

**TITLE OF INVENTION: CHARACTERISTIC SPECTRUM CONSTRUCTION
METHOD FOR CYRTOMIUM FORTUNE, CARBONIZED CYRTOMIUM FORTUNE
DECOCTION PIECES, STANDARD DECOCTION AND FORMULA GRANULES**

5 **CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the priority of Chinese Patent Application No. 202311154624.4, filed with the China National Intellectual Property Administration on September 7, 2023, and titled with “STANDARD DECOCTION CHARACTERISTIC SPECTRUM METHOD AND IDENTIFICATION FOR WOODWARDIA UNIGEMMATA AND CARBONIZED
10 WOODWARDIA UNIGEMMATA DECOCTION PIECES, STANDARD DECOCTIONS, AND FORMULA GRANULES”, which is hereby incorporated by reference in its entirety.

FIELD

[0002] The present disclosure relates to the field of analytical detection, especially relates to a
15 standard decoction characteristic spectrum method and identification for *Woodwardia unigemmata* and carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules.

BACKGROUND

[0003] Guan Zhong refers to the dried rhizome of *Woodwardia unigemmata* (Makino) Nakai, a plant of the family Blechnaceae, and is commonly known as “Gouji Guan Zhong.” It is harvested in spring and autumn, with the petioles removed, fibrous roots and soil removed, washed clean, and sun-dried; alternatively, the fresh material may be sliced and then sun-dried. Guan Zhong has the effects of clearing heat and detoxifying, stopping bleeding, and promoting sore healing and
25 tissue regeneration. *Woodwardia unigemmata* standard decoction is a freeze-dried powder prepared from the medicinal material after processing according to a fixed preparation process. *Woodwardia unigemmata* formula granules are prepared by the *Woodwardia unigemmata* medicinal material processed according to the main quality indicators of the standard decoction.

Guan Zhong is a relatively commonly used traditional Chinese medicinal material, and its medicinal use was first recorded in “Shen Nong Ben Cao Jing”, where it was described as having effects such as clearing heat, detoxifying, and expelling parasites. Records in successive herbal classics regarding the morphology, habitat, and producing areas of Guan Zhong indicate that there are numerous source plant species. According to literature reports, as many as 58 species from 18 genera belonging to 11 families have been used as source plants of Guan Zhong in China. Investigations conducted by Ai Tiemin et al. showed that, in the 1980s, 27 plant species were used as medicinal sources of Guan Zhong. The “Dictionary of Traditional Chinese Medicine” records seven source plant species of Guan Zhong. Yang Chunyu et al. identified a total of 38 source plant species of Guan Zhong through investigation, among which 18 species were widely collected and used as medicinal materials. Moreover, the provisions regarding the original plant species of Guan Zhong in the “Chinese Pharmacopoeia” have undergone multiple revisions: the 1977 edition included *Dryopteris crassirhizoma* and *Osmunda japonica* as sources; the 1995 edition included only *Dryopteris crassirhizoma*; and the 2010 edition again added *Osmunda japonica*. This demonstrates that medicinal materials classified as Guan Zhong are highly complex.

[0004] Carbonized *Woodwardia unigemmata* is prepared by stir-frying the medicinal material of *Woodwardia unigemmata* according to the simple stir-frying method (General Rule 0213) until the outer surface turns black and the interior becomes brown, thereby enhancing the hemostatic effect. Carbonized *Woodwardia unigemmata* standard decoction is a freeze-dried powder prepared from the medicinal material after processing according to a fixed preparation process. Carbonized *Woodwardia unigemmata* formula granules are prepared by the *Woodwardia unigemmata* medicinal material processed according to the main quality indicators of the standard decoction. The object is to ensure the uniformity and stability of the quality of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules.

[0005] It is therefore necessary to establish a novel characteristic spectrum method for quality control of carbonized *Woodwardia unigemmata*, which is capable of clearly identifying carbonized *Woodwardia unigemmata* from *Woodwardia unigemmata* and preparations thereof.

[0006] SUMMARY

[0007] In view of this, the technical problem to be solved in the present disclosure is to provide a UPLC characteristic spectrum construction method for carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules. The UPLC characteristic spectrum method for carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules constructed by the present disclosure is stable and reliable, and is capable of controlling the quality of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules.

[0008] A UPLC characteristic spectrum construction method for carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules, comprising:

[0009] A) dissolving a carbonized *Woodwardia unigemmata* raw material in a solvent to obtain a test solution;

[0010] B) subjecting the test solution to high-performance liquid chromatography to obtain a UPLC characteristic spectrum of the carbonized *Woodwardia unigemmata* raw material;

[0011] wherein the chromatographic conditions of the high-performance liquid chromatography are: a C18 chromatographic column; acetonitrile as mobile phase A; 0.1% formic acid as mobile phase B; and gradient elution.

[0012] In the UPLC characteristic fingerprint construction method for carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules provided by the present disclosure, the carbonized *Woodwardia unigemmata* raw material is first taken and dissolved with a solvent to obtain the test solution. Preferably, the solvent is 70% methanol.

[0013] The extraction solvent used in the present disclosure provides a large amount of chromatographic peak information and good analytical performance.

[0014] Specifically, the carbonized *Woodwardia unigemmata* raw material is first boiled in water, and then filtered; the filter residue is subsequently treated with a solvent, subjected to ultrasonic extraction, mixed thoroughly, and filtered to obtain a test solution.

[0015] In the present disclosure, the extraction method is water boiling followed by ultrasonic extraction or direct ultrasonic extraction.

[0016] The parameters for the water boiling followed by ultrasonic extraction are specifically: the mass ratio of carbonized *Woodwardia unigemmata* to water is preferably 1:50; the boiling duration is preferably 20 min to 30 min; the ultrasonic power is preferably 600 W, and the frequency is preferably 40 kHz; the extraction duration is preferably 15 min to 45 min, more preferably 15 min to 30 min.

[0017] The parameters for direct ultrasonic extraction are specifically: ultrasonic treatment with 70% methanol for 10 min to 15 min.

[0018] For medicinal material, decoction pieces, and reference medicinal material, the water boiling followed by ultrasonic extraction method is applied; for standard decoctions and formula granules, the direct ultrasonic extraction method is applied.

[0019] The mass of carbonized *Woodwardia unigemmata* raw material (g) to the volume of solvent (mL) ratio is preferably (0.1-1) : (10-50), more preferably 0.2:25.

[0020] The carbonized *Woodwardia unigemmata* raw material may be medicinal material, decoction pieces, standard decoctions, or formula granules. The present disclosure does not limit the specific form, and all the above materials may be subjected to quality control and qualitative analysis using the method of the present disclosure.

[0021] The present disclosure further comprises preparing a reference solution: respectively taking protocatchuic acid and 5-hydroxymethylfurfural, boiling in water, then filtering, and dissolving the filter residue in 70% methanol to obtain the reference solution. In the present disclosure, the extraction method is water boiling followed by ultrasonic extraction or direct ultrasonic extraction, wherein the mass ratio of the *Woodwardia unigemmata* medical material to water is preferably 1:50; the boiling duration is preferably 20 min to 30 min; the ultrasonic power is preferably 600 W, and the frequency is preferably 40 kHz; the extraction duration is preferably 15 min to 45 min; more preferably 15 min to 30 min; the ratio of the mass (g) of the carbonized *Woodwardia unigemmata* reference medical material to the volume (mL) of the solvent is preferably (0.1-1):(10-50); more preferably 0.2:25.

[0022] The reference substance solution is determined by high-performance liquid chromatography (HPLC) to obtain a chromatogram of the reference substance; and the components in the UPLC characteristic spectrum of carbonized *Woodwardia unigemmata*

decoction pieces, standard decoctions, and formula granules are qualitatively determined based on the chromatogram of the reference substance.

[0023] Mobile phase A is acetonitrile, and mobile phase B is 0.1% formic acid, with gradient elution applied.

5 **[0024]** In the present disclosure, the gradient elution of is preferably specifically as follows:

[0025] 0 min to 8 min, A phase: 1%, B phase: 99%;

[0026] 8 min to 18 min, A phase: 1%-15%, B phase: 99%-85%;

[0027] 18 min to 35 min, A phase: 15%-25%, B phase: 85%-75%;

[0028] 35 min to 50 min, A phase: 25%-26%, B phase: 75%-74%.

10 **[0029]** The present disclosure exhibits good baseline separation, good peak separation, and stable baseline under the above elution gradient conditions.

[0030] 1.6 μ m, 2.1 $\times$ 150mm; the column temperature is 35 $^{\circ}$ C.

[0031] In the present disclosure, at the column temperature of 35 $^{\circ}$ C, the chromatographic peaks are symmetric with good resolution..

15 **[0032]** The flow rate of the mobile phase is preferably 0.3 mL/min.

[0033] The present inventors found that, a flow rate of 0.3 mL/min achieves better peak separation and more symmetric peak shape, and is thus the most preferred embodiment.

[0034] In the present disclosure, the detection wavelength is preferably 250 nm.

20 **[0035]** The inventors found that this wavelength provides abundant chromatographic information, good absorption for each component, moderate response, satisfactory peak resolution, and stable baseline.

[0036] In the present disclosure, the injection volume is 1 μ L.

25 **[0037]** The beneficial effects of the present disclosure are that, under a single liquid chromatography condition, the chemical constituent profiles of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules are controlled by fingerprint chromatograms, and the fingerprint chromatograms are positioned using

protocatechuic acid; thereby greatly reducing detection costs and achieving qualitative detection.

[0038] A Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System is used to evaluate the similarity of the UPLC characteristic spectrum of the carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules, thereby obtaining a UPLC standard characteristic spectrum of the carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules composed of 13 characteristic peaks, wherein peak 1 is hydroxymethylfurfural and peak 3 is protocatechuic acid.

[0039] In the characteristic spectrum of the carbonized *Woodwardia unigemmata* standard decoctions, protocatechuic acid is used as a reference peak S, and the relative retention times of each characteristic peak to peak S are calculated. The relative retention times are within $\pm 10\%$ of the specified values, wherein the specified values are respectively: 0.77 (peak 1), 0.87 (peak 2), 1.51 (peak 4), 2.75 (peak 5), 2.81 (peak 6), 3.34 (peak 7), 3.43 (peak 8), 3.50 (peak 9), 3.67 (peak 10), 4.57 (peak 11), 4.72 (peak 12), and 4.77 (peak 13).

[0040] In the characteristic spectrum of the carbonized *Woodwardia unigemmata* decoction pieces, protocatechuic acid is used as a reference peak S, and the relative retention times of each characteristic peak to peak S are calculated. The relative retention times are within $\pm 10\%$ of the specified values, wherein the specified values are respectively: 0.77 (peak 1), 0.86 (peak 2), 1.48 (peak 4), 2.67 (peak 5), 2.72 (peak 6), 3.24 (peak 7), 3.32 (peak 8), 3.39 (peak 9), 3.55 (peak 10), 4.44 (peak 11), 4.57 (peak 12), and 4.61 (peak 13).

[0041] In the characteristic spectrum of the carbonized *Woodwardia unigemmata* formula granules, protocatechuic acid is used as a reference peak S, and the relative retention times of each characteristic peak to peak S are calculated. The relative retention times are within $\pm 10\%$ of the specified values, wherein the specified values are respectively: 0.77 (peak 1), 0.87 (peak 2), 1.50 (peak 4), 2.72 (peak 5), 2.77 (peak 6), 3.30 (peak 7), 3.39 (peak 8), 3.46 (peak 9), 3.62 (peak 10), 4.53 (peak 11), 4.65 (peak 12), and 4.70 (peak 13).

[0042] According to the detection results of the present disclosure, *Woodwardia unigemmata* and preparations thereof exhibit only 11 chromatographic peaks, lacking peaks 1 and 2 compared to carbonized *Woodwardia unigemmata*, thereby enabling clear identification between *Woodwardia unigemmata* and carbonized *Woodwardia unigemmata*.

[0043] Quality evaluation criteria: Samples of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules are taken and operated according to the same method as described above to obtain characteristic spectrum of the carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules. The standard
5 characteristic spectrum and the sample characteristic spectrum of the carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules are analyzed using the “Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2012 edition)” issued by the National Pharmacopoeia Commission, and the similarity is greater than 0.90.

[0044] By adopting the method provided by the present disclosure, the quality of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules from different batches can be effectively monitored, so as to ensure stable quality. The method has high precision and good reproducibility, and is beneficial for comprehensively monitoring product quality.

[0045] In the present disclosure, the characteristic spectrum of the carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules uses protocatechuic acid and 5-hydroxymethylfurfural as reference substances, and emphasizes the order of each characteristic peak and the correlation thereof with the medical material and intermediate products, and are capable of comprehensively evaluating the overall quality profile characteristics
20 of the products. The method is scientific and reliable.

[0046] The newly established characteristic fingerprint method of the present disclosure can detect components with relatively high polarity in *Woodwardia unigemmata* and its standard decoctions. In addition, the preparation method of the test solution is simple and easy to operate, and a relatively large number of characteristic peaks can be identified. Therefore, accurate and
25 reliable characteristic spectrum detection can be performed on *Woodwardia unigemmata* and preparations thereof. The authenticity, quality consistency, and stability of *Woodwardia unigemmata* and preparations thereof can all be effectively detected and controlled, thereby providing a basis for effectively controlling and comprehensively evaluating the quality of the *Woodwardia unigemmata* standard decoctions, and ensuring uniformity and stability of the
30 quality of *Woodwardia unigemmata* and its standard decoctions.

[0047] The present disclosure provides a method for constructing UPLC characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules, comprising: A) dissolving carbonized *Woodwardia unigemmata* raw material in a solvent to obtain a test solution; and B) subjecting the test solution to high-performance liquid chromatography to obtain a UPLC characteristic spectrum of the carbonized *Woodwardia unigemmata* raw material; wherein the chromatographic conditions of the high-performance liquid chromatography are: a C18 chromatographic column; acetonitrile as mobile phase A; 0.1% formic acid as mobile phase B; and gradient elution. The present disclosure employs high-performance liquid chromatography with acetonitrile-0.1% formic acid as the mobile phase for gradient elution, using protocatechuic acid as a reference, to establish UPLC characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules. The method exhibits good reproducibility, precision, stability, reliability, and can be used to control the quality of the carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules.

BRIEF DESCRIPTION OF DRAWINGS

[0048] FIG. 1-1 is a reference characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces disclosed in Example 1 of the present disclosure;

[0049] FIG. 1-2 is a reference characteristic spectrum of *Woodwardia unigemmata* medical material under the same determination conditions;

[0050] FIG. 2 is a diagram showing investigation results of extraction methods for carbonized *Woodwardia unigemmata* decoction pieces;

[0051] FIG. 3 is a chromatogram of carbonized *Woodwardia unigemmata* decoction pieces extracted with different solvents;

[0052] FIG. 4 is a chromatogram of carbonized *Woodwardia unigemmata* decoction pieces extracted for different durations;

[0053] FIG. 5 is a chromatogram obtained by extracting with different volumes of solvent;

[0054] FIG. 6 is a diagram showing identification results of chromatographic peaks;

[0055] FIG. 7 is a diagram showing investigation results obtained using different instruments;

[0056] FIG. 8 is a diagram showing investigation results obtained using different chromatographic columns;

[0057] FIG. 9-1 is a characteristic spectrum of carbonized *Woodwardia unigemmata* decoction
5 pieces;

[0058] FIG. 9-2 is a characteristic spectrum of carbonized *Woodwardia unigemmata* decoction
pieces;

[0059] FIG. 9-3 is a characteristic spectrum of *Woodwardia unigemmata* medical material;

[0060] FIG. 9-4 is a characteristic spectrum of *Woodwardia unigemmata* medical material;

[0061] FIG. 9-5 is a reference characteristic spectrum of *Woodwardia unigemmata* medical
10 material;

[0062] FIG. 10 is an investigation of extraction methods for a carbonized *Woodwardia unigemmata* decoction;

[0063] FIG. 11 is an investigation of extraction solvents for the carbonized *Woodwardia unigemmata* decoction;
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[0064] FIG. 12 is a chromatogram obtained by extracting for different durations;

[0065] FIG. 13 is a chromatogram obtained by extracting with different volumes of solvent;

[0066] FIG. 14 is a diagram showing identification of chromatographic peaks;

[0067] FIG. 15 is a diagram showing investigation results obtained using different instruments;

[0068] FIG. 16 is a diagram showing investigation results obtained using different
20 chromatographic columns;

[0069] FIG. 17 is a characteristic spectrum of a carbonized *Woodwardia unigemmata* standard
decoction;

[0070] FIG. 18 is a characteristic spectrum of the carbonized *Woodwardia unigemmata*
25 standard decoction;

[0071] FIG. 19 is a reference characteristic spectrum of a *Woodwardia unigemmata* standard

decoction;

[0072] FIG. 20 is an investigation of mobile phase types;

[0073] FIG. 21 is a chromatogram showing investigation of column temperature;

[0074] FIG. 22 is an investigation of flow rate;

5 **[0075]** FIG. 23 is an investigation of delay performance;

[0076] FIG. 24 is chromatograms obtained by using different extraction methods;

[0077] FIG. 25 is chromatograms obtained by extracting with different solvents;

[0078] FIG. 26 is chromatograms obtained by extracting for different durations;

[0079] FIG. 27 is chromatograms obtained by extracting by different volumes of solvent;

10 **[0080]** FIG. 28 is a diagram showing identification of chromatographic peaks;

[0081] FIG. 29 is a diagram showing investigation results obtained using different instruments;

[0082] FIG. 30 is a diagram showing investigation results obtained using different chromatographic columns;

15 **[0083]** FIG. 31 is a verification diagram of characteristic spectrum of three batches of *Woodwardia unigemmata* formula granules;

[0084] FIG. 32 is a reference characteristic spectrum of carbonized *Woodwardia unigemmata* formula granules;

[0085] FIG. 33 is a characteristic spectrum of *Woodwardia unigemmata* medical material;

[0086] FIG. 34 is a characteristic spectrum of *Woodwardia unigemmata* medical material;

20 **[0087]** FIG. 35 is a reference characteristic spectrum of *Woodwardia unigemmata* medical material;

[0088] FIG. 36 is a characteristic spectrum of a *Woodwardia unigemmata* standard decoction;

[0089] FIG. 37 is a characteristic spectrum of the *Woodwardia unigemmata* standard decoction;

25 **[0090]** FIG. 38 is a reference characteristic spectrum of the *Woodwardia unigemmata* standard decoction;

[0091] FIG. 39 is a characteristic spectrum of *Woodwardia unigemmata* formula granules;

[0092] FIG. 40 is a reference characteristic spectrum of the *Woodwardia unigemmata* formula granules; and

[0093] FIG. 41 is a diagram showing results under different gradient conditions of Comparative

5 Example 4.

DETAILED DESCRIPTION

[0094] In order to further illustrate the present disclosure, the method for constructing UPLC characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces, standard
10 decoctions, and formula granules provided by the present disclosure is described in detail below in conjunction with the examples.

[0095] High-performance liquid chromatographs: Agilent 1290; Shimadzu LC-40B X3; Thermo Fisher Vanquish Flex.

[0096] Electronic balances: ME204E/02, MS205DU, XP26 (Mettler-Toledo Instruments Co.,
15 Ltd.).

[0097] Ultrapure water system: Cell-type 1810A (Shanghai Mole Scientific Instrument Co., Ltd.).

[0098] Ultrasonic cleaner: model KQ600DB (600 W, 40 kHz; Kunshan Ultrasonic Instrument Co., Ltd.).

[0099] Chromatographic columns: CORTECS® UPLC® T3, 2.1 × 150 mm, 1.6 μm;
20 Phenomenex Luna® Omega PS C18 100 A, 2.1 × 150 mm, 1.6 μm; ACQUITY UPLC® BEH Shield RP18, 2.1 × 150 mm, 1.7 μm.

[00100] Methanol and acetonitrile are chromatographic grade; water is ultrapure water; all other reagents are analytical grade.

[00101] Protocatechuic acid reference substance (National Institutes for Food and Drug Control,
25 batch No. 110809-201906).

[00102] 5-Hydroxymethylfurfural reference substance (National Institutes for Food and Drug

Control, batch No. 111626-202215).

[00103] *Woodwardia unigemmata* reference medical material (Sichuan Institute for Drug Control, batch No. NO.SCZD003-201905).

[00104] Carbonized *Woodwardia unigemmata* decoction pieces (batch Nos. YP-01, YP-02, YP-03, YP-04, YP-05, YP-06, YP-07, YP-08, YP-09, YP-10, YP-11, YP-12, YP-13, YP-14, YP-15, YP-16, YP-17, YP-18, YP-19, YP-20);

[00105] Freeze-dried powder of carbonized *Woodwardia unigemmata* standard decoction (prepared by Sichuan Neo-green Pharmaceutical Technology Development Co., Ltd.; batch Nos. BT-01, BT-02, BT-03, BT-04, BT-05, BT-06, BT-07, BT-08, BT-09, BT-10, BT-11, BT-12, BT-13, BT-14, BT-15, BT-16, BT-17, BT-18, BT-19, BT-20);

[00106] Carbonized *Woodwardia unigemmata* formula granules (batch Nos. KL-01, KL-001, KL-002, KL-003).

[00107] Example 1: Screening of chromatographic conditions for carbonized *Woodwardia unigemmata* medical materials and decoction pieces

[00108] Chromatographic conditions and system suitability test: Octadecylsilane chemically bonded silica was used as the packing material (column length: 150 mm, inner diameter: 2.1 mm, particle size: 1.6 μm); acetonitrile was used as mobile phase A; 0.1% formic acid solution was used as mobile phase B; gradient elution was performed according to the conditions specified in the table below; the column temperature was 35 $^{\circ}\text{C}$; the flow rate was 0.3 mL/min; and the detection wavelength was 250 nm. The number of theoretical plates, calculated based on the protocatechuic acid peak, should not be less than 5000.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-8	1	99
8-18	1→15	99→85
18-35	15→25	85→75
35-50	25→26	75→74

[00109] Preparation of reference solution: 1 g of *Woodwardia unigemmata* reference medical material was weighed, placed into a stoppered conical flask, and added with 50 mL of water, followed by boiling for 30 minutes. After filtration, the filtrate was evaporated to dryness. The

residue was added with 25 mL of 70% methanol and subjected to ultrasonic treatment (power: 600 W, frequency: 40 kHz) for 15 minutes, shaken up, and filtered. The subsequent filtrate was collected to obtain a reference medical material reference solution. In addition, an appropriate amount of protocatechuic acid reference substance was accurately weighed and precisely diluted
 5 with methanol to prepare a solution containing 20 µg of protocatechuic acid per 1 mL, which was used as a reference substance solution.

[00110] About 1 g of the sample powder (passed through a No. 3 sieve) was weighed, placed into a stoppered conical flask, and added with 50 mL of water, followed by boiling for 30 minutes. After filtration, the filtrate was evaporated to dryness. The residue was added with 25 mL of 70%
 10 methanol and subjected to ultrasonic treatment (power: 600 W, frequency: 40 kHz) for 15 minutes, shaken up, and filtered. The subsequent filtrate was collected to obtain the test solution.

[00111] Determination method: 1 µL of each of the test solution and the reference substance solution was precisely aspirated and injected into the liquid chromatograph for determination.

[00112] Thirteen characteristic peaks should be present in the chromatogram of the test solution.

15 Except for peak 1 and peak 2, all peaks should correspond in retention time to the eleven characteristic peaks in the chromatogram of the reference medical material reference solution. The peak corresponding to the protocatechuic acid reference substance was designated as reference peak S. The relative retention times of each characteristic peak to peak S were calculated, and the relative retention times should be within $\pm 10\%$ of the specified values. The
 20 specified values were: 0.77 (peak 1), 0.86 (peak 2), 1.48 (peak 4), 2.67 (peak 5), 2.72 (peak 6), 3.24 (peak 7), 3.32 (peak 8), 3.39 (peak 9), 3.55 (peak 10), 4.44 (peak 11), 4.57 (peak 12), and 4.61 (peak 13). The results are shown in FIG. 1, wherein FIG. 1-1 is a reference characteristic spectrum of the carbonized *Woodwardia unigemmata* decoction pieces disclosed in Example 1 of the disclosure, and FIG. 1-2 is a reference characteristic spectrum of the *Woodwardia*
 25 *unigemmata* medical material under the same determination conditions. The method of the present disclosure can clearly identify carbonized *Woodwardia unigemmata* from *Woodwardia unigemmata* medical material.

[00113] Example 2: Investigation of extraction methods

[00114] Extraction methods including water boiling followed by ultrasonic extraction, reflux

extraction, and ultrasonic extraction were compared. It was found that the chromatographic peak information obtained by water boiling followed by ultrasonic extraction was consistent with that of the carbonized *Woodwardia unigemmata* standard decoction. Therefore, water boiling followed by ultrasonic extraction was finally selected as the extraction method. The results are shown in FIG. 2, which is a diagram showing the investigation results of extraction methods for carbonized *Woodwardia unigemmata* decoction pieces.

[00115] Example 3: Investigation of extraction solvents

[00116] Methanol, water, 50% methanol, and 70% methanol were investigated as extraction solvents. The results showed that when 70% methanol was used as the extraction solvent, a larger amount of chromatographic peak information and better resolution were obtained. Therefore, 70% methanol was selected as the extraction solvent. The results are shown in FIG. 3, which is a chromatogram of carbonized *Woodwardia unigemmata* decoction pieces extracted with different solvents.

[00117] Example 4: Investigation of extraction duration

[00118] Ultrasonic extraction times of 15 minutes, 30 minutes, and 60 minutes were compared. The chromatograms were substantially consistent. Therefore, the extraction time was determined to be 15 minutes. The results are shown in FIG. 4, which is a chromatogram of carbonized *Woodwardia unigemmata* decoction pieces extracted for different extraction durations.

[00119] Example 5: Investigation of extraction solvent volume

[00120] Extraction solvent volumes of 25 mL, 50 mL, and 100 mL were compared. When the extraction solvent volume was 25 mL, the chromatographic peak sizes were more appropriate. Therefore, the extraction solvent volume was determined to be 25 mL. The results are shown in FIG. 5, which is a chromatogram obtained by extracting with different volumes of solvent.

[00121] Example 6: Methodological investigation

[00122] 6.1 Identification of chromatographic peaks

[00123] Preparation of the test solution: The test solution of *Woodwardia unigemmata* medicinal material was prepared according to the finally determined preparation method. Specifically, approximately 1 g of the powdered sample (passed through No. 3 sieve) was weighed and placed

into a stoppered conical flask, and 50 mL of water was added and boiled for 30 minutes. After filtration, the filtrate was evaporated to dryness. The residue was added with 25 mL of 70% methanol, subjected to ultrasonic treatment (power: 600 W; frequency: 40 kHz) for 15 minutes, shaken up, and filtered. The subsequent filtrate was collected to obtain the test solution.

- 5 **[00124]** Preparation of the reference substance solution: An appropriate amount of protocatechuic acid reference substance was accurately weighed and dissolved in methanol to prepare a reference substance solution containing 20 µg of protocatechuic acid per 1 mL.

- [00125]** Preparation of the reference medicinal material solution: 1 g of *Woodwardia unigemmata* reference medicinal material was weighed and placed into a stoppered conical flask,
10 and 50 mL of water was added and boiled for 30 minutes. After filtration, the filtrate was evaporated to dryness. The residue was added with 25 mL of 70% methanol, subjected to ultrasonic treatment (power: 600 W; frequency: 40 kHz) for 15 minutes, shaken up, and filtered. The subsequent filtrate was collected and used as the reference medicinal material solution.

- [00126]** Preparation of the negative control solution: According to the above experimental
15 conditions, a negative control solution lacking *Woodwardia unigemmata* medicinal material was prepared. The characteristic chromatographic peaks of *Woodwardia unigemmata* were located. As shown in FIG. 6, which is the results of chromatographic peak identification.

[00127] 6.2 Investigation of precision

- [00128]** The test solution of carbonized *Woodwardia unigemmata* decoction pieces (batch No.
20 YP-01) was taken and continuously injected six times according to the established experimental method, with an injection volume of 1 µL each time. The retention times and peak areas of the characteristic peaks were calculated. The results are shown in Table 1-1.

[00129] Table 1-1 Investigation of precision - retention time

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	7.138	8.003	9.339	13.799	24.832	25.248	30.172	30.906	31.502	32.989	41.241	42.429	42.867
2	7.144	8.007	9.352	13.807	24.828	25.238	30.175	30.902	31.496	32.983	41.236	42.432	42.869
3	7.159	8.019	9.371	13.819	24.843	25.247	30.186	30.908	31.500	32.984	41.218	42.423	42.859

4	7.16	8.022	9.384	13.824	24.842	25.248	30.181	30.901	31.493	32.980	41.223	42.420	42.857
5	7.142	8.007	9.355	13.811	24.834	25.244	30.182	30.91	31.504	32.995	41.221	42.418	42.856
6	7.151	8.014	9.369	13.817	24.833	25.238	30.168	30.888	31.479	32.965	41.184	42.384	42.821
RSD(%)	0.13	0.10	0.18	0.07	0.03	0.02	0.03	0.03	0.03	0.04	0.05	0.05	0.05

[00130] Table 1-2 Investigation of precision - peak area

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	72.377	81.088	80.886	35.327	4.952	15.617	5.157	15.077	8.945	15.286	9.091	5.743	3.496
2	72.851	81.653	81.269	35.529	4.974	15.751	5.153	15.137	9.031	15.426	9.050	5.808	3.472
3	72.165	80.981	81.278	35.553	4.712	15.688	5.148	15.047	8.954	15.307	4.345	5.786	3.550
4	72.902	81.665	82.136	35.900	4.779	15.816	5.184	15.146	9.064	15.450	4.536	5.887	3.615
5	73.185	82.039	81.803	35.823	4.950	15.826	5.206	15.352	9.059	15.484	4.583	5.855	3.636
6	72.557	81.580	81.595	35.683	4.893	15.721	5.123	15.140	9.023	15.385	4.328	5.833	3.884
RSD(%)	0.13	0.10	0.18	0.07	0.03	0.02	0.03	0.03	0.03	0.04	0.05	0.05	0.05

[00131] The results showed that the RSDs of the retention times of the characteristic peaks in the precision test ranged from 0.02% to 0.18%, and the RSDs of the peak areas ranged from 0.02% to 0.18%. The instrument exhibited good precision.

[00132] 6.3 Investigation of repeatability

[00133] Six portions of carbonized *Woodwardia unigemmata* decoction pieces (batch No. YP-01) were taken and prepared and determined according to the established experimental method. The results are shown in Table 2-1 and Table 2-2.

[00134] Table 2-1 Investigation of repeatability - relative retention time ratio

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62
2	0.77	0.86	1.00	1.48	2.66	2.71	3.24	3.32	3.38	3.54	4.43	4.55	4.60
3	0.77	0.86	1.00	1.48	2.66	2.71	3.23	3.31	3.38	3.54	4.42	4.55	4.60
4	0.77	0.86	1.00	1.48	2.66	2.71	3.23	3.31	3.38	3.54	4.42	4.55	4.60

5	0.76	0.86	1.00	1.48	2.66	2.70	3.23	3.31	3.37	3.53	4.41	4.54	4.59
6	0.77	0.86	1.00	1.48	2.66	2.71	3.24	3.32	3.38	3.54	4.43	4.55	4.60
RSD(%)	0.54	0.00	0.00	0.00	0.16	0.24	0.26	0.25	0.30	0.28	0.31	0.22	0.22

[00135] Table 2-2 Investigation of repeatability - relative peak area ratio

No.	Characteristic peaks										Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9					
1	0.90	1.01	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.11	0.07	0.04	
2	0.90	1.01	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.11	0.07	0.04	
3	0.90	1.01	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.12	0.07	0.04	
4	0.90	1.01	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.11	0.07	0.04	
5	0.90	1.00	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.11	0.07	0.04	
6	0.91	1.02	1.00	0.44	0.06	0.20	0.06	0.19	0.11	0.20	0.09	0.07	0.04	
RSD(%)	0.46	0.63	0.00	0.00	0.00	2.13	0.00	0.00	0.00	2.13	9.08	0.00	0.00	

[00136] The results showed that the RSDs of the relative retention times of the characteristic peaks in the repeatability test ranged from 0.00% to 0.54%, and the RSDs of the relative peak areas ranged from 0.00% to 9.08%. The method exhibited good repeatability.

[00137] 6.4 Investigation of intermediate precision

[00138] 6.4.1 Different people at different times

[00139] Based on the above experimental conditions, carbonized *Woodwardia unigemmata* decoction pieces (batch No. YP-01) were taken, and test solutions were prepared by different people at different times for determination. The results are shown in Table 3-1 and Table 3-2.

[00140] Table 3-1 Investigation of intermediate precision - relative retention time ratio

No.	Characteristic peaks										Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9					
Person 1	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62	
Person 2	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.44	4.57	4.62	

Time 2	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.44	4.57	4.62
RSD(%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.17	0.13	0.00	0.00

[00141] Table 3-2 Investigation of intermediate precision - relative peak area ratio

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Person 1	0.90	1.01	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.11	0.07	0.04
Person 2	0.91	1.02	1.00	0.44	0.06	0.20	0.06	0.19	0.11	0.19	0.08	0.07	0.04
Time 2	0.90	1.01	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.11	0.07	0.04
RSD(%)	0.64	0.57	0.00	0.00	0.00	2.99	0.00	0.00	0.00	0.00	17.33	0.00	0.00

[00142] The results showed that when the same sample was determined by different people at different times, the method exhibited good precision.

[00143] 6.5 Different instruments

[00144] Based on the above experimental conditions, investigations were respectively conducted using Instrument 1, Instrument 2, and Instrument 3. As shown in FIG. 7, FIG. 7 is the results of the investigation using different instruments, and the results are also shown in Table 4-1 and Table 4-2.

[00145] Table 4-1 Investigation of different instruments - relative retention time

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Instrument 1	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62
Instrument 2	0.69	0.86	1.00	1.82	3.38	3.41	4.02	4.04	4.16	4.35	5.33	5.47	5.51
Instrument 3	0.83	0.87	1.00	1.46	2.45	2.50	2.95	3.03	3.08	3.22	4.13	4.26	4.31
RSD(%)	9.21	0.67	0.00	12.76	17.16	16.51	16.21	14.97	15.65	15.63	13.41	13.19	12.95

[00146] Table 4-2 Investigation of different instruments - relative peak area

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Instrument 1	0.90	1.01	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.11	0.07	0.04
Instrument 2	0.89	1.07	1.00	0.45	0.06	0.20	0.06	0.19	0.10	0.19	0.06	0.07	0.05
Instrument 3	0.90	1.13	1.00	0.43	0.06	0.21	0.06	0.20	0.11	0.19	0.05	0.07	0.06
RSD(%)	0.65	5.61	0.00	2.28	0.00	5.00	0.00	2.99	5.42	0.00	43.84	0.00	20.00

[00147] The results showed that when the above three instruments were used for detection, the RSDs of the relative retention times of the characteristic peaks ranged from 0.67% to 17.16%, and the RSDs of the relative peak areas ranged from 0.00% to 43.84%, indicating poor durability among different instruments.

[00148] 6.6 Investigation of chromatographic column durability

[00149] Based on the above experimental conditions, investigations were respectively conducted using Column 1, Column 2, and Column 3. As shown in FIG. 8, FIG. 8 is the results of the investigation using different chromatographic columns, and the results are also shown in Table 5-1 and Table 5-2.

[00150] Table 5-1 Chromatographic column durability - relative retention time

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Column 1	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62
Column 2	0.67	0.97	1.00	1.33	2.41	2.42	2.88	2.92	2.98	3.10	4.01	4.10	4.16
Column 3	0.43	0.60	1.00	1.61	2.66	2.69	3.34	3.30	3.34	3.48	4.44	4.53	4.59
RSD(%)	28.04	23.46	0.00	9.52	5.71	6.34	7.73	7.18	7.02	7.28	5.85	5.93	5.78

[00151] Table 5-2 Chromatographic column durability - relative peak area

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13

Column 1	0.90	1.01	1.00	0.44	0.06	0.19	0.06	0.19	0.11	0.19	0.11	0.07	0.04
Column 2	0.63	0.45	1.00	0.33	0.08	0.85	0.09	0.58	0.30	0.60	0.13	0.21	0.12
Column 3	0.56	0.60	1.00	0.33	0.14	0.71	0.03	0.52	0.27	0.52	0.18	0.04	0.08
RSD(%)	25.78	42.22	0.00	17.33	44.61	59.62	50.00	48.84	45.07	49.78	25.76	85.07	50.00

[00152] As shown in FIG. 8, the chromatogram obtained using the CORTECS® UPLC® T3 column 2.1 × 150 mm, 1.6 μm (Column 1) exhibited better separation. Therefore, the CORTECS® UPLC® T3 column 2.1 × 150 mm, 1.6 μm was recommended for use.

5 **[00153]** 6.7 Investigation of stability

[00154] Based on the above experimental conditions, the same test solution was taken and determined at 0 h, 3 h, 6 h, 9 h, 12 h, 18 h, and 24 h, respectively. The results are shown in Table 6-1 and Table 6-2.

[00155] Table 6-1 Investigation of stability - retention time

Time (h)	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
0	7.114	7.984	9.275	13.764	24.797	25.232	30.145	30.901	31.503	32.990	41.249	42.423	42.869
3	7.121	7.990	9.290	13.775	24.817	25.246	30.168	30.914	31.512	32.995	41.269	42.451	42.895
6	7.148	8.014	9.340	13.804	24.830	25.242	30.172	30.908	31.503	32.988	41.255	42.441	42.882
9	7.126	7.995	9.305	13.782	24.817	25.241	30.164	30.907	31.504	32.990	41.260	42.441	42.885
12	7.132	7.999	9.315	13.786	24.818	25.241	30.161	30.901	31.500	32.988	41.256	42.432	42.874
18	7.143	8.009	9.344	13.806	24.828	25.242	30.165	30.898	31.495	32.984	41.242	42.429	42.870
24	7.112	7.979	9.279	13.764	24.797	25.234	30.143	30.892	31.492	32.979	41.247	42.420	42.865
RSD(%)	0.19	0.16	0.30	0.13	0.06	0.02	0.04	0.03	0.03	0.02	0.03	0.03	0.03

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[00156] Table 6-2 Investigation of stability - peak area

Time (h)	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
0	69.382	77.664	76.773	34.109	4.642	14.957	4.881	14.443	8.723	14.725	8.510	5.567	3.363

3	69.797	78.294	77.359	34.087	4.635	15.078	4.915	14.494	8.739	14.840	8.829	5.575	2.904
6	69.843	77.869	77.567	33.848	4.716	15.039	4.910	14.432	8.688	14.762	8.992	5.553	3.192
9	71.506	80.316	79.876	34.818	4.913	15.558	5.070	15.097	9.017	15.211	8.946	5.699	3.200
12	73.837	83.432	83.007	36.044	5.083	16.149	5.240	15.658	9.311	15.838	9.570	5.974	3.540
18	70.709	79.318	78.942	34.474	4.682	15.358	5.031	14.714	8.838	15.077	8.801	5.643	3.389
24	71.042	79.751	78.856	34.743	4.771	15.320	5.059	14.982	8.863	15.081	8.687	5.687	3.457
RSD(%)	2.14	2.50	2.67	2.13	3.46	2.67	2.51	3.04	2.47	2.54	3.76	2.58	6.48

[00157] The results showed that the RSDs of the retention times of the corresponding characteristic peaks ranged from 0.02% to 0.19%, and the RSDs of the peak areas ranged from 2.13% to 6.48%. The sample solution was demonstrated to be relatively stable within 24 hours. In summary, the RSDs of the relative retention times of the characteristic peaks in the above investigations all met the requirements, indicating that the method was good. The above 13 characteristic peaks were included in subsequent investigations.

[00158] 6.8 Verification of the characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces

[00159] Using the present method, characteristic spectrum analysis was performed on 20 batches of carbonized *Woodwardia unigemmata* decoction pieces. The relative retention times and relative peak area ratios were calculated. The results are shown in FIG. 9-1 and FIG. 9-2 and Table 7-1 and Table 7-2.

[00160] FIG. 9-1 shows the characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces, with batch numbers from bottom to top being YP-01, YP-02, YP-03, YP-04, YP-05, YP-06, YP-07, YP-08, YP-09, and YP-10.

[00161] FIG. 9-2 shows the characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces, with batch numbers from bottom to top being YP-11, YP-12, YP-13, YP-14, YP-15, YP-16, YP-17, YP-18, YP-19, and YP-20.

[00162] Table 7-1 Relative retention time of characteristic spectrum of 20 batches carbonized *Woodwardia unigemmata* decoction pieces

Batch No.	Characteristic peaks												
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	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
YP-01	0.77	0.86	1.00	1.48	2.66	2.71	3.24	3.32	3.38	3.54	4.43	4.55	4.60
YP-02	0.77	0.86	1.00	1.48	2.67	2.71	3.24	3.32	3.39	3.55	4.44	4.57	4.61
YP-03	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.44	4.57	4.62
YP-04	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.45	4.57	4.62
YP-05	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62
YP-06	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.58	4.62
YP-07	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62
YP-08	0.77	0.86	1.00	1.49	2.68	2.72	3.25	3.33	3.40	3.56	4.45	4.58	4.63
YP-09	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62
YP-10	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.45	4.57	4.62
YP-11	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.44	4.57	4.62
YP-12	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.45	4.57	4.62
YP-13	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.44	4.57	4.61
YP-14	0.77	0.86	1.00	1.48	2.66	2.71	3.23	3.31	3.38	3.54	4.42	4.55	4.60
YP-15	0.77	0.86	1.00	1.48	2.66	2.71	3.24	3.32	3.38	3.54	4.43	4.56	4.60
YP-16	0.77	0.86	1.00	1.48	2.66	2.71	3.23	3.31	3.38	3.54	4.42	4.55	4.60
YP-17	0.77	0.86	1.00	1.48	2.66	2.71	3.24	3.31	3.38	3.54	4.42	4.55	4.60
YP-18	0.77	0.86	1.00	1.48	2.66	2.71	3.24	3.32	3.38	3.54	4.43	4.56	4.60
YP-19	0.77	0.86	1.00	1.48	2.67	2.71	3.24	3.32	3.39	3.55	4.43	4.56	4.61
YP-20	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.44	4.57	4.62
RSD(%)	0.00	0.00	0.00	0.16	0.21	0.19	0.22	0.23	0.23	0.22	0.26	0.21	0.22
Average	0.77	0.86	-	1.48	2.67	2.72	3.24	3.32	3.39	3.55	4.44	4.57	4.61
90%	0.69	0.77	-	1.33	2.40	2.45	2.92	2.99	3.05	3.20	4.00	4.11	4.15
110%	0.85	0.95	-	1.63	2.94	2.99	3.56	3.65	3.73	3.90	4.88	5.03	5.07

[00163] Table 7-2 Relative peak area of characteristic spectrum of 20 batches carbonized *Woodwardia unigemmata* decoction pieces

Batch No.	Characteristic peaks										Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9					

YP-01	0.90	1.07	1.00	0.46	0.06	0.18	0.05	0.16	0.10	0.17	0.09	0.06	0.04
YP-02	0.88	1.07	1.00	0.45	0.06	0.17	0.05	0.15	0.10	0.16	0.09	0.06	0.04
YP-03	0.88	1.10	1.00	0.45	0.05	0.16	0.05	0.14	0.09	0.15	0.08	0.05	0.04
YP-04	0.87	1.10	1.00	0.45	0.05	0.15	0.05	0.14	0.09	0.15	0.08	0.05	0.03
YP-05	0.89	1.08	1.00	0.44	0.06	0.17	0.05	0.15	0.10	0.16	0.06	0.06	0.04
YP-06	0.88	1.08	1.00	0.45	0.06	0.17	0.05	0.15	0.09	0.16	0.08	0.06	0.04
YP-07	0.88	1.06	1.00	0.45	0.06	0.17	0.05	0.15	0.10	0.16	0.09	0.06	0.04
YP-08	0.87	1.08	1.00	0.45	0.06	0.16	0.05	0.14	0.09	0.15	0.08	0.05	0.04
YP-09	0.92	1.04	1.00	0.44	0.06	0.18	0.06	0.16	0.10	0.17	0.09	0.06	0.04
YP-10	0.92	1.04	1.00	0.45	0.05	0.17	0.05	0.15	0.10	0.16	0.09	0.06	0.04
YP-11	0.92	1.05	1.00	0.45	0.05	0.16	0.05	0.14	0.09	0.15	0.09	0.06	0.04
YP-12	0.92	1.05	1.00	0.45	0.05	0.16	0.05	0.14	0.09	0.15	0.09	0.06	0.04
YP-13	0.89	1.10	1.00	0.45	0.05	0.14	0.04	0.12	0.08	0.13	0.07	0.05	0.03
YP-14	0.92	1.10	1.00	0.45	0.05	0.14	0.04	0.12	0.08	0.13	0.07	0.05	0.03
YP-15	0.91	1.10	1.00	0.45	0.05	0.14	0.04	0.12	0.08	0.13	0.07	0.05	0.03
YP-16	0.95	1.07	1.00	0.46	0.05	0.13	0.04	0.11	0.07	0.12	0.07	0.04	0.03
YP-17	0.95	1.08	1.00	0.46	0.05	0.13	0.04	0.11	0.07	0.12	0.06	0.04	0.03
YP-18	1.11	1.08	1.00	0.46	0.07	0.19	0.07	0.18	0.11	0.19	0.08	0.07	0.05
YP-19	0.66	0.54	1.00	0.24	0.15	0.64	0.08	0.44	0.26	0.47	0.12	0.15	0.10
YP-20	0.69	0.55	1.00	0.25	0.15	0.64	0.07	0.44	0.25	0.46	0.10	0.14	0.09
RSD(%)	10.14	16.08	0.00	14.80	46.24	71.74	21.16	55.04	48.34	54.06	17.09	44.89	43.41

[00164] According to the principles of stable relative retention times, detectability in all batches, and relatively high peak intensities, a total of 13 peaks with good repeatability were selected as characteristic peaks. The results showed that when Peak 3 was used as the S peak, the RSDs of the relative retention times of the characteristic peaks of 20 batches of carbonized *Woodwardia unigemmata* decoction pieces ranged from 0.00% to 0.26%. And the RSDs of the relative retention times of the 13 characteristic peaks in the 20 batches carbonized *Woodwardia unigemmata* decoction pieces were all less than 10%.

[00165] It was finally specified that 13 characteristic peaks should be present in the chromatogram of the test sample. Except for Peak 1 and Peak 2, all peaks should correspond in retention time to the 11 characteristic peaks in the chromatogram of the reference medicinal

material. The peak corresponding to the protocatechuic acid reference peak was designated as the S peak. The relative retention times of each characteristic peak to the S peak were calculated, and the relative retention times were required to fall within $\pm 10\%$ of the specified values. The specified values were: 0.77 (Peak 1), 0.86 (Peak 2), 1.48 (Peak 4), 2.67 (Peak 5), 2.72 (Peak 6), 3.24 (Peak 7), 3.32 (Peak 8), 3.39 (Peak 9), 3.55 (Peak 10), 4.44 (Peak 11), 4.57 (Peak 12), and 4.61 (Peak 13).

[00166] 6.9 Results of the characteristic spectrum of *Woodwardia unigemmata* medicinal material

[00167] The characteristic spectrums of 20 batches of *Woodwardia unigemmata* medicinal material are shown in FIG. 9-3 and FIG. 9-4. FIG. 9-3 shows the characteristic spectrum of *Woodwardia unigemmata* medicinal material (batch numbers from bottom to top being XLS202107277, XLS202107278, XLS202107279, XLS202107280, XLS202107281, XLS202107282, XLS202107283, XLS202107284, XLS202107285, and XLS202107286). FIG. 9-4 shows the characteristic spectrum of *Woodwardia unigemmata* medicinal material (batch numbers from bottom to top being XLS202107287, XLS202107288, XLS202107289, XLS202107290, XLS202107291, XLS202107292, XLS202107293, XLS202107294, XLS202107295, and 010176-2012001).

[00168] FIG. 9-5 shows the reference characteristic spectrum of *Woodwardia unigemmata* medicinal material. Peak 1 (S): protocatechuic acid. Note: chromatographic column: CORTECS® UPLC® T3 2.1 $\times$ 150 mm, 1.6 μ m.

[00169] Example 7: Selection of chromatographic conditions of carbonized *Woodwardia unigemmata* standard decoction

[00170] The chromatographic conditions and system suitability tests of the characteristic spectrum of the standard decoction of *Woodwardia unigemmata* were determined as follows: octadecylsilane chemically bonded silica was used as the packing material (column length: 150 mm, inner diameter: 2.1 mm, particle size: 1.6 μ m); acetonitrile was used as mobile phase A; 0.1% formic acid solution was used as mobile phase B; gradient elution was performed according to the conditions specified in the table below; the column temperature was 35 $^{\circ}$ C; the flow rate was 0.3 mL/min; and the detection wavelength was 250 nm. The number of theoretical plates,

calculated based on the protocatchuic acid peak, should not be less than 5000.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-8	1	99
8-18	1→15	99→85
18-35	15→25	85→75
35-50	25→26	75→74

[00171] Preparation of reference solution

[00172] 1 g of *Woodwardia unigemmata* reference medical material was weighed, placed into a stoppered conical flask, and added with 50 mL of water, followed by boiling for 30 minutes. After filtration, the filtrate was evaporated to dryness. The residue was added with 25 mL of 70% methanol and subjected to ultrasonic treatment (power: 600 W, frequency: 40 kHz) for 30 minutes, shaken up, and filtered. The subsequent filtrate was collected to obtain a reference medical material reference solution. In addition, an appropriate amount of protocatchuic acid reference substance was accurately weighed and precisely diluted with methanol to prepare a solution containing 20 µg of protocatchuic acid per 1 mL, which was used as a reference substance solution.

[00173] Preparation of test solution

[00174] About 0.2 g of the carbonized *Woodwardia unigemmata* standard decoction was weighed, placed into a stoppered conical flask, and added with 50 mL of 70% methanol and subjected to ultrasonic treatment (power: 600 W, frequency: 40 kHz) for 30 minutes, taken out, and cooled. The subsequent filtrate was collected to obtain the test solution.

[00175] Determination method

[00176] 1 µL of each of the test solution and the reference substance solution was precisely aspirated and injected into the liquid chromatograph for determination.

[00177] Selection of detection wavelength, column temperature, and flow rate: The material composition of the standard decoction and the formula granules was fundamentally consistent, and the characteristic chromatographic conditions and system suitability tests were kept

consistent with those used for the characteristic spectrum method of the granules.

[00178] 7.1 Investigation of extraction method

[00179] Reflux extraction and ultrasonic extraction were compared. The chromatographic peak information obtained by the two extraction methods was generally similar. Therefore, ultrasonic
5 extraction, which was simpler to operate, was finally selected. The results are shown in FIG. 10. FIG. 10 is the investigation of extraction methods for the carbonized *Woodwardia unigemmata* decoction.

[00180] 7.2 Investigation of extraction solvent

[00181] Methanol, water, 50% methanol, and 70% methanol were investigated as extraction
10 solvents. The results showed that the chromatographic peak information obtained using 70% methanol as the extraction solvent was more abundant and exhibited better resolution. Therefore, 70% methanol was selected as the extraction solvent. The results are shown in FIG. 11. FIG. 11 is the investigation of extraction solvents for the carbonized *Woodwardia unigemmata* decoction.

[00182] 7.3 Investigation of extraction duration

[00183] Ultrasonic extraction for 15 minutes, 30 minutes, and 60 minutes was compared. The
15 chromatograms were essentially consistent. Therefore, the extraction duration was determined to be 15 minutes. The results are shown in FIG. 12. FIG. 12 is chromatograms obtained by extracting for different durations.

[00184] 7.4 Investigation of extraction solvent volume

[00185] Extraction solvent volumes of 25 mL, 50 mL, and 100 mL were compared. When the
20 extraction solvent volume was 25 mL, the chromatographic peak sizes were more appropriate. Therefore, the extraction solvent volume was determined to be 25 mL. The results are shown in FIG. 13. FIG. 13 is chromatograms obtained by extracting with different volumes of solvent.

[00186] 7.5 Finally determined test sample preparation method

[00187] Approximately 0.2 g of the sample was weighed and placed into a stoppered conical
25 flask, 25 mL of 70% methanol was added, and subjected to ultrasonic treatment (power: 600 W; frequency: 40 kHz) for 15 minutes, shaken up and filtered. The subsequent filtrate was collected to obtain the test solution.

[00188] Example 8: Methodological investigation of *Woodwardia unigemmata* standard decoction

[00189] 8.1 Identification of chromatographic peaks

[00190] Preparation of the test solution: The test solution of carbonized *Woodwardia unigemmata* standard decoction was prepared according to the finally determined preparation method.

[00191] Preparation of the reference substance solution: An appropriate amount of protocatchuic acid reference substance was accurately weighed and dissolved in methanol to prepare a reference substance solution containing 20 µg of protocatchuic acid per 1 mL.

[00192] Preparation of the reference medicinal material solution: 1 g of *Woodwardia unigemmata* reference medicinal material was weighed and placed into a stoppered conical flask, and 50 mL of water was added and boiled for 30 minutes. After filtration, the filtrate was evaporated to dryness. The residue was added with 25 mL of 70% methanol, subjected to ultrasonic treatment (power: 600 W; frequency: 40 kHz) for 15 minutes, shaken up, and filtered. The subsequent filtrate was collected and used as the reference medicinal material solution.

[00193] Preparation of the negative control solution: According to the above experimental conditions, a negative control solution lacking *Woodwardia unigemmata* standard decoction was prepared. The characteristic chromatographic peaks of *Woodwardia unigemmata* standard decoction were located. As shown in FIG. 14, FIG. 14 is the results of chromatographic peak identification.

[00194] 8.2 Investigation of precision

[00195] The test solution of carbonized *Woodwardia unigemmata* standard decoction (batch No. BT-01) was taken and continuously injected six times according to the established experimental method, with an injection volume of 1 µL each time. The retention times and peak areas of the characteristic peaks were calculated. The results are shown in Table 8 and Table 9.

[00196] Table 8 Investigation of precision - retention time

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak	Peak	Peak	Peak

										10	11	12	13
1	7.159	8.003	9.388	13.826	24.817	25.217	30.154	30.871	31.461	32.948	41.351	42.356	42.794
2	7.135	8.007	9.352	13.805	24.804	25.212	30.143	30.868	31.462	32.952	41.323	42.360	42.799
3	7.122	8.019	9.325	13.788	24.792	25.213	30.135	30.868	31.465	32.945	41.294	42.354	42.796
4	7.099	8.022	9.292	13.770	24.783	25.210	30.128	30.865	31.463	32.949	41.278	42.337	42.781
5	7.099	8.007	9.290	13.770	24.789	25.222	30.135	30.872	31.469	32.954	41.274	42.346	42.791
6	7.08	8.014	9.265	13.756	24.764	25.205	30.11	30.853	31.453	32.941	41.236	42.343	42.788
RSD(%)	0.41	0.10	0.49	0.19	0.08	0.03	0.05	0.03	0.02	0.02	0.10	0.03	0.02

[00197] Table 9 Investigation of precision - peak area

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	86.389	81.088	70.658	39.372	4.535	10.075	4.994	11.022	5.082	11.861	23.819	4.340	2.741
2	87.208	81.653	71.402	39.970	4.537	10.189	5.029	11.104	5.146	11.533	24.160	4.422	2.822
3	86.960	80.981	71.572	40.510	4.431	10.220	5.070	11.262	5.175	11.461	24.156	4.353	2.739
4	86.964	81.665	71.645	40.886	4.525	10.246	5.107	11.147	5.205	11.538	24.022	4.216	2.699
5	86.009	82.039	71.157	40.719	4.466	10.115	5.077	11.282	5.114	11.728	24.059	4.231	2.703
6	85.690	81.580	70.085	40.287	4.534	10.136	5.059	11.261	5.160	11.484	23.768	4.186	2.654
RSD(%)	0.70	0.49	0.86	1.38	1.01	0.65	0.79	0.95	0.86	1.37	0.70	2.18	2.08

[00198] The results showed that the RSDs of the retention times of the characteristic peaks in the precision test ranged from 0.02% to 0.41%, and the RSDs of the peak areas ranged from 0.49% to 2.18%. The instrument exhibited good precision.

[00199] 8.3 Investigation of repeatability

[00200] Six portions of carbonized *Woodwardia unigemmata* standard decoction (batch No. BT-01) were accurately weighed, and test solutions were prepared and determined according to the established experimental method. The results are shown in Table 10-1 and Table 10-2.

[00201] Table 10-1 Investigation of repeatability - relative retention time ratio

No.	Characteristic peaks
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	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	0.77	0.86	1.00	1.49	2.68	2.73	3.26	3.34	3.40	3.57	4.46	4.59	4.64
2	0.77	0.86	1.00	1.48	2.67	2.71	3.24	3.32	3.39	3.55	4.44	4.56	4.61
3	0.77	0.86	1.00	1.48	2.67	2.72	3.24	3.33	3.39	3.55	4.44	4.57	4.61
4	0.77	0.86	1.00	1.48	2.67	2.72	3.24	3.32	3.39	3.55	4.44	4.57	4.61
5	0.77	0.86	1.00	1.49	2.68	2.73	3.26	3.34	3.41	3.57	4.47	4.59	4.64
6	0.77	0.86	1.00	1.49	2.68	2.73	3.26	3.34	3.40	3.56	4.46	4.58	4.63
RSD(%)	0.00	0.00	0.00	0.37	0.21	0.30	0.34	0.30	0.25	0.28	0.30	0.27	0.33

[00202] Table 10-2 Investigation of repeatability - relative peak area ratio

No.	Characteristic peaks									Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	1.26	1.46	1.00	0.58	0.07	0.15	0.07	0.16	0.08	0.17	0.34	0.06	0.03
2	1.23	1.43	1.00	0.56	0.06	0.14	0.07	0.16	0.07	0.16	0.33	0.06	0.04
3	1.23	1.42	1.00	0.56	0.06	0.14	0.07	0.16	0.07	0.16	0.34	0.06	0.03
4	1.23	1.42	1.00	0.56	0.06	0.14	0.07	0.16	0.07	0.16	0.33	0.06	0.03
5	1.27	1.46	1.00	0.58	0.07	0.15	0.07	0.16	0.08	0.17	0.34	0.06	0.04
6	1.27	1.46	1.00	0.58	0.07	0.15	0.07	0.16	0.08	0.17	0.27	0.06	0.04
RSD(%)	1.64	1.42	0.00	1.93	8.43	3.78	0.00	0.00	7.31	3.32	8.43	0.00	15.65

[00203] The results showed that the RSDs of the relative retention times of the characteristic peaks in the repeatability test ranged from 0.00% to 0.37%, and the RSDs of the relative peak areas ranged from 0.00% to 15.65%. The method exhibited good repeatability.

[00204] 8.4 Investigation of intermediate precision

[00205] 8.4.1 Different people at different times

[00206] Based on the above experimental conditions, *Woodwardia unigemmata* standard decoction (batch No. BT-01) were accurately weighed, and test solutions were prepared by different people at different times for determination. The results are shown in Table 11-1 and

Table11-2.

[00207] Table 11-1 Investigation of intermediate precision - relative retention time ratio

No.	Characteristic peaks										Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9					
Person A	0.77	0.86	1.00	1.49	2.68	2.73	3.26	3.34	3.40	3.57	4.46	4.59	4.64	
Person B	0.77	0.86	1.00	1.48	2.67	2.72	3.24	3.33	3.39	3.55	4.45	4.56	4.61	
Time 2	0.77	0.86	1.00	1.48	2.67	2.71	3.24	3.32	3.39	3.55	4.44	4.56	4.61	
RSD(%)	0.00	0.00	0.00	0.39	0.22	0.37	0.36	0.31	0.18	0.33	0.23	0.38	0.38	

[00208] Table 11-2 Investigation of intermediate precision - relative peak area ratio

No.	Characteristic peaks										Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9					
Person A	1.26	1.46	1.00	0.58	0.07	0.15	0.07	0.16	0.08	0.17	0.34	0.06	0.03	
Person B	1.23	1.42	1.00	0.56	0.06	0.14	0.07	0.16	0.07	0.16	0.33	0.06	0.04	
Time 2	1.23	1.42	1.00	0.55	0.06	0.14	0.07	0.16	0.07	0.16	0.34	0.06	0.03	
RSD(%)	1.40	1.62	0.00	2.72	9.12	4.03	0.00	0.00	7.89	3.54	1.72	0.00	17.33	

5

[00209] The results showed that when the same sample was determined by different people at different times, the method exhibited good precision.

[00210] 8.4.2 Investigation of different instruments

[00211] Based on the above experimental conditions, investigations were respectively conducted using Instrument 1, Instrument 2, and Instrument 3. As shown in FIG. 15, Table 12-1 and Table 12-2, FIG. 15 is the investigation using different instruments.

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[00212] Table 12-1 Investigation of different instruments - relative retention time

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Instrument 1	0.77	0.86	1.00	1.49	2.68	2.73	3.26	3.34	3.40	3.57	4.46	4.59	4.64

Instrument 2	0.69	0.86	1.00	1.82	3.37	3.41	4.01	4.09	4.16	4.34	5.32	5.46	5.51
Instrument 3	0.83	0.87	1.00	1.46	2.46	2.51	2.97	3.04	3.10	3.23	4.08	4.29	4.34
RSD(%)	9.21	0.67	0.00	12.57	16.74	16.28	15.73	15.50	15.38	15.32	13.76	12.72	12.59

[00213] Table 12-2 Investigation of different instruments - relative peak area

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Instrument 1	1.26	1.46	1.00	0.58	0.07	0.15	0.07	0.16	0.08	0.17	0.34	0.06	0.03
Instrument 2	1.21	1.53	1.00	0.56	0.06	0.15	0.07	0.15	0.08	0.15	0.05	0.06	0.04
Instrument 3	1.13	1.57	1.00	0.53	0.07	0.15	0.08	0.18	0.07	0.15	0.27	0.06	0.06
RSD(%)	5.47	3.67	0.00	4.53	8.67	0.00	7.88	9.36	7.54	7.38	68.79	0.00	35.26

[00214] The results showed that when the above three instruments were used for detection, the

- 5 RSDs of the relative retention times of the characteristic peaks ranged from 9.21% to 16.74%, and the RSDs of the relative peak areas ranged from 0.00% to 68.79%, indicating poor durability among different instruments.

[00215] 8.5 Investigation of durability

[00216] 8.5.1 Investigation of chromatographic column durability

- 10 **[00217]** Based on the above experimental conditions, investigations were respectively conducted using Column 1, Column 2, and Column 3. As shown in FIG. 16, Table 13-1 and Table 13-2, FIG. 16 is the investigation using different chromatographic columns.

[00218] Table 13-1 Investigation of chromatographic column durability - relative retention time

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Column 1	0.77	0.86	1.00	1.49	2.68	2.73	3.26	3.34	3.40	3.57	4.46	4.59	4.64
Column 2	0.67	0.97	1.00	1.33	2.41	2.43	2.89	2.92	2.99	3.10	4.01	4.09	4.16
Column 3	0.43	0.61	1.00	1.62	2.68	2.72	3.27	3.33	3.38	3.52	4.48	4.57	4.64
RSD(%)	28.04	22.69	0.00	9.82	6.02	6.49	6.90	7.50	7.10	7.60	6.16	6.41	6.19

[00219] Table 13-2 Investigation of chromatographic column durability - relative peak area

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Column 1	1.26	1.46	1.00	0.58	0.07	0.15	0.07	0.16	0.08	0.17	0.34	0.06	0.03
Column 2	1.08	1.25	1.00	0.67	0.05	0.19	0.07	0.17	0.08	0.18	0.05	0.34	0.04
Column 3	1.05	1.53	1.00	0.25	0.07	0.17	0.01	0.17	0.07	0.17	0.12	0.09	0.02
RSD(%)	10.06	10.32	0.00	44.23	18.24	11.77	69.29	3.47	7.54	3.34	89.02	94.13	33.34

[00220] As shown in FIG. 16, the chromatogram obtained using the CORTECS® UPLC® T3 column 2.1 × 150 mm, 1.6 μm (Column 1) exhibited better separation. Therefore, the CORTECS® UPLC® T3 column 2.1 × 150 mm, 1.6 μm was recommended for use.

[00221] 8.5.2 Investigation of stability

[00222] Based on the above experimental conditions, the same test solution was taken and determined at 0 h, 3 h, 6 h, 9 h, 12 h, 18 h, and 24 h, respectively. The results are shown in Table 14-1 and Table 14-2.

[00223] Table 14-1 Investigation of stability - retention time

Time (h)	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
0	7.098	7.970	9.252	13.753	24.792	25.232	30.140	30.898	31.501	32.989	41.286	42.451	42.898
3	7.134	8.003	9.319	13.791	24.822	25.242	30.169	30.909	31.506	32.995	41.352	42.446	42.889
6	7.132	8.002	9.322	13.792	24.826	25.248	30.167	30.906	31.502	32.990	41.339	42.437	42.882
9	7.125	7.994	9.306	13.783	24.821	25.245	30.165	30.909	31.505	32.994	41.337	42.444	42.888
12	7.129	7.998	9.316	13.790	24.821	25.242	30.162	30.902	31.498	32.989	41.348	42.447	42.890
18	7.134	8.001	9.325	13.794	24.682	25.247	30.173	30.913	31.510	32.994	41.360	42.442	42.883
24	7.107	7.974	9.269	13.759	24.802	25.236	30.150	30.902	31.500	32.986	41.287	42.425	42.868
RSD(%)	0.21	0.18	0.32	0.13	0.21	0.03	0.04	0.02	0.02	0.02	0.08	0.03	0.03

[00224] Table 14-2 Investigation of stability - peak area

Time (h)	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
0	86.882	100.985	69.221	40.075	4.596	10.140	5.036	11.262	5.263	12.024	23.779	4.231	2.094
3	86.505	100.089	70.518	39.216	4.513	10.062	4.951	11.134	5.220	11.536	23.682	4.177	2.243
6	87.092	100.736	70.965	39.375	4.558	10.102	4.969	11.213	5.220	11.505	23.882	4.246	2.370
9	86.706	100.356	70.673	39.385	4.536	10.063	4.985	11.185	5.224	11.446	23.705	4.194	2.259
12	87.092	100.864	71.015	39.394	4.555	10.133	5.022	11.185	5.255	11.502	23.799	4.166	2.243
18	87.008	100.876	71.083	39.435	4.560	10.147	5.027	11.213	5.235	11.507	23.997	4.253	2.343
24	86.661	100.695	70.555	39.582	4.578	10.123	5.047	11.292	5.258	11.502	23.647	4.215	2.488
RSD(%)	0.27	0.32	0.91	0.71	0.60	0.36	0.74	0.47	0.37	1.73	0.52	0.81	5.41

[00225] The results showed that the RSDs of the retention times of the corresponding characteristic peaks ranged from 0.02% to 0.21%, and the RSDs of the peak areas ranged from 0.27% to 5.41%. The sample solution was demonstrated to be relatively stable within 24 hours.

[00226] In summary, the RSDs of the relative retention times of the characteristic peaks in the above investigations all met the requirements, indicating that the method was good. The above 13 characteristic peaks were included in subsequent investigations.

[00227] Example 9: Determination of characteristic peaks and establishment of reference spectrum

[00228] 9.1 Verification of the characteristic spectrum of 20 batches of carbonized *Woodwardia unigemmata* standard decoction. Using the present method, characteristic spectrum analysis was performed on 20 batches standard decoction. The relative retention times and relative peak area ratios were calculated. The results are shown in FIG. 17, FIG. 18, Table 15-1 and Table 15-2. FIG. 17 shows the characteristic spectrum of carbonized *Woodwardia unigemmata* standard decoction (batch numbers from bottom to top were: BT-01, BT-02, BT-03, BT-04, BT-05, BT-06, BT-07, BT-08, BT-09, and BT-10). FIG. 18 shows the characteristic spectrum of carbonized *Woodwardia unigemmata* standard decoction (batch numbers from bottom to top were: BT-11, BT-12, BT-13, BT-14, BT-15, BT-16, BT-17, BT-18, BT-19, and BT-20).

[00229] Table 15-1 Relative retention time of 20 batches carbonized *Woodwardia unigemmata* standard decoction

Batch No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
BT-01	0.77	0.87	1.00	1.53	2.79	2.85	3.39	3.48	3.55	3.72	4.63	4.79	4.84
BT-02	0.77	0.87	1.00	1.53	2.78	2.84	3.38	3.48	3.55	3.71	4.62	4.78	4.83
BT-03	0.77	0.87	1.00	1.52	2.77	2.83	3.37	3.47	3.54	3.70	4.60	4.76	4.81
BT-04	0.77	0.87	1.00	1.52	2.78	2.84	3.38	3.47	3.54	3.71	4.61	4.77	4.82
BT-05	0.77	0.87	1.00	1.52	2.76	2.81	3.35	3.44	3.51	3.68	4.58	4.73	4.78
BT-06	0.77	0.89	1.00	1.52	2.76	2.82	3.36	3.45	3.52	3.68	4.58	4.74	4.79
BT-07	0.77	0.87	1.00	1.52	2.77	2.83	3.37	3.46	3.53	3.69	4.60	4.75	4.80
BT-08	0.77	0.87	1.00	1.51	2.75	2.80	3.34	3.43	3.50	3.67	4.56	4.71	4.76
BT-09	0.77	0.87	1.00	1.51	2.73	2.79	3.32	3.41	3.48	3.64	4.54	4.69	4.74
BT-10	0.77	0.86	1.00	1.51	2.75	2.80	3.34	3.43	3.50	3.66	4.57	4.71	4.76
BT-11	0.77	0.86	1.00	1.51	2.74	2.79	3.33	3.42	3.48	3.65	4.55	4.69	4.74
BT-12	0.77	0.86	1.00	1.50	2.72	2.77	3.31	3.39	3.46	3.62	4.52	4.66	4.71
BT-13	0.77	0.86	1.00	1.50	2.72	2.77	3.30	3.39	3.46	3.62	4.52	4.66	4.71
BT-14	0.77	0.87	1.00	1.50	2.73	2.79	3.32	3.41	3.47	3.64	4.54	4.68	4.73
BT-15	0.76	0.86	1.00	1.51	2.74	2.79	3.33	3.41	3.48	3.64	4.55	4.69	4.74
BT-16	0.77	0.86	1.00	1.50	2.72	2.77	3.31	3.39	3.46	3.62	4.52	4.66	4.71
BT-17	0.76	0.86	1.00	1.51	2.74	2.80	3.33	3.42	3.49	3.66	4.56	4.70	4.75
BT-18	0.77	0.86	1.00	1.51	2.74	2.80	3.34	3.43	3.49	3.66	4.56	4.71	4.76
BT-19	0.77	0.87	1.00	1.51	2.75	2.81	3.35	3.44	3.50	3.67	4.57	4.72	4.77
BT-20	0.77	0.87	1.00	1.52	2.76	2.82	3.36	3.46	3.52	3.69	4.59	4.75	4.80
RSD(%)	0.40	0.85	0.00	0.62	0.77	0.86	0.78	0.86	0.86	0.85	0.72	0.85	0.85
Average	0.77	0.87	0.00	1.51	2.75	2.81	3.34	3.43	3.50	3.67	4.57	4.72	4.77
90%	0.69	0.78	0.00	1.36	2.48	2.53	3.01	3.09	3.15	3.30	4.11	4.25	4.29
110%	0.85	0.96	0.00	1.66	3.03	3.09	3.67	3.77	3.85	4.04	5.03	5.19	5.25

[00230] Table 15-2 Relative peak area of 20 batches carbonized *Woodwardia unigemmata* standard decoction

Batch No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
BT-01	1.30	1.47	1.00	0.57	0.07	0.16	0.07	0.15	0.09	0.14	0.28	0.06	0.05
BT-02	1.29	1.45	1.00	0.57	0.06	0.16	0.07	0.16	0.08	0.15	0.34	0.05	0.04
BT-03	1.29	1.43	1.00	0.57	0.06	0.17	0.06	0.14	0.08	0.14	0.34	0.03	0.02
BT-04	1.28	1.45	1.00	0.56	0.07	0.16	0.06	0.16	0.08	0.15	0.33	0.05	0.05
BT-05	1.31	1.39	1.00	0.58	0.06	0.16	0.07	0.16	0.08	0.15	0.33	0.05	0.05
BT-06	1.29	1.39	1.00	0.58	0.06	0.15	0.06	0.15	0.09	0.14	0.34	0.05	0.05
BT-07	1.29	1.44	1.00	0.58	0.07	0.15	0.06	0.16	0.09	0.15	0.32	0.05	0.04
BT-08	1.34	1.41	1.00	0.58	0.06	0.16	0.06	0.16	0.08	0.16	0.33	0.04	0.02
BT-09	1.43	1.49	1.00	0.62	0.07	0.16	0.06	0.17	0.10	0.17	0.36	0.05	0.04
BT-10	1.35	1.44	1.00	0.58	0.07	0.16	0.05	0.17	0.09	0.15	0.34	0.03	0.01
BT-11	1.44	1.50	1.00	0.62	0.07	0.16	0.04	0.17	0.09	0.15	0.38	0.04	0.04
BT-12	1.66	1.73	1.00	0.72	0.09	0.19	0.04	0.21	0.11	0.18	0.44	0.07	0.06
BT-13	1.65	1.71	1.00	0.71	0.08	0.19	0.05	0.19	0.10	0.18	0.42	0.04	0.02
BT-14	1.50	1.56	1.00	0.64	0.07	0.16	0.05	0.19	0.09	0.17	0.39	0.06	0.04
BT-15	1.45	1.52	1.00	0.62	0.07	0.16	0.04	0.19	0.09	0.16	0.36	0.06	0.05
BT-16	1.63	1.70	1.00	0.70	0.08	0.19	0.05	0.20	0.10	0.17	0.41	0.06	0.06
BT-17	1.32	1.44	1.00	0.59	0.07	0.15	0.04	0.16	0.08	0.14	0.35	0.03	0.03
BT-18	1.34	1.41	1.00	0.58	0.07	0.15	0.05	0.16	0.08	0.14	0.34	0.05	0.04
BT-19	1.30	1.38	1.00	0.56	0.07	0.15	0.05	0.16	0.08	0.12	0.28	0.03	0.02
BT-20	1.25	1.40	1.00	0.56	0.06	0.15	0.05	0.15	0.07	0.13	0.06	0.04	0.03
RSD(%)	9.42	7.30	0.00	8.40	11.43	8.16	18.43	11.03	11.05	10.59	22.83	24.99	37.80

[00231] According to the principles of stable relative retention times, detectability in all batches, and relatively high peak intensities, a total of 13 peaks with good repeatability were selected as characteristic peaks. The results showed that when Peak 3 was used as the S peak, the RSDs of the relative retention times of the characteristic peaks of 20 batches of carbonized *Woodwardia unigemmata* standard decoction ranged from 0.40% to 0.86%, and the RSDs of the relative retention times of the 13 characteristic peaks in the 20 batches carbonized *Woodwardia unigemmata* standard decoction were all less than 10%.

[00232] It was finally specified that 13 characteristic peaks should be present in the chromatogram of the test sample. Except for Peak 1 and Peak 2, all peaks should correspond in retention time to the 11 characteristic peaks in the chromatogram of the reference medicinal

material. The peak corresponding to the protocatechuic acid reference substance was designated as the S peak. The relative retention times of each characteristic peak to the S peak were calculated, and the relative retention times were required to fall within $\pm 10\%$ of the specified values. The specified values were: 0.77 (Peak 1), 0.87 (Peak 2), 1.51 (Peak 4), 2.75 (Peak 5), 2.81 (Peak 6), 3.34 (Peak 7), 3.43 (Peak 8), 3.50 (Peak 9), 3.67 (Peak 10), 4.57 (Peak 11), 4.72 (Peak 12), and 4.77 (Peak 13). FIG. 19 shows the reference characteristic spectrum of *Woodwardia unigemmata* standard decoction. Peak 1: 5-hydroxymethylfurfural; Peak 3 (S): protocatechuic acid. Note: chromatographic column: CORTECS® UPLC® T3 2.1 $\times$ 150 mm, 1.6 μ m.

[00233] Example 10: Chromatographic conditions of carbonized *Woodwardia unigemmata* formula granules

[00234] In the chromatographic conditions and system suitability tests, octadecylsilane chemically bonded silica was used as the packing material (column length: 150 mm, inner diameter: 2.1 mm, particle size: 1.6 μ m); acetonitrile was used as mobile phase A; 0.1% formic acid solution was used as mobile phase B; gradient elution was performed according to the conditions specified in the table below; the column temperature was 35 $^{\circ}$ C; the flow rate was 0.3 mL/min; and the detection wavelength was 250 nm. The number of theoretical plates, calculated based on the protocatechuic acid peak, should not be less than 5000.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-8	1	99
8-18	1 $\rightarrow$ 15	99 $\rightarrow$ 85
18-35	15 $\rightarrow$ 25	85 $\rightarrow$ 75
35-50	25 $\rightarrow$ 26	75 $\rightarrow$ 74

[00235] 10.1 Preparation of reference solution

[00236] 1 g of *Woodwardia unigemmata* reference medical material was weighed, placed into a stoppered conical flask, and added with 50 mL of water, followed by boiling for 30 minutes. After filtration, the filtrate was evaporated to dryness. The residue was added with 25 mL of 70% methanol and subjected to ultrasonic treatment (power: 600 W, frequency: 40 kHz) for 30 minutes, shaken up, and filtered. The subsequent filtrate was collected to obtain a reference

medical material solution. In addition, an appropriate amount of protocatechuic acid reference substance was accurately weighed and precisely diluted with methanol to prepare a solution containing 20 µg of protocatechuic acid per 1 mL, which was used as a reference substance solution.

5 **[00237]** 10.2 Preparation of test solution

[00238] Carbonized *Woodwardia unigemmata* formula granules was taken and ground into fine powder. About 0.2 g was weighed, placed into a stoppered conical flask, and added with 50 mL of 70% methanol and subjected to ultrasonic treatment (power: 600 W, frequency: 40 kHz) for 30 minutes, taken out, and cooled. The subsequent filtrate was collected to obtain the test solution.

10 **[00239]** 10.3 Determination method

[00240] 1 µL of each of the test solution and the reference substance solution was precisely aspirated and injected into the liquid chromatograph for determination.

[00241] 10.4 Selection of detection wavelength

[00242] The wavelength was selected at 250 nm.

15 **[00243]** 10.5 Investigation of mobile phase composition

[00244] Based on the above experimental conditions, mobile phases of acetonitrile-water, acetonitrile-0.1% formic acid, and acetonitrile-0.1% phosphoric acid were respectively investigated, as shown in FIG. 20. FIG. 20 is the investigation of mobile phase composition. The results indicated that when acetonitrile-0.1% formic acid was used as the mobile phase, good chromatographic peak separation was achieved. Therefore, acetonitrile-0.1% formic acid was selected as the mobile phase.

[00245] 10.6 Investigation of column temperature

[00246] Based on the above experimental conditions, column temperatures of 25 °C, 30 °C, and 35 °C were respectively investigated, as shown in FIG. 21. FIG. 21 is the chromatograms obtained from the investigation of column temperature.

[00247] The results indicated that good chromatographic peak separation was obtained at a column temperature of 35 °C. Accordingly, the column temperature was temporarily set at 35 °C.

[00248] 10.7 Investigation of flow rate

[00249] Based on the above-established experimental conditions, flow rates of 0.25 mL/min, 0.30 mL/min, and 0.35 mL/min were respectively investigated, as shown in FIG. 22. FIG. 22 is the investigation of flow rate.

- 5 **[00250]** The results indicated that when the flow rates were 0.25 mL/min, 0.30 mL/min, and 0.35 mL/min, the information content of the chromatographic peaks of each characteristic peak was substantially consistent. When the flow rate was 0.30 mL/min, better chromatographic peak separation was obtained. Therefore, the flow rate was determined to be 0.30 mL/min.

[00251] 10.8 Investigation of delay

- 10 **[00252]** Based on the above experimental conditions, the chromatogram acquisition time was set to twice the original time. The results are shown in FIG. 23. FIG. 23 is the investigation of delay. The results indicated that no chromatographic peaks appeared after 50 minutes.

- [00253]** In summary, the chromatographic conditions and system suitability test for the characteristic spectrum of *Woodwardia unigemmata* formula granules were determined as follows:
- 15 octadecylsilane chemically bonded silica was used as the packing material (column length: 150 mm, inner diameter: 2.1 mm, particle size: 1.6 μ m); acetonitrile was used as mobile phase A; 0.1% formic acid solution was used as mobile phase B; gradient elution was performed according to the conditions specified in the table below; the column temperature was 35 $^{\circ}$ C; the flow rate was 0.3 mL/min; and the detection wavelength was 250 nm. The number of theoretical plates,
- 20 calculated based on the protocatchuic acid peak, should not be less than 5000.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-8	6	94
8-18	6→15	94→85
18-35	15→25	85→75
35-50	25→26	75→74

[00254] Example 11: Optimization of pretreatment method for *Woodwardia unigemmata* formula granules

[00255] 11.1 Investigation of extraction method

[00256] Reflux extraction and ultrasonic extraction were compared. The chromatographic peak information obtained by the two extraction methods was generally similar. Therefore, ultrasonic extraction, which was simpler to operate, was finally selected. The results are shown in FIG. 24. FIG. 24 is a chromatogram obtained by using different extraction methods.

5 **[00257]** 11.2 Investigation of extraction solvent

[00258] Methanol, water, 50% methanol, and 70% methanol were investigated as extraction solvents. The results showed that the chromatographic peak information obtained using 70% methanol as the extraction solvent was more abundant and exhibited better resolution. Therefore, 70% methanol was selected as the extraction solvent. The results are shown in FIG. 25. FIG. 25 is the chromatograms obtained by extracting with different solvents.

[00259] 11.3 Investigation of extraction duration

[00260] Ultrasonic extraction for 15 minutes, 30 minutes, and 60 minutes was compared. The chromatograms were essentially consistent. Therefore, the extraction duration was determined to be 15 minutes. The results are shown in FIG. 26. FIG. 26 is the chromatograms obtained by extracting for different durations.

[00261] 11.4 Investigation of extraction solvent volume

[00262] Extraction solvent volumes of 25 mL, 50 mL, and 100 mL were compared. When the extraction solvent volume was 25 mL, the chromatographic peak sizes were more appropriate. Therefore, the extraction solvent volume was determined to be 25 mL. The results are shown in FIG. 27. FIG. 27 is the chromatograms obtained by extracting with different volumes of solvent.

[00263] 11.5 Finally determined test sample preparation method

[00264] An appropriate amount of the sample was taken and ground into fine powder. About 0.2 g was weighed, placed into a stoppered conical flask, 25 mL of 70% methanol was added, and subjected to ultrasonic treatment (power: 600 W; frequency: 40 kHz) for 15 minutes, shaken up and filtered. The subsequent filtrate was collected to obtain the test solution.

[00265] Example 12: Methodological investigation of *Woodwardia unigemmata* formula granules

[00266] 12.1 Identification of chromatographic peaks

[00267] Preparation of the test solution: The test solution of carbonized *Woodwardia unigemmata* formula granules was prepared according to the finally determined preparation method.

[00268] Preparation of the reference standard solution: An appropriate amount of protocathechuic acid reference substance was accurately weighed and dissolved in methanol to prepare a reference substance solution containing 20 µg of protocathechuic acid per 1 mL. And an appropriate amount of 5-hydroxymethylfurfural reference substance was accurately weighed and dissolved in methanol to prepare a reference substance solution containing 50 µg of 5-hydroxymethylfurfural per 1 mL.

[00269] 1 g of *Woodwardia unigemmata* reference medicinal material was weighed and placed into a stoppered conical flask, and 50 mL of water was added and boiled for 30 minutes. After filtration, the filtrate was evaporated to dryness. The residue was added with 25 mL of 70% methanol, subjected to ultrasonic treatment (power: 600 W; frequency: 40 kHz) for 15 minutes, shaken up, and filtered. The subsequent filtrate was collected and used as the reference medicinal material solution.

[00270] Preparation of the negative control solution: According to the above experimental conditions, a negative control solution lacking *Woodwardia unigemmata* formula granules was prepared.

[00271] The characteristic chromatographic peaks of *Woodwardia unigemmata* formula granules were located. As shown in FIG. 28, FIG. 28 is the results of chromatographic peak identification.

[00272] 12.2 Investigation of precision

[00273] The test solution of *Woodwardia unigemmata* formula granules (batch No. KL-01) was taken and continuously injected six times according to the established experimental method, with an injection volume of 1 µL each time. The retention times and peak areas of the characteristic peaks were calculated. The results are shown in Table 16-1 and Table 16-2.

[00274] Table 16-1 Investigation of precision - retention time

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak	Peak	Peak	Peak

										10	11	12	13
1	7.075	7.920	9.251	13.746	24.772	25.219	30.122	30.867	31.467	32.950	41.191	42.351	42.795
2	7.078	7.922	9.257	13.752	24.774	25.215	30.116	30.860	31.458	32.942	41.166	42.326	42.770
3	7.079	7.924	9.261	13.753	24.771	25.211	30.125	30.866	31.464	32.947	41.172	42.331	42.777
4	7.078	7.923	9.261	13.756	24.768	25.209	30.118	30.862	31.463	32.947	41.169	42.328	42.771
5	7.069	7.915	9.243	13.741	24.757	25.206	30.110	30.861	31.463	32.949	41.177	42.336	42.780
6	7.071	7.915	9.239	13.738	24.752	25.202	30.1	30.852	31.454	32.937	41.172	42.335	42.779
RSD(%)	0.06	0.06	0.11	0.06	0.04	0.03	0.04	0.02	0.02	0.02	0.03	0.03	0.03

[00275] Table 16-2 Investigation of precision - peak area

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	52.748	69.318	81.030	29.835	3.863	33.085	6.450	27.417	15.340	25.390	16.640	9.416	6.444
2	52.390	69.786	81.643	29.805	3.848	32.638	6.370	27.031	15.128	24.889	15.878	9.343	6.347
3	52.761	70.352	82.433	29.972	3.904	32.869	6.457	27.299	15.181	25.098	16.351	9.302	6.408
4	52.724	70.473	82.620	29.955	3.858	32.787	6.429	27.222	15.197	25.055	16.046	9.357	6.388
5	52.735	67.782	81.980	29.902	3.864	32.793	6.362	27.202	15.119	25.088	15.675	9.323	6.344
6	52.740	68.011	78.651	29.845	3.915	32.762	6.184	27.240	15.128	24.987	15.619	9.346	6.330
RSD(%)	0.28	1.67	1.8	0.23	0.71	0.46	1.6	0.47	0.56	0.68	2.49	0.42	0.7

[00276] The results showed that the RSDs of the retention times of the characteristic peaks in the precision test ranged from 0.02% to 0.11%, and the RSDs of the peak areas ranged from 0.23% to 2.49%. The instrument exhibited good precision.

[00277] 12.3 Investigation of repeatability

[00278] Six portions of carbonized *Woodwardia unigemmata* formula granules (batch No. KL-01) were accurately weighed, and test solutions were prepared and determined according to the established experimental method. The results are shown in Table 17-1 and Table 17-2.

[00279] Table 17-1 Investigation of repeatability - relative retention time ratio

No.	Characteristic peaks
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	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	0.77	0.86	1.00	1.49	2.68	2.72	3.25	3.34	3.40	3.56	4.45	4.58	4.63
2	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.58	4.63
3	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62
4	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.58	4.62
5	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.40	3.56	4.45	4.57	4.62
6	0.77	0.86	1.00	1.48	2.67	2.72	3.25	3.33	3.39	3.55	4.44	4.57	4.62
RSD(%)	0.00	0.00	0.00	0.28	0.16	0.00	0.00	0.13	0.13	0.12	0.10	0.12	0.12

[00280] Table 17-2 Investigation of repeatability - relative peak area ratio

No.	Characteristic peaks									Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9				
1	0.65	0.87	1.00	0.35	0.05	0.39	0.08	0.33	0.19	0.30	0.19	0.11	0.06
2	0.64	0.87	1.00	0.35	0.05	0.40	0.08	0.33	0.18	0.30	0.15	0.11	0.07
3	0.65	0.87	1.00	0.35	0.05	0.39	0.08	0.32	0.19	0.30	0.18	0.11	0.07
4	0.64	0.87	1.00	0.35	0.05	0.39	0.08	0.32	0.18	0.30	0.19	0.11	0.07
5	0.60	0.83	1.00	0.34	0.05	0.42	0.08	0.35	0.20	0.32	0.20	0.12	0.07
6	0.64	0.86	1.00	0.34	0.05	0.39	0.08	0.32	0.18	0.30	0.18	0.11	0.07
RSD(%)	2.93	1.86	0.00	1.49	0.00	3.06	0.00	3.57	4.38	2.70	9.49	3.66	5.98

[00281] The results showed that the RSDs of the relative retention times of the characteristic peaks in the repeatability test ranged from 0.00% to 0.28%, and the RSDs of the relative peak areas ranged from 0.00% to 9.49%. The method exhibited good repeatability.

[00282] 12.4 Investigation of intermediate precision

[00283] 12.4.1 Different people at different times

[00284] Based on the above experimental conditions, *Woodwardia unigemmata* formula granules were taken, and test solutions were prepared by different people at different times for determination. The results are shown in Table 18-1 and Table 18-2.

[00285] Table 18-1 Investigation of intermediate precision - relative retention time ratio

No.	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Person A	0.77	0.86	1.00	1.49	2.68	2.72	3.25	3.34	3.40	3.56	4.45	4.58	4.63
Person B	0.77	0.86	1.00	1.48	2.67	2.71	3.24	3.32	3.38	3.54	4.43	4.56	4.61
Time 2	0.76	0.86	1.00	1.48	2.66	2.71	3.24	3.32	3.38	3.54	4.43	4.55	4.60
RSD(%)	0.76	0.00	0.00	0.39	0.38	0.22	0.18	0.35	0.35	0.33	0.27	0.34	0.33

[00286] Table 18-2 Investigation of intermediate precision - relative peak area ratio

No.	Characteristic peaks										Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9					
Person A	0.65	0.87	1.00	0.35	0.05	0.39	0.08	0.33	0.19	0.30	0.19	0.11	0.06	
Person B	0.63	0.85	1.00	0.34	0.05	0.39	0.08	0.32	0.18	0.30	0.19	0.11	0.07	
Time 2	0.64	0.87	1.00	0.34	0.05	0.39	0.08	0.32	0.18	0.30	0.19	0.11	0.07	
RSD(%)	1.57	1.34	0.00	1.69	0.00	0.00	0.00	1.79	3.15	0.00	0.00	0.00	8.67	

[00287] The results showed that when the same sample was determined by different people at different times, the method exhibited good precision.

[00288] 12.4.2 Investigation of durability of instruments

[00289] Based on the above experimental conditions, investigations were respectively conducted using Instrument 1, Instrument 2, and Instrument 3. As shown in FIG. 29, Table 19-1 and Table 19-2, FIG. 29 is the investigation using different instruments.

[00290] Table 19-1 Investigation of chromatographic column durability - relative retention time

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Instrument 1	0.77	0.86	1.00	1.49	2.68	2.72	3.25	3.34	3.40	3.56	4.45	4.58	4.63
Instrument 2	0.69	0.86	1.00	1.82	3.38	3.41	4.02	4.10	4.17	4.35	5.33	5.47	5.52
Instrument 3	0.83	0.87	1.00	1.46	2.45	2.50	2.95	3.03	3.09	3.22	4.06	4.27	4.31
RSD(%)	9.21	0.67	0.00	12.57	17.08	16.51	16.21	15.78	15.65	15.63	14.11	13.06	13.01

[00291] Table 19-2 Investigation of chromatographic column durability - relative peak area

Instrument	Characteristic peaks									Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9				
Instrument 1	0.65	0.87	1.00	0.35	0.05	0.39	0.08	0.33	0.19	0.30	0.19	0.11	0.06
Instrument 2	0.63	0.92	1.00	0.35	0.04	0.39	0.08	0.32	0.17	0.29	0.09	0.11	0.08
Instrument 3	0.60	0.93	1.00	0.38	0.05	0.40	0.08	0.35	0.18	0.30	0.10	0.11	0.07
RSD(%)	4.02	3.55	0.00	4.82	12.38	1.47	0.00	4.59	5.56	1.95	43.49	0.00	14.29

[00292] The results showed that when the above three instruments were used for detection, the RSDs of the relative retention times of the characteristic peaks ranged from 0.67% to 17.08%, and the RSDs of the relative peak areas ranged from 0.00% to 43.49%, indicating poor durability among different instruments.

[00293] 12.5 Investigation of durability

[00294] 12.5.1 Investigation of chromatographic column durability

[00295] Based on the above experimental conditions, investigations were respectively conducted using Column 1, Column 2, and Column 3. As shown in FIG. 30, Table 20-1 and Table 20-2, FIG. 30 is the investigation using different chromatographic columns.

[00296] Table 20-1 Investigation of chromatographic column durability - relative retention time

Instrument	Characteristic peaks									Peak 10	Peak 11	Peak 12	Peak 13
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9				
Column 1	0.77	0.86	1.00	1.49	2.68	2.72	3.25	3.34	3.40	3.56	4.45	4.58	4.63
Column 2	0.67	0.96	1.00	1.33	2.41	2.43	2.89	2.93	2.99	3.11	4.02	4.12	4.17
Column 3	0.43	0.61	1.00	1.62	2.69	2.74	3.28	3.34	3.53	3.39	4.50	4.59	4.66
RSD(%)	28.04	22.26	0.00	9.82	6.13	6.60	6.92	7.39	8.53	6.78	6.11	6.07	6.13

[00297] Table 20-2 Investigation of chromatographic column durability - relative peak area

Instrument	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Column 1	0.65	0.87	1.00	0.35	0.05	0.39	0.08	0.33	0.19	0.30	0.19	0.11	0.06
Column 2	0.55	0.86	1.00	0.49	0.08	0.53	0.10	0.42	0.21	0.38	0.11	0.26	0.09
Column 3	0.50	0.98	1.00	0.24	0.04	0.46	0.01	0.38	0.19	0.34	0.14	0.06	0.06
RSD(%)	13.48	7.38	0.00	34.81	36.74	15.22	74.62	11.98	5.88	11.77	27.56	72.62	24.75

[00298] As shown in FIG. 30, the chromatogram obtained using the CORTECS® UPLC® T3 column 2.1 × 150 mm, 1.6 μm (Column 1) exhibited better separation. Therefore, the CORTECS® UPLC® T3 column 2.1 × 150 mm, 1.6 μm was recommended for use.

5 **[00299]** 12.6 Investigation of stability

[00300] Based on the above experimental conditions, the same test solution was taken and determined at 0 h, 3 h, 6 h, 9 h, 12 h, 18 h, and 24 h, respectively. The results are shown in Table 21-1 and Table 21-2.

[00301] Table 21-1 Investigation of stability - retention time

Time (h)	Characteristic peaks												
	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
0	7.109	7.979	9.266	13.761	24.806	25.243	30.153	30.905	31.508	32.996	41.278	42.455	42.901
3	7.132	8.000	9.316	13.790	24.826	25.245	30.175	30.915	31.511	32.999	41.264	42.444	42.886
6	7.129	7.996	9.308	13.784	24.809	25.234	30.158	30.900	31.498	32.986	41.266	42.443	42.887
9	7.129	7.996	9.309	13.784	24.821	25.247	30.166	30.906	31.504	32.992	41.262	42.440	42.884
12	7.133	8.000	9.318	13.792	24.820	25.243	30.172	30.912	31.507	32.992	41.264	42.443	42.887
18	7.135	7.999	9.320	13.791	24.821	25.242	30.167	30.905	31.502	32.988	41.254	42.433	42.874
24	7.106	7.973	9.274	13.764	24.803	25.238	30.151	30.899	31.498	32.986	41.233	42.406	42.851
RSD(%)	0.17	0.14	0.24	0.10	0.04	0.02	0.04	0.02	0.02	0.02	0.04	0.04	0.04

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[00302] Table 21-2 Investigation of stability - peak area

Time (h)	Characteristic peaks												
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	Peak 1	Peak 2	Peak 3 (S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
0	53.000	71.509	81.807	28.944	3.911	32.230	6.268	26.703	15.367	24.752	15.662	9.241	4.751
3	53.322	72.234	83.928	28.837	3.834	32.906	6.369	27.081	15.647	25.217	16.491	9.434	5.347
6	53.085	71.789	83.315	28.634	3.873	32.551	6.329	26.848	15.423	24.963	16.242	9.321	5.326
9	53.268	72.013	83.607	28.639	3.861	32.613	6.326	26.897	15.460	24.919	16.320	9.285	5.395
12	52.908	71.598	83.131	28.500	3.828	32.223	6.301	26.607	15.300	24.629	16.083	9.196	5.284
18	53.445	72.398	83.875	28.882	3.910	32.593	6.339	26.932	15.367	24.889	16.159	9.322	5.651
24	53.426	72.342	83.276	28.902	3.879	32.683	6.396	26.924	15.345	24.950	15.732	9.381	6.056
RSD(%)	0.40	0.50	0.86	0.59	0.86	0.76	0.67	0.59	0.75	0.74	1.89	0.87	7.32

[00303] The results showed that the RSDs of the retention times of the corresponding characteristic peaks ranged from 0.02% to 0.24%, and the RSDs of the peak areas ranged from 0.40% to 7.32%. The sample solution was demonstrated to be relatively stable within 24 hours.

- 5 **[00304]** In summary, the RSDs of the relative retention times of the characteristic peaks in the above investigations all met the requirements, indicating that the method was good. The above 13 characteristic peaks were included in subsequent investigations.

[00305] Example 13 : Determination of characteristic peaks and establishment of reference spectrum

- 10 **[00306]** 13.1 Verification of the characteristic spectrum of batches of *Woodwardia unigemmata* formula granules

- [00307]** Using the present method, characteristic spectrum determination was performed on 3 batches sample. The relative retention times and relative peak area were calculated. The results are shown in FIG. 31, Table 22-1 and Table 22-2. FIG. 31 shows the characteristic spectrum of *Woodwardia unigemmata* formula granules of 3 batches.

[00308] Table 22-1 3 batches carbonized *Woodwardia unigemmata* formula granules - relative retention time

No.	Batch No.	Characteristic peaks											
		Peak 1	Peak 2	Peak 3(S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12

1	Validation 1	0.77	0.87	1.00	1.50	2.71	2.76	3.30	3.38	3.45	3.61	4.51	4.64	4.69
2	Validation 2	0.77	0.87	1.00	1.50	2.72	2.77	3.30	3.39	3.46	3.62	4.52	4.65	4.70
3	Validation 3	0.77	0.87	1.00	1.50	2.72	2.78	3.32	3.40	3.47	3.63	4.54	4.67	4.72
	RSD%	0.00	0.00	0.00	0.00	0.22	0.37	0.35	0.30	0.29	0.28	0.34	0.33	0.33
	Average	0.77	0.87	-	1.50	2.72	2.77	3.30	3.39	3.46	3.62	4.53	4.65	4.70
	90%	0.69	0.78	-	1.35	2.45	2.49	2.97	3.05	3.11	3.26	4.08	4.19	4.23
	110%	0.85	0.96	-	1.65	2.99	3.05	3.63	3.73	3.81	3.98	4.98	5.12	5.17

[00309] Table 22-2 3 batches carbonized *Woodwardia unigemmata* formula granules - relative peak area

No.	Batch No.	Characteristic peaks												
		Peak 1	Peak 2	Peak 3(S)	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
1	Validation 1	0.49	0.74	1.00	0.31	0.03	0.43	0.08	0.31	0.19	0.30	0.15	0.11	0.08
2	Validation 2	0.49	0.74	1.00	0.31	0.03	0.44	0.08	0.31	0.19	0.30	0.17	0.11	0.08
3	Validation 3	0.49	0.74	1.00	0.31	0.03	0.44	0.08	0.31	0.19	0.30	0.15	0.11	0.08
	RSD%	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	7.38	0.00	0.00

5 **[00310]** According to the principles of stable relative retention times, detectability in all batches, and relatively high peak intensities, a total of 13 peaks with good repeatability were selected as characteristic peaks. The results showed that when Peak 3 was used as the S peak, the RSDs of the relative retention times of the characteristic peaks of 3 batches of carbonized *Woodwardia unigemmata* formula granules ranged from 0.00% to 0.37%, and the RSDs of the relative retention times of the 13 characteristic peaks in the 3 batches carbonized *Woodwardia unigemmata* formula granules were all less than 10%.

15 **[00311]** It was finally specified that 13 characteristic peaks should be present in the chromatogram of the test sample. Except for Peak 1 and Peak 2, all peaks should correspond in retention time to the 11 characteristic peaks in the chromatogram of the reference medicinal material. The peak corresponding to the protocatechuic acid reference substance was designated as the S peak. The relative retention times of each characteristic peak to the S peak were

calculated, and the relative retention times were required to fall within $\pm 10\%$ of the specified values. The specified values were: 0.77 (Peak 1), 0.87 (Peak 2), 1.50 (Peak 4), 2.72 (Peak 5), 2.77 (Peak 6), 3.30 (Peak 7), 3.39 (Peak 8), 3.46 (Peak 9), 3.62 (Peak 10), 4.53 (Peak 11), 4.65 (Peak 12), and 4.70 (Peak 13).

5 **[00312]** Using the Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2012 edition), 3 batches of carbonized *Woodwardia unigemmata* formula granules were combined, and a reference chromatogram of the characteristic spectrum of the carbonized *Woodwardia unigemmata* formula granules was established. The results are shown in FIG. 32. FIG. 32 is the reference characteristic spectrum of carbonized *Woodwardia unigemmata*
10 formula granules. Peak 1: 5-hydroxymethylfurfural; Peak 3 (S): protocatechuic acid; chromatographic column: CORTECS® UPLC® T3 2.1 $\times$ 150 mm, 1.6 μ m.

[00313] Comparative Example 1

[00314] *Woodwardia unigemmata* medical material was determined under the same conditions as those in Example 1. The results are shown in FIG. 33 to FIG. 35, wherein FIG. 33 and FIG. 34
15 are the characteristic spectrum of the *Woodwardia unigemmata* medical material, and FIG. 35 is a reference characteristic spectrum of the *Woodwardia unigemmata* medical material. Peak 1 (S): protocatechuic acid. Note: chromatographic column: CORTECS® UPLC® T3, 2.1 mm $\times$ 150 mm, 1.6 μ m. From the above results, it was demonstrated that, by using the method of the present disclosure, *Woodwardia unigemmata* medical material decoction pieces and carbonized
20 *Woodwardia unigemmata* medical material decoction pieces could be effectively identified.

[00315] Comparative Example 2

[00316] A *Woodwardia unigemmata* standard decoction was determined under the same conditions as those in Example 1. The results are shown in FIG. 36 to FIG. 38, wherein FIG. 36 is the characteristic spectrum of the standard decoction of *Woodwardia unigemmata* (batch
25 numbers from bottom to top were: BT-01, BT-02, BT-03, BT-04, BT-05, BT-06, BT-07, BT-08, BT-09, and BT-10), FIG. 37 is the characteristic spectrum of the standard decoction of *Woodwardia unigemmata* (batch numbers from bottom to top were: BT-11, BT-12, BT-13, BT-14, BT-15, BT-16, BT-17, BT-18, BT-19, and BT-20) and FIG. 38 is a reference characteristic spectrum of the standard decoction of *Woodwardia unigemmata*. Peak 1 (S): protocatechuic acid.

Note: chromatographic column: CORTECS® UPLC® T3, 2.1 mm × 150 mm, 1.6 μm. From the above results, it was demonstrated that, by using the method of the present disclosure, the *Woodwardia unigemmata* standard decoction and the carbonized *Woodwardia unigemmata* standard decoction could be effectively identified.

5 **[00317]** Comparative Example 3

[00318] *Woodwardia unigemmata* formula granules were determined under the same conditions as those in Example 1. The results are shown in FIG. 39 to FIG. 40, wherein FIG. 39 is a characteristic spectrum of *Woodwardia unigemmata* formula granules (from bottom to top: KL-001, KL-002, and KL-003), and FIG. 40 is a reference characteristic spectrum of *Woodwardia unigemmata* formula granules. Peak 1 (S): protocathechuic acid. Note: chromatographic column: CORTECS® UPLC® T3, 2.1 mm × 150 mm, 1.6 μm. From the above results, it was demonstrated that, by using the method of the present disclosure, the *Woodwardia unigemmata* formula granules and the carbonized *Woodwardia unigemmata* formula granules could be effectively identified.

15 **[00319]** Compared with the carbonized *Woodwardia unigemmata*, *Woodwardia unigemmata* and the preparations thereof exhibited only 11 peaks, lacking Peak 1 and Peak 2, thereby enabling clear identification between *Woodwardia unigemmata* and the carbonized *Woodwardia unigemmata*.

[00320] Comparative Example 4

20 **[00321]** The comparative example 4 was performed under the same conditions as those in Example 1 except that different gradient elution conditions were adopted:

[00322] Mobile phase gradient:

[00323] Gradient 1:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-10	1→15	99→85
10-35	15→25	85→75
35-50	25	75

[00324] The results are shown in FIG. 41. As could be seen from the figure, Peaks 1 and 2 were

absent, Peak 5 was split, and the resolution between Peaks 12 and 13 was poor.

[00325] Gradient 2:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-10	1→5	99→95
10-18	5→15	95→85
18-35	15→25	85→75
35-50	25	75

[00326] The results are shown in FIG. 41. As could be seen from the figure, Peaks 2 and 3 were not separated, and the resolution between Peaks 12 and 13 was poor.

5 **[00327]** Gradient 3:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0-5	1→5	99→95
5-20	5→20	95→80
20-35	20→25	80→75
35-50	25	75

[00328] The results are shown in FIG. 41. As could be seen from the figure, Peaks 11, 12, and 13 were absent.

[00329] From the above results, it was demonstrated that only the gradient elution conditions of the present disclosure were capable of achieving good chromatographic peak separation.

10 **[00330]** The above descriptions were merely preferred embodiments of the present disclosure. It should be noted that, for those skilled in the art, various modifications and refinements could be made without departing from the principles of the present disclosure, and such modifications and refinements should also be regarded as falling within the scope of protection of the present disclosure.

CLAIMS

[Claim 1] A method for constructing a standard decoction characteristic spectrum of *Woodwardia unigemmata* and carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules, comprising:

A) dissolving a carbonized *Woodwardia unigemmata* raw material in a solvent to obtain a test solution;

B) subjecting the test solution to high-performance liquid chromatography to obtain a UPLC characteristic spectrum of the carbonized *Woodwardia unigemmata* raw material;

wherein chromatographic conditions of the high-performance liquid chromatography are: a C18 chromatographic column; acetonitrile as mobile phase A; 0.1% formic acid as mobile phase B; and gradient elution.

[Claim 2] The method according to claim 1, wherein the method further comprises preparing a reference solution: respectively taking protocatechuic acid and 5-hydroxymethylfurfural, boiling in water, then filtering, and dissolving filter residue with 70% methanol to obtain the reference solution; wherein a concentration of the reference substance solution is 20 $\mu\text{g/mL}$;

determining the reference substance solution by high-performance liquid chromatography to obtain a chromatogram of the reference substance; and based on the chromatogram of the reference substance, qualitatively determining the components in the UPLC characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules.

[Claim 3] The method according to claim 1, wherein the gradient elution is specifically as follows:

0-8 min, mobile phase A: 1%, mobile phase B: 99%;

8-18 min, mobile phase A: 1%-15%, mobile phase B: 99%-85%;

18-35 min, mobile phase A: 15%-25%, mobile phase B: 85%-75%;

35-50 min, mobile phase A: 25%-26%, mobile phase B: 75%-74%.

[Claim 4] The method according to claim 3, wherein the chromatographic column is a C18
5 column with a specification of 1.6 μm , 2.1 $\times$ 150 mm, and a column temperature is 35 $^{\circ}\text{C}$.

[Claim 5] The method according to claim 4, wherein a flow rate of the mobile phase is 0.3 mL/min, a detection wavelength is 250 nm, and an injection volume is 1 μL .

10 [Claim 6] The method according to claim 1, wherein a Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System is used to evaluate similarity of the UPLC characteristic spectrum of carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, and formula granules, to obtain a UPLC standard characteristic spectrum composed of 13 characteristic peaks, wherein Peak 1 is hydroxymethylfurfural and Peak 3 is protocatechuic
15 acid.

[Claim 7] The method according to claim 6, wherein, in the characteristic spectrum of the carbonized *Woodwardia unigemmata* standard decoctions, protocatechuic acid is used as a reference peak S, relative retention times of the characteristic peaks relative to the S peak are
20 calculated, and the relative retention times fall within $\pm 10\%$ of specified values, wherein the specified values are respectively: 0.77 (Peak 1), 0.87 (Peak 2), 1.51 (Peak 4), 2.75 (Peak 5), 2.81 (Peak 6), 3.34 (Peak 7), 3.43 (Peak 8), 3.50 (Peak 9), 3.67 (Peak 10), 4.57 (Peak 11), 4.72 (Peak 12), and 4.77 (Peak 13);

wherein in the characteristic spectrum of carbonized *Woodwardia unigemmata* decoction
25 pieces, protocatechuic acid is used as the reference peak S, relative retention times of the characteristic peaks relative to the S peak are calculated, and the relative retention times falling within $\pm 10\%$ of specified values, wherein the specified values are respectively: 0.77 (Peak 1), 0.86 (Peak 2), 1.48 (Peak 4), 2.67 (Peak 5), 2.72 (Peak 6), 3.24 (Peak 7), 3.32 (Peak 8), 3.39

(Peak 9), 3.55 (Peak 10), 4.44 (Peak 11), 4.57 (Peak 12), and 4.61 (Peak 13);

wherein in the characteristic spectrum of carbonized *Woodwardia unigemmata* formula granules, protocatechuic acid is used as the reference peak S, relative retention times of the characteristic peaks relative to the S peak are calculated, and relative retention times fall within
5 $\pm 10\%$ of specified values, wherein the specified values are respectively: 0.77 (Peak 1), 0.87 (Peak 2), 1.50 (Peak 4), 2.72 (Peak 5), 2.77 (Peak 6), 3.30 (Peak 7), 3.39 (Peak 8), 3.46 (Peak 9), 3.62 (Peak 10), 4.53 (Peak 11), 4.65 (Peak 12), and 4.70 (Peak 13).

[Claim 8] The method according to claim 1, wherein in step A), the solvent is 70%
10 methanol; the extraction method is water boiling followed by ultrasonic extraction; an ultrasonic power is 600 W; a frequency is 40 kHz; and an extraction duration is 15-45 min.

[Claim 9] The method according to claim 8, wherein in step A), a mass (g) of the carbonized *Woodwardia unigemmata* raw material to a volume (mL) of the solvent is in a ratio of
15 (0.1-1):(10-50).

[Claim 10] The method according to claim 1, wherein in step A), the carbonized *Woodwardia unigemmata* raw material is carbonized *Woodwardia unigemmata* decoction pieces, standard decoctions, or formula granules.