

**TITLE OF INVENTION: THIN LAYER IDENTIFICATION METHOD FOR
FORMULATION GRANULES OF *LYCOPODIASTRUM CASUARINOIDES* AND
*LYCOPODIUM JAPONICUM***

5 **[0001]** This application claims the priority of Chinese Patent Application No. 202311397059.4, filed with the China National Intellectual Property Administration on October 25, 2023, and titled with “THIN LAYER IDENTIFICATION METHOD FOR FORMULATION GRANULES OF *LYCOPODIASTRUM CASUARINOIDES* AND *LYCOPODIUM JAPONICUM*”, which is hereby incorporated by reference in its entirety.

10 **FIELD**

[0002] The present disclosure relates to the technical field of analytical detection, especially relates to a method for identifying *Lycopodium casuarinoides* formula granules and *Lycopodium japonicum* formula granules by thin-layer chromatography.

15 **BACKGROUND**

[0003] Hanging clubmoss herba is the dried aerial part of *Lycopodium casuarinoides* (Spring) Holub, a plant of family Lycopodiaceae. It has the effects of dispelling wind and eliminating dampness, and relaxing tendons and promoting blood circulation. It is used for treating rheumatic numbness, traumatic injury, musculoskeletal pain, irregular menstruation, and the like.

[0004] Lycopodium herba is the dried whole herb of *Lycopodium japonicum* Thunb, a plant of family Lycopodiaceae. It has the effects of dispelling wind and eliminating dampness, and relaxing tendons and activating collaterals. It is used for treating joint soreness and pain, difficulty in flexion and extension, and the like.

25 **[0005]** In clinical practice, *Lycopodium casuarinoides* is mainly used for treating rheumatic diseases, whereas *Lycopodium japonicum* is mainly used for treating muscle and skeletal injuries; therefore, the two should not be used interchangeably. However, the method for identifying *Lycopodium casuarinoides* formula granules by thin-layer chromatography in the current

standards is not yet well established, and there is no effective means to distinguish them from the easily confused drug, i.e., *Lycopodium japonicum* formula granules.

[0006] Accordingly, it is necessary to establish a method for effectively identifying *Lycopodiastrum casuarinoides* formula granules by thin-layer chromatography and distinguishing
5 it from the easily confused formulation, i.e., *Lycopodium japonicum* formula granules.

[0007] SUMMARY

[0008] In view of this, the technical problem to be solved by the present disclosure is to provide a method for identifying *Lycopodiastrum casuarinoides* formula granules and *Lycopodium*
10 *japonicum* formula granules by thin-layer chromatography.

[0009] In the present disclosure, the terms “comprise”, “include”, and “have” are used interchangeably and are intended to indicate a comprisable meaning, implying that the embodiment may include elements other than those listed. It should also be understood that the use of the terms “comprise”, “include”, and “have” herein also provides an embodiment involving “consisting of”.

15 [0010] In the present disclosure, the term “and/or” is used to describe an association relationship between associated objects, indicating that three relationships may exist. For example, “A and/or B” may represent: A alone, A and B together, or B alone. A and B may be singular or plural.

[0011] In the present disclosure, “at least one” refers to one or more, and “a plurality of” refers to two or more. “At least one of” or similar expressions refers to any combination of the following
20 items, including any combination of a single item or a plurality of items.

[0012] It should be understood that, in various embodiments of the present disclosure, the numerical order of the processes described below does not necessarily indicate the order of execution. Some or all of the steps may be executed in parallel or sequentially. The execution order of each process should be determined according to its functions and internal logic and shall not
25 constitute any limitation to the implementation process of the embodiments of the present disclosure.

[0013] The present disclosure provide a method for identifying *Lycopodiastrum casuarinoides* formula granules and *Lycopodium japonicum* formula granules by thin-layer chromatography,

comprising:

[0014] A) taking a sample to be identified, pretreating to obtain a sample solution;

[0015] B) taking *Lycopodiastrum casuarinoides* reference medicinal material, pretreating to obtain a reference medicinal material solution;

5 [0016] C) performing thin layer chromatographic detection on the sample solution and the reference medicine material solution, wherein a thin layer plate is a silica gel G thin layer plate, and a developing solvent is cyclohexane - ethyl acetate; and

[0017] D) observing under ultraviolet light, wherein in the case that a blue fluorescent spot is present at an R_f value of 0.31 to 0.464, the sample is *Lycopodiastrum casuarinoides* formula
10 granules; and in case that no blue fluorescent spot is present at an R_f value of 0.31 to 0.464, the sample is *Lycopodium japonicum* formula granules.

[0018] In the present disclosure, in step A), the pretreatment specifically comprises: taking a sample to be identified, dissolving the sample in petroleum ether, performing heating for reflux extraction, discarding petroleum ether solution, performing decoction by adding water, filtering,
15 performing ultrasonic treatment by adding methanol, filtering, and dissolving residue in methanol to obtain the sample solution.

[0019] Specifically, a mass-to-volume ratio of the sample to be identified, water, and methanol is 1 g : 100 mL : 40 mL.

[0020] In some embodiments, the heating for reflux extraction is performed for a duration of 2h
20 to 3h; the decoction is performed for a duration of 1.5h to 2h; the ultrasonic treatment is performed for a duration of 30 min to 40 min; and the petroleum ether has a boiling point of 60 °C to 90 °C.

[0021] In some embodiments, the heating for reflux extraction is performed for a duration of 3h; the decoction is performed for a duration of 2h; the ultrasonic treatment is performed for a duration of 30 min; and the petroleum ether has a boiling point of 60 °C to 90 °C.

25 [0022] *Lycopodiastrum casuarinoides* reference medicinal material is taken and pretreated to obtain the reference medical material solution. The pretreatment specifically comprises taking the *Lycopodiastrum casuarinoides* reference medicinal material, dissolving the material in petroleum ether, performing heating for reflux extraction, discarding petroleum ether solution, performing

decoction by adding water, filtering, performing ultrasonic treatment by adding methanol, filtering, and dissolving residue in methanol to obtain the test solution.

[0023] Specifically, a mass-to-volume ratio of the sample to be identified, water, and methanol is 1 g : 100 mL : 40 mL.

5 [0024] In some embodiments, the heating for reflux extraction is performed for a duration of 1h to 2h; the decoction is performed for a duration of 1.5h to 2h; the ultrasonic treatment is performed for a duration of 30 min to 40 min; and the petroleum ether has a boiling point of 60 °C to 90 °C.

[0025] In some embodiments, the heating reflux extraction is performed for a duration of 2h; the decoction is performed for a duration of 2h; the ultrasonic treatment is performed for a duration of
10 30 min; and the petroleum ether has a boiling point of 60 °C to 90 °C.

[0026] The sample solution and the reference medical material solution are tested by thin-layer chromatography.

[0027] Preferably, the sample solution and the reference medicinal material solution are respectively loaded on the same silica gel G thin-layer plate. The plate is developed with a
15 developing solvent, taken out and dried, and then observed under ultraviolet light.

[0028] In the present disclosure, the ultraviolet light has a wavelength of 365 nm.

[0029] In the present disclosure, the thin-layer plate is a silica gel G thin-layer plate. The plate may be selected from the group consisting of a cuttable thin-layer chromatography plate available from Tianjin Silida Technology Co., Ltd., a pre-coated silica gel G plate available from Merck, or
20 a pre-coated silica gel G plate available from Qingdao Ocean Chemical Factory Branch.

[0030] The results showed that, among the three thin-layer plates, a blue fluorescent spot was present at a distance of 3.193 cm to 5.130 cm from the origin and retention factor (Rf) values were 0.424, 0.310, and 0.354, respectively, all of which met the identification requirements and can be used for identifying *Lycopodiastrium casuarinoides* formula granules.

25 [0031] Upon observation under ultraviolet light, if a blue fluorescent spot is present at an Rf value of 0.31 to 0.464 in the chromatogram of a sample, the sample is identified as *Lycopodiastrium casuarinoides* formula granules; if no blue fluorescent spot is present at an Rf value of 0.31 to 0.464, the sample is identified as *Lycopodium japonicum* formula granules.

[0032] The Rf value may be 0.31 to 0.464, and specifically may be 0.421, 0.437, 0.424, 0.310, 0.354, 0.417, 0.428, 0.481, 0.460, 0.468, 0.459, or 0.409.

[0033] In the present disclosure, the loading amount of the sample solution is 1 μ L to 5 μ L, and the loading amount of the reference medicinal material solution is 1 μ L to 3 μ L.

5 [0034] In some preferred embodiments of the present disclosure, a mass ratio of cyclohexane to ethyl acetate is 5:4, and the petroleum ether has a boiling point of 60°C to 90 °C.

[0035] In the present disclosure, the observation is performed at a temperature of 4 °C to 35 °C. The method of the present disclosure shows good adaptability to different temperatures. A blue fluorescent spot is present for *Lycopodiastrum casuarinoides* formula granules at retention factors
10 of 0.417, 0.428, and 0.481, whereas no fluorescent spot is present for *Lycopodium japonicum* formula granules.

[0036] In the present disclosure, the observation is performed at a humidity of 32% RH to 75% RH. The method of the present disclosure shows good adaptability to different humidities. A blue fluorescent spot is present for *Lycopodiastrum casuarinoides* formula granules at retention factors
15 of 0.584, 0.613, and 0.599, whereas no fluorescent spot is present for *Lycopodium japonicum* formula granules.

[0037] In the present disclosure, the sample to be identified is *Lycopodiastrum casuarinoides* formula granules and/or *Lycopodium japonicum* formula granules.

[0038] The present disclosure provide a method for identifying *Lycopodiastrum casuarinoides*
20 formula granules and *Lycopodium japonicum* formula granules by thin-layer chromatography, comprising: A) taking a sample to be identified, pretreating to obtain a sample solution; B) taking *Lycopodiastrum casuarinoides* reference medicinal material, pretreating to obtain a reference medicinal material solution; C) performing thin layer chromatographic detection on the sample solution and the reference medicine material solution, wherein a thin layer plate is a silica gel G
25 thin layer plate, and a developing solvent is cyclohexane - ethyl acetate; and D) observing under ultraviolet light, wherein in the case that a blue fluorescent spot is present at an Rf value of 0.31 to 0.464, the sample is *Lycopodiastrum casuarinoides* formula granules; and in case that no blue fluorescent spot is present at an Rf value of 0.31 to 0.464, the sample is *Lycopodium japonicum* formula granules. The present disclosure establishes a method for identifying *Lycopodiastrum*

casuarinoides formula granules and *Lycopodium japonicum* formula granules by thin-layer chromatography. By observing whether a fluorescent spot is present within a specified retention factor range in the thin-layer chromatogram, an identification point is established, thereby enabling rapid and effective identification of *Lycopodiastrum casuarinoides* formula granules and
5 *Lycopodium japonicum* formula granules.

BRIEF DESCRIPTION OF DRAWINGS

- [0039] FIG. 1 is the investigation of sample loading amount;
- [0040] FIG. 2 is the investigation of specificity;
- 10 [0041] FIG. 3 is the investigation of the thin layer plate from Tianjin Silida;
- [0042] FIG. 4 is the investigation of the thin layer plate from Merck;
- [0043] FIG. 5 is the investigation of thin layer plate from Qingdao Ocean;
- [0044] FIG. 6 is the investigation of the temperature of 4°C;
- [0045] FIG. 7 is the investigation of the temperature of 25°C;
- 15 [0046] FIG. 8 is the investigation of the temperature of 35°C;
- [0047] FIG. 9 is the investigation of the humidity of 32%;
- [0048] FIG. 10 is the investigation of the humidity of 51%;
- [0049] FIG. 11 is the investigation of the humidity of 75%;
- [0050] FIG. 12 is the verification of multi-batches;
- 20 [0051] FIG. 13 is the result of the method of comparative example 1;
- [0052] FIG. 14 is the result of the method of comparative example 2;
- [0053] FIG. 15 is the result of the method of comparative example 2.

DETAILED DESCRIPTION

- 25 [0054] In order to further illustrate the present disclosure, the thin-layer chromatography

identification method for *Lycopodiastrum casuarinoides* formula granules and *Lycopodium japonicum* formula granules provided by the present disclosure is described in detail below with reference to the following examples.

[0055] Water bath; Soxhlet extractor; heating plate; thin-layer imaging system: CAMAG TLC

5 Visualizer; silica gel G thin-layer plates (Qingdao Ocean Chemical Factory, batch No. 20180527; Tianjin Silida Technology Co., Ltd., batch No. 191016; Merck, batch No. HX87183353).

[0056] Petroleum ether (60~90°C), methanol, cyclohexane, ethyl acetate were all of analytical grade and water was ultrapure water (prepared in the laboratory).

[0057] *Lycopodiastrum casuarinoides* reference medicinal material (National Institutes for Food
10 and Drug Control, batch No. 380003-202104); *Lycopodiastrum casuarinoides* formula granules (Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd., batch Nos. 2302078, 2302079 and 2302080); *Lycopodium japonicum* formula granules (Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd., batch Nos. 2002001, 2002002 and 2002003).

[0058] Example 1

15 **[0059]** Preparation of the sample solution

[0060] 1 g of the formula granules was taken, ground into fine powder, and an appropriate amount of petroleum ether (60-90°C) was added. The mixture was placed in a Soxhlet extractor and subjected to heating under reflux for 3 h. The petroleum ether solution was discarded, and the residue was evaporated to dryness. 100 mL of water was then added, and the mixture was decocted
20 for 2 h, followed by filtration. The filtrate was evaporated to dryness, and 40 mL of methanol was added to the residue. The mixture was subjected to ultrasonic treatment for 30 min and filtered. The filtrate was evaporated to dryness, and the residue was dissolved in 1 mL of methanol to obtain a sample solution. Thin-layer chromatography was carried out according to the method described in the 2020 edition of the Chinese Pharmacopoeia, General Chapter 0502. 1 µL of the sample
25 solution and 1 µL of the reference medicinal material solution were respectively loaded on the same silica gel G thin-layer plate. Cyclohexane - ethyl acetate (5:4) was used as a developing solvent. The plate was developed, removed, dried, and then observed under ultraviolet light (365 nm). In the chromatogram of the sample, a fluorescent spot having the same color was present at the position corresponding to that of the chromatogram of the reference medicinal material.

[0061] Preparation of the reference and the reference medicinal material solution

[0062] 2 g of the crude medicinal material powder was taken, and an appropriate amount of petroleum ether (60-90°C) was added. The mixture was placed in a Soxhlet extractor and subjected to heating under reflux for 2 h. The petroleum ether solution was discarded, and the residue was evaporated to dryness. 100 mL of water was then added, and the mixture was decocted for 2 h and filtered. The filtrate was evaporated to dryness, and 40 mL of methanol was added to the residue. The mixture was subjected to ultrasonic treatment for 30 min and filtered. The filtrate was evaporated to dryness, and the residue was dissolved in 1 mL of methanol to obtain the reference medicinal material solution.

[0063] Example 2: methodological investigation

[0064] 2.1 Investigation of loading amount

[0065] Under the above-determined experimental conditions, 1 µL, 3 µL, 5 µL, 10 µL, 15 µL, and 20 µL of the *Lycopodium casuarinoides* reference medicinal material solution and the *Lycopodium casuarinoides* sample solution were respectively loaded on the same silica gel G thin-layer plate. The fluorescent spots in the thin-layer chromatogram were clearly developed. Accordingly, the loading amount was determined to be 1-3µL for the reference medicinal material solution and 1-5 µL for the sample solution. A spot having the same color was present at an R_f value of 0.421, which was used as the identification spot for *Lycopodium casuarinoides* formula granules.

[0066] 2.2 Investigation of specificity

[0067] According to the above preparation method for the sample solution, a *Lycopodium casuarinoides* reference medicinal material solution, a *Lycopodium casuarinoides* formula granule solution, and a negative solution were prepared respectively. A test of identifying by thin-layer chromatography was carried out and developed under the determined chromatographic conditions. The results are shown in FIG. 2. As can be seen from the figure, the negative sample showed no interference with the sample of *Lycopodium casuarinoides* formula granules, indicating that the method has good specificity and can be used for identifying *Lycopodium casuarinoides* formula granules.

[0068] 2.3 Investigation of durability

[0069] 2.3.1 Comparison of different thin-layer plates

[0070] A cuttable thin-layer chromatography plate from Tianjin Silida Technology Co., Ltd., a pre-coated silica gel G plate from Merck, and a pre-coated silica gel G plate from Qingdao Ocean Chemical Factory Branch were selected. The tests were performed according to the determined experimental method, and the results are shown in FIGs. 3-5. The results showed that a blue fluorescent spot was present at positions 3.193-5.130 cm from the origin on the three thin-layer plates, with retention factors (R_f) of 0.424, 0.310, and 0.354, respectively. All of these met the identification requirements and could be used for identifying *Lycopodium casuarinoides* formula granules.

[0071] The results are shown in FIG. 3. FIG. 3 is the investigation of the thin layer plate from Tianjin Silida. 1, *Lycopodium casuarinoides* reference medicinal material solution; 2-4, *Lycopodium casuarinoides* formula granule solution.

[0072] FIG. 4 is the investigation of the thin layer plate from Merck. 1, *Lycopodium casuarinoides* reference medicinal material solution; 2-4, *Lycopodium casuarinoides* formula granule solution.

[0073] FIG. 5 is the investigation of thin layer plate from Qingdao Ocean. 1, *Lycopodium casuarinoides* reference medicinal material solution; 2-4, *Lycopodium casuarinoides* formula granule solution.

[0074] 2.3.2 Comparison of different temperatures

[0075] The loaded thin-layer plates were developed under different temperature conditions respectively: a low temperature of 4 °C, a room temperature of 25 °C, and a high temperature of 35 °C. As shown in FIGs. 6-8, the method exhibited good adaptability to different temperature conditions. Blue fluorescent spots were present with retention factor (R_f) of 0.417, 0.428, and 0.481, respectively, which could be used for identifying *Lycopodium casuarinoides* formula granules.

[0076] FIG. 6 is the investigation of the temperature of 4°C. 1, *Lycopodium casuarinoides* reference medicinal material solution; 2-4, *Lycopodium casuarinoides* formula granule solution.

[0077] FIG. 7 is the investigation of the temperature of 25°C. 1, *Lycopodium casuarinoides* reference medicinal material solution; 2-4, *Lycopodium casuarinoides* formula granule solution.

[0078] FIG. 8 is the investigation of the temperature of 35°C. 1, *Lycopodiastrum casuarinoides* reference medicinal material solution; 2-4, *Lycopodiastrum casuarinoides* formula granule solution.

[0079] 2.3.3 Comparison of different humilities

[0080] The loaded thin-layer plates were developed under 32%RH, 51%RH and 75%RH humidity conditions, respectively. As shown in FIGs. 9-11, the method exhibited good adaptability to different temperature conditions. Blue fluorescent spots were present with retention factor (Rf) of 0.584, 0.613, and 0.599, respectively, which could be used for identifying *Lycopodiastrum casuarinoides* formula granules.

[0081] FIG. 9 is the investigation of the humidity of 32%.1, *Lycopodiastrum casuarinoides* reference medicinal material solution; 2-4, *Lycopodiastrum casuarinoides* formula granule solution.

[0082] FIG. 10 is the investigation of the humidity of 51%.1, *Lycopodiastrum casuarinoides* reference medicinal material solution; 2-4, *Lycopodiastrum casuarinoides* formula granule solution.

[0083] FIG. 11 is the investigation of the humidity of 75%.1, *Lycopodiastrum casuarinoides* reference medicinal material solution; 2-4, *Lycopodiastrum casuarinoides* formula granule solution.

[0084] 2.3.4 Verification

[0085] Thin-layer chromatographic verification was carried out on three batches of *Lycopodiastrum casuarinoides* formula granules. A blue fluorescent spot was present at a position 5.03 cm from the origin, with a retention factor (Rf) of 0.409. The experimental results are shown in FIG. 12.

[0086] FIG. 12 is the verification result of multi-batches. 1, *Lycopodiastrum casuarinoides* reference medicinal material solution; 2-4, *Lycopodiastrum casuarinoides* formula granule solution.

[0087] 2.4 Identification of *Lycopodiastrum casuarinoides* formula granules and *Lycopodium japonicum* formula granules

[0088] According to the above preparation method for the sample solution, a *Lycopodiastrum casuarinoides* formula granule solution and a *Lycopodium japonicum* formula granule solution were prepared respectively, and thin-layer chromatographic identification tests were carried out. For *Lycopodiastrum casuarinoides* formula granules, a blue fluorescent spot was present at a position 5.2 cm from the origin with a retention factor (Rf) of 0.591, whereas no spot was present

at the corresponding position for *Lycopodium japonicum* formula granules. The results are shown in FIG. 13. 1, 2, *Lycopodiastrium casuarinoides* formula granules; 3, 4, *Lycopodium japonicum* formula granules. As can be seen from the figure, the method can be used to identify *Lycopodiastrium casuarinoides* formula granules from *Lycopodium japonicum* formula granules.

5 [0089] 2.5 Determination of Rf value of the identification spot for *Lycopodiastrium casuarinoides* formula granules and *Lycopodium japonicum* formula granules

[0090] All retention factor data obtained from the thin-layer chromatographic methodological investigation were summarized, and the results are shown in Table 1. According to the summarized results, the retention factor (Rf) of the identification spot for *Lycopodiastrium casuarinoides*
10 formula granules was determined to fall within a 10% range, and the specified value was set at 0.482.

[0091] Table 1 Summary of retention factor data from methodological investigation

Test	Identification spot 1			
	Retention factors	Average value	RSD%	Range
Loading amount	0.421	0.422	11.520	0.310-0.464
Specificity	0.437			
Silida thin-layer plate	0.424			
Merck thin-layer plate	0.310			
Qingdao Ocean thin-layer plate	0.354			
Temperature of 4°C	0.417			
Temperature of 22°C	0.428			
Temperature of 35°C	0.481			
Humidity of 32%RH	0.460			
Humidity of 51%RH	0.468			
Humidity of 75%RH	0.459			
Verification of multi-batches	0.409			

[0092] Comparative Example 1

15 [0093] 2 g of the formula granules was taken, ground into fine powder, and 40 mL of water was added. The mixture was decocted for 3 h and filtered. The filtrate was evaporated to dryness, and 20 mL of methanol was added. The mixture was subjected to ultrasonic treatment for 30 min and filtered. The filtrate was evaporated to dryness, and the residue was dissolved in 2 mL of methanol

to obtain a sample solution. In addition, 1 g of *Lycopodium casuarinoides* reference medicinal material and 2 g of a negative control sample without *Lycopodium casuarinoides* were taken and prepared in the same manner to obtain a reference medicinal material solution and a negative control solution, respectively. 5 µL of each of the above solutions were respectively loaded on the same silica gel G thin-layer plate. Cyclohexane - ethyl acetate (8:5) was used as the developing solvent. The plate was developed, removed, dried, and then observed under ultraviolet light (365 nm). In the chromatogram of the test sample, no red spot was present at the position corresponding to that of the chromatogram of the reference medicinal material, and the negative control sample showed no interference. The results are shown in FIG. 14. FIG. 14 is the results of Comparative Example 1. 1-2, *Lycopodium casuarinoides* reference medicinal material solution; 3-4, *Lycopodium casuarinoides* formula granule solutions; 5, negative control solution. The results indicated that, using the above thin-layer chromatographic identification method, the spots of the *Lycopodium casuarinoides* reference medicinal material were inconsistent with those of the formula granules, and thus the two could not be identified.

[0094] Comparative Example 2

[0095] 2 g of the formula granules was taken, ground into a fine powder, and 5 mL of ammonia solution was added and mixed uniformly. Then, 20 mL of chloroform was added, and the mixture was subjected to heating under reflux for 2 h and filtered. The filtrate was transferred into a separatory funnel, and 20 mL of a 2% sulfuric acid solution was added and shaken. The acidic aqueous layer was collected, adjusted to pH 8-9 with a sodium hydroxide solution, and transferred into a separatory funnel. Chloroform extraction was carried out twice with 10 mL each time. The combined chloroform extracts were concentrated to 2 mL to obtain a sample solution. In addition, 2 g of *Lycopodium casuarinoides* reference medicinal material and 2 g of a negative control sample without *Lycopodium casuarinoides* were taken and prepared in the same manner to obtain a reference medicinal material solution and a negative control solution, respectively. 10 µL of each of the above solutions were respectively loaded on the same silica gel G thin-layer plate. Chloroform - methanol - acetone - ammonia (40:5:5:2) was used as the developing solvent. The plate was developed, removed, and dried. Color development was carried out using an equal mixture of iodine - potassium iodide solution and potassium bismuth iodide solution. The plate was then observed under daylight. In the chromatogram of the sample, a yellow-brown spot was present

at the position corresponding to that of the chromatogram of the reference medicinal material, and the negative control showed no interference. The results are shown in FIG. 15.

[0096] FIG. 15 is the result of Comparative Example 2. 1, negative control solution; 2, *Lycopodiastrum casuarinoides* reference medicinal material solution; 3-5, *Lycopodiastrum casuarinoides* formula granule solutions.

[0097] The results indicated that, using the above thin-layer chromatographic identification method, the spots of *Lycopodiastrum casuarinoides* formula granules were too few and not sufficiently well-defined, and thus the two could not be identified.

[0098] The above descriptions are merely preferred embodiments of the present disclosure. It should be noted that, for those skilled in the art, various modifications and improvements may be made without departing from the principle of the present disclosure, and such modifications and improvements shall also fall within the scope of protection of the present disclosure.

CLAIMS

[Claim 1] A method for identifying *Lycopodiastrum casuarinoides* formula granules and *Lycopodium japonicum* formula granules by thin-layer chromatography, comprising:

5 A) taking a sample to be identified, pretreating to obtain a sample solution;

 B) taking *Lycopodiastrum casuarinoides* reference medicinal material, pretreating to obtain a reference medicinal material solution;

 C) performing thin layer chromatographic detection on the sample solution and the reference medicine material solution, wherein a thin layer plate is a silica gel G thin layer plate, and a
10 developing solvent is cyclohexane - ethyl acetate; and

 D) observing under ultraviolet light, wherein in the case that a blue fluorescent spot is present at an Rf value of 0.31 to 0.464, the sample is *Lycopodiastrum casuarinoides* formula granules; and in case that no blue fluorescent spot is present at an Rf value of 0.31 to 0.464, the sample is *Lycopodium japonicum* formula granules.

15 [Claim 2] The method according to claim 1, wherein in step A), the pretreatment specifically comprises:

 taking a sample to be identified, dissolving the sample in petroleum ether, performing heating for reflux extraction, discarding petroleum ether solution, performing decoction by adding water, filtering, performing ultrasonic treatment by adding methanol, filtering, and dissolving residue in
20 methanol to obtain the sample solution.

 [Claim 3] The method according to claim 2, wherein a mass-to-volume ratio of the sample to be identified, water, and methanol is 1 g : 100 mL : 40 mL;

 wherein the heating for reflux extraction is performed for a duration of 2h to 3h; the decoction is performed for a duration of 1.5h to 2h; the ultrasonic treatment is performed for a duration of 30
25 min to 40 min; and the petroleum ether has a boiling point of 60 °C to 90 °C.

 [Claim 4] The method according to claim 1, wherein in step B), the pretreatment specifically comprises:

 taking the *Lycopodiastrum casuarinoides* reference medicinal material, dissolving the

material in petroleum ether, performing heating for reflux extraction, discarding petroleum ether solution, performing decoction by adding water, filtering, performing ultrasonic treatment by adding methanol, filtering, and dissolving residue in methanol to obtain the test solution;

5 wherein the heating for reflux extraction is performed for a duration of 1h to 2h; the decoction is performed for a duration of 1.5h to 2h; the ultrasonic treatment is performed for a duration of 30 min to 40 min; and the petroleum ether has a boiling point of 60 °C to 90 °C.

[Claim 5] The method according to claim 1, wherein a loading amount of the sample solution is 1μL to 5μL, and a loading amount of the reference medicinal material solution is 1μL to 3μL.

10 [Claim 6] The method according to claim 1, wherein in step D), a mass ratio of cyclohexane to ethyl acetate is 5:4.

[Claim 7] The method according to claim 1, wherein in step E), the ultraviolet light has a wavelength of 365 nm.

[Claim 8] The method according to claim 1, wherein in step E), the observation is performed at a temperature of 4 °C to 35 °C.

15 [Claim 9] The method according to claim 8, wherein in step E), the observation is performed at a humidity of 32% RH to 75% RH.

[Claim 10] The method according to claim 1, wherein in step A), the sample to be identified is *Lycopodiastrum casuarinoides* formula granules and/or *Lycopodium japonicum* formula granules.

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