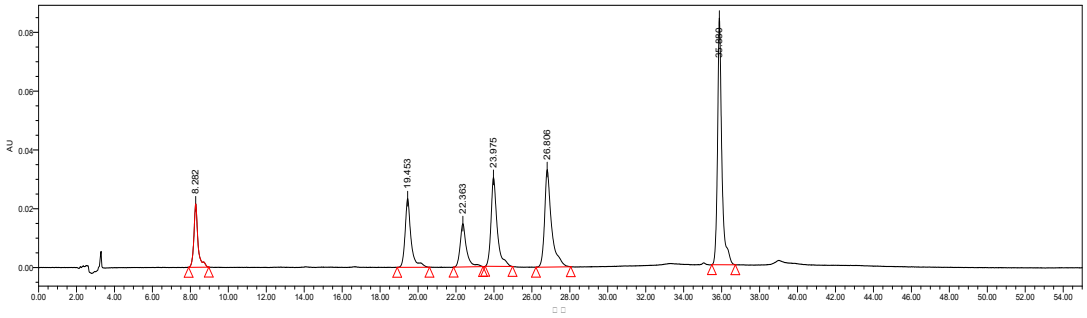

Figure 2. Chromatogram of separation effect of representative samples (the peak sequence is as follows: Sandalwood glycoside of clover bean, anthocyanin of prickly awn stalk, gaolihuaisu, matriol, matrine and isomatrine).

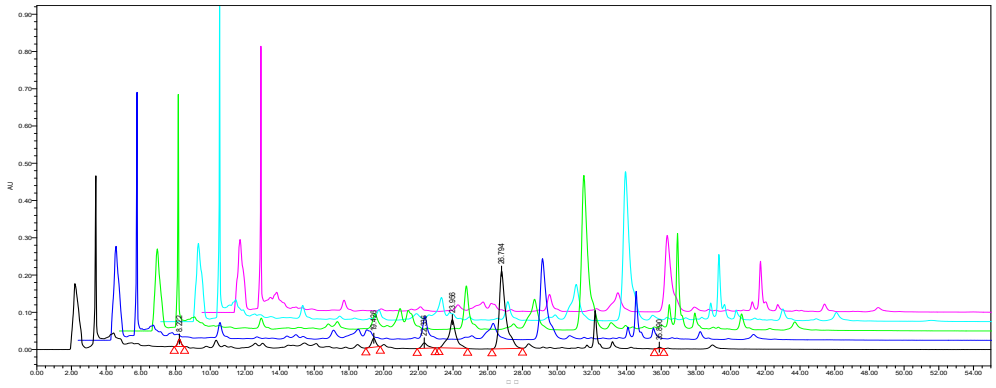

Figure 3. Chromatogram of separation effect of 5 batches of samples (the order of peaks is as follows: Sandalwood glycoside of clover bean, anthocyanin of prickly awn stalk, gaolihuaisu, matriol, matrine and isomatrine).

3.2. Preparation of solution

3.2.1. Preparation of the reference solution

Take a proper amount of the reference substance of anthocyanin, matrine, isomatrine, matriol I, sophoricin and tripterygium rosewood glycoside, accurately weigh, and add methanol to prepare a solution containing 1 mg of isomatrine per 1 mL.

3.2.2. Preparation of test solution

Preparation of test solution: take about 11.0 g of powder (passing No. 3 sieve), weigh accurately, put it into a conical flask with a stopper, precisely add 20 mL of methanol, close the stopper, weigh the weight, conduct ultrasonic treatment (power 250 W, frequency 33 kHz) for 60 minutes, cool, weigh again, make up the lost weight with methanol, shake well, filter, and take the continuous filtrate [4].

3.2.3. Negative control test

Prepare the negative control without Sophora flavescens in strict accordance with the prescription of agaru-15 powder, and prepare the negative control solution according to the preparation method of the test solution. Under the above chromatographic conditions, accurately absorb 10% of the control solution, 10% of the test solution and 10% of the negative control solution, respectively, and inject them into the liquid chromatograph. The measured results are as follows: in the negative control chromatogram, the colorless peak appears at the retention time corresponding to the

chromatograms of the above six controls and test samples, indicating that other drug components in the formulation do not interfere with the determination of the above six components [5].

3.3. Investigation of linear relationship

Precision absorption of isomatrine standard solution (the content of isomatrine is 284 μg/mL): 1 μL, 2 μL, 6 μL, 8 μL, 10 μL injected into the liquid chromatograph, measured according to the chromatographic conditions, and the peak area. The injection volume was analyzed by linear regression with peak area. The range of isomatrine was 0.284–2.84 μL. The regression equation is y = 25.811x - 0.1969, r = 0.99990.

3.4. Precision test

Take the same isomatrine reference solution (the content of isomatrine is 284 μg / mL), repeat the injection 6 times according to the chromatographic conditions under item 2.1, and the RSD of the peak area of isomatrine is 1.2%. The results show that the precision of the instrument is good [6].

3.5. Repeatability test

Take 6 samples of known isomatrine content in the same batch (66.16 μg / g), prepare the test solution according to the proposed method, determine the content of each sample, and calculate the relative standard deviation of the content value, as shown in **Table 1**.

Table 1. Repeatability of methods

Sampling quantity(g)	Isomatrine peak area	Isomatrine content	Average content of isomatrine	RSD(%)
11.0013	1.352	66.07μg/g	66.68μ/g	0.85
11.0516	1.419	66.03μg/g		
11.1029	1.499	66.51μg/g		
11.0094	1.381	66.95μg/g		
11.0533	1.451	67.41μg/g		
11.0125	1.388	67.08μg/g		

3.6. Sample adding recovery test

Take 5 known isomatrine contents of the same batch (66.16 μg / g), accurately weigh, and accurately add the spiked standard solution (the content of isomatrine is 28.4 μg / mL), prepare the test solution according to the proposed method, determine the content of each, and calculate the recovery (%), as shown in **Table 2**.

Table 2. Test results of standard addition and recovery of isomatrine

Sampling quantity (g)	Content of isomatrine in test sample (μg)	Addition amount of reference substance (μg)	Measured content (μg)	Rate of recovery (%)	Average recovery (%)	RSD (%)
11.0113	66.91	28.4	92.78	91.09	91.05	1.23
11.0024	66.32	28.4	91.89	90.07		
11.0035	66.39	28.4	92.31	91.27		
11.0021	66.30	28.4	92.65	92.78		
11.0106	66.86	28.4	92.43	90.04		

3.7. Determination of sample content

Take about 11.0 g of five batches of agaru-15 powder samples, accurately weigh them, prepare the test solution according to the method discussed earlier, inject samples and analyze them respectively according to the chromatographic conditions discussed earlier, and calculate the content of isomatrine. The results are shown in **Table 3**. The experimental results show that the content of isomatrine in agaru-15 powder is generally in the same range, and can be measured accurately and stably, which is of certain significance to drug quality control and quality supervision.

Table 3. Content determination results

Isomatrine content (μg/g)	RSD (%)
66.16	1.2
59.78	0.83
72.03	0.65
51.37	1.1
55.11	1.1

4. Discussion

4.1. Selection of mobile phase

Under the condition of isocratic elution, even if the proportion of organic phase in the mobile phase is compressed to 40%, the above six components cannot be well separated within a certain time. After trying to use gradient elution, isomatrine can be well separated from other components. Under this mobile phase, isomatrine has a higher separation degree, a higher number of theoretical plates and a more appropriate retention time.

4.2. Investigation of extraction time

Using methanol as extraction solvent, ultrasonic extraction was performed on two batches of samples for 15 min, 30 min, 60 min and 90 min, respectively. Through data analysis, it was found that the extraction amount of isomatrine increased with the extension of extraction time within 60 min. However, the extraction amount of 60 min isomatrine is roughly the same as that of 90 min isomatrine, so the final extraction time is 60 min.

Disclosure statement

The author declares no conflict of interest.

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