

THIN-LAYER CHROMATOGRAPHY IDENTIFICATION METHOD FOR RHIZOMA DIOSCOREAE HYPOGLAUCAE FORMULA GRANULES

[0001] This application claims the priority of Chinese Patent Application No. 202310812079.7,
5 filed with the China National Intellectual Property Administration on July 4, 2023, and titled with
“THIN-LAYER CHROMATOGRAPHY IDENTIFICATION METHOD FOR RHIZOMA
DIOSCOREAE HYPOGLAUCAE FORMULA GRANULES”, which is hereby incorporated by
reference in its entirety.

10 FIELD

[0002] The present disclosure relates to the technical field of analytical detection, and
particularly to a method for identifying Bixie formula granules by thin layer chromatography
(TLC).

15 BACKGROUND

[0003] Bixie refers to the dried rhizome of *Smilax glauco-china* Warb or *Smilax ferox* Wall. ex
Kunth of the genus *Smilax* in the family Liliaceae. It has the efficacies of dispelling wind and
removing dampness, detoxifying and reducing swelling. It is used for rheumatic arthralgia,
impaired joint movement, lumbago and back pain, sores and carbuncles, cutaneous wind tinea,
20 dysentery, and the like.

[0004] According to the “Technical Requirements for Quality Control and Standard Formulation
of Traditional Chinese Medicine Formula granules (Draft for Comment)” issued by the National
Medical Products Administration, the raw medicine materials used for traditional Chinese medicine
formula granules shall have fixed taxonomic origins. Macroscopic identification is an effective
25 method for identifying *Smilax glauco-china* Warb and *Smilax ferox* Wall. ex Kunth. However,
traditional Chinese medicine formula granules are prepared from medicine materials through the
processes of processing, decoction, concentration, drying, mixing, and granulation. After decoction,
the original macroscopic characteristics of the medicine materials are destroyed, and thus

macroscopic identification methods are not suitable to identify the traditional Chinese medicine formula granules.

[0005] At present, there is no effective method for identifying Bixie (*Smilax glauco-china* Warb) formula granules and Bixie (*Smilax ferox* Wall. ex Kunth) formula granules. Therefore, it is necessary to establish a method capable of distinguishing Bixie (*Smilax glauco-china* Warb) formula granules from Bixie (*Smilax ferox* Wall. ex Kunth) formula granules.

SUMMARY

[0006] In view of this, the technical problem to be solved by the present disclosure is to provide a method for identifying Bixie traditional Chinese medicine formula granules by thin layer chromatography.

[0007] As used herein, the terms “comprise,” “include” and “have” are used interchangeably to denote inclusiveness of a solution, meaning that the solution may include elements other than those listed. It should also be understood that the description using “comprise,” “include,” and “have” also provides a solution defined by “consist of.”

[0008] In the present disclosure, the term “and/or” describes the associative relationship of the related objects and indicates three possible relationships. For example, A and/or B may refer to: A alone, A and B together, or B alone, wherein A and B may be singular or plural.

[0009] In the present disclosure, “at least one” refers to one or more, and “more” refers to two or more. The expression “at least one of the following” or similar expressions refer to any combination of the listed items, including any single item or any combination of multiple items.

[0010] It should be understood that, in various embodiments of the present disclosure, the sequence numbers of the steps described herein do not imply the order of conduction. Some or all steps may be conducted in parallel or sequentially. The conduction order of the steps shall be determined by their functions and inherent logic, and shall not be construed as limiting the embodiments of the present disclosure.

[0011] The present disclosure is intended to establish a method for identifying Bixie (*Smilax glauco-china* Warb) formula granules and Bixie (*Smilax ferox* Wall. ex Kunth) formula granules by

thin layer chromatography. By observing whether a fluorescent spot is present within a specified Rf value range on the TLC plate as an identification spot, it can rapidly and effectively identify Bixie (*Smilax glauco-china* Warb) formula granules and Bixie (*Smilax ferox* Wall. ex Kunth) formula granules.

5 **[0012]** The present disclosure provides a method for identifying Bixie formula granules by thin layer chromatography, comprising:

[0013] A) taking a sample to be identified, dissolving with a solvent to obtain a sample solution; wherein the sample to be identified is *Smilax glauco-china* Warb formula granules or *Smilax ferox* Wall. ex Kunth formula granules;

10 **[0014]** B) taking resveratrol as reference substance, dissolving with a solvent to obtain a reference substance solution;

[0015] C) taking *Smilax ferox* Wall. ex Kunth as reference medicine material, dissolving with a solvent to obtain a reference medicine material solution;

15 **[0016]** D) performing thin layer chromatographic detection on the sample solution, the reference substance solution and the reference medicine material solution, wherein a TLC plate is a silica gel G TLC plate, and a developing solvent is toluene-ethyl acetate-formic acid; and,

[0017] E) observing the TLC plate under ultraviolet light, wherein the presence of a blue fluorescent spot in the TLC plate of the sample solution at an Rf value of $0.829 \pm 10\%$ indicates the sample is *Smilax ferox* Wall. ex Kunth; and the absence of a blue fluorescent spot in the TLC plate of the sample solution at an Rf value of $0.829 \pm 10\%$ indicates the sample is *Smilax glauco-china* Warb.

[0018] The present disclosure provides a method for identifying Bixie formula granules by thin layer chromatography, comprising taking a sample to be identified, dissolving with a solvent to obtain a sample solution.

25 **[0019]** Specifically, the step A) of the present disclosure comprises: grinding the sample to be identified, performing ultrasonic extraction with methanol, and filtering to obtain the sample solution.

[0020] In particular, a mass-to-volume ratio of the sample to be identified to methanol is 1g : 5

to 15mL.

[0021] The ultrasonic extraction is performed for 10 to 20 minutes.

[0022] Taking resveratrol as reference substance, dissolving with a solvent, and obtaining a reference substance solution. A mass concentration of the resveratrol reference substance solution is 0.1 to 2.0 mg/mL.

[0023] Taking *Smilax ferox* Wall. ex Kunth as reference medicine material, dissolving with a solvent, and obtaining a reference medicine material solution.

[0024] Preferably, the present disclosure specifically comprises:

[0025] taking *Smilax ferox* Wall. ex Kunth as reference medicine, decocting with water, then adding methanol for extraction, ultrasonically filtering, and obtaining the reference medicine material solution;

[0026] specifically, a mass ratio of the *Smilax ferox* Wall. ex Kunth as reference medicine material, water, and methanol is 1g : 10 to 20mL : 5 to 10mL.

[0027] The sample solution, the reference substance solution, and the reference medicine material solution are subjected to thin layer chromatography detection.

[0028] Preferably, the step comprises: taking the sample solution, the reference substance solution, and the reference medicine material solution, and respectively loading them on the same silica gel G TLC plate, developing with a developing solvent, taking out, drying, and detecting under ultraviolet light; wherein a wavelength of the ultraviolet light is 365 nm.

[0029] According to the present disclosure, the developing solvent is toluene-ethyl acetate-formic acid.

[0030] In a preferred embodiment of the present disclosure, a mass ratio of the toluene, ethyl acetate and formic acid is 4:3:0.2.

[0031] The TLC plate in the present disclosure is a silica gel G TLC plate. The TLC plate may be selected from a cuttable thin layer chromatography plate provided by Tianjin Silida Technology Co., Ltd., a plate provided by Merck, or a precast silica gel G plate provided by Branch Factory Qingdao Ocean Chemical Factory. The results show that the method exhibits good durability and

can meet the requirements for identification. At Rf values of 0.885, 0.800, and 0.833, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibit fluorescent spots, while Bixie (*Smilax glauco-china* Warb) formula granules exhibit no fluorescent spot, through which Bixie (*Smilax ferox* Wall. ex Kunth) formula granules and Bixie (*Smilax glauco-china* Warb) formula granules can be identified.

5 **[0032]** Under ultraviolet light detection, if the sample solution chromatogram exhibits a blue fluorescent spot at an Rf value of $0.829 \pm 10\%$, the sample is identified as *Smilax ferox* Wall. ex Kunth; if the sample solution chromatogram exhibits no blue fluorescent spot at an Rf value of $0.829 \pm 10\%$, the sample is identified as *Smilax glauco-china* Warb.

10 **[0033]** The Rf value is $0.829 \pm 10\%$; preferably, the Rf value is 0.747-0.885. Specifically, the Rf value may be 0.747, 0.778, 0.766, 0.800, 0.833, 0.856, 0.875, 0.883, or 0.885.

[0034] A sample loading amount of the thin layer chromatography of the present disclosure is 5 to 15 μ L.

15 **[0035]** The detection of the present disclosure is performed at a temperature of 4°C to 25°C. The method of the present disclosure shows good adaptability to different temperatures. At Rf values of 0.856 and 0.875, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibit fluorescent spots, while Bixie (*Smilax glauco-china* Warb) formula granules exhibit no fluorescent spot, through which Bixie (*Smilax ferox* Wall. ex Kunth) formula granules and Bixie (*Smilax glauco-china* Warb) formula granules can be identified.

20 **[0036]** The detection of the present disclosure is performed at a humidity of 32% rh to 75% rh. The method of the present disclosure exhibits good adaptability to different humidity conditions. At Rf values of 0.883 and 0.862, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibit fluorescent spots, while Bixie (*Smilax glauco-china* Warb) formula granules exhibit no fluorescent spot, through which Bixie (*Smilax ferox* Wall. ex Kunth) formula granules and Bixie (*Smilax glauco-china* Warb) formula granules can be identified.

25 **[0037]** The sample to be identified is *Smilax glauco-china* Warb formula granules or *Smilax ferox* Wall. ex Kunth formula granules.

[0038] The present disclosure provides a method for identifying Bixie formula granules by thin layer chromatography, comprising: A) taking a sample to be identified, dissolving with a solvent to obtain a sample solution; wherein the sample to be identified is *Smilax glauco-china* Warb

formula granules or *Smilax ferox* Wall. ex Kunth formula granules; B) taking resveratrol as reference substance, dissolving with a solvent to obtain a reference substance solution; C) taking *Smilax ferox* Wall. ex Kunth as reference medicine material, dissolving with a solvent to obtain a reference medicine material solution; D) performing thin layer chromatographic detection on the sample solution, the reference substance solution and the reference medicine material solution, wherein a TLC plate is a silica gel G TLC plate, and a developing solvent is toluene-ethyl acetate-formic acid; and E) observing the TLC plate under ultraviolet light, wherein the presence of a blue fluorescent spot in the TLC plate of the sample solution at an Rf value of $0.829 \pm 10\%$ indicates the sample is *Smilax ferox* Wall. ex Kunth; and the absence of a blue fluorescent spot in the TLC plate of the sample solution at an Rf value of $0.829 \pm 10\%$ indicates the sample is *Smilax glauco-china* Warb. The present disclosure establishes a method for identifying Bixie formula granules by thin layer chromatography. By observing whether a fluorescent spot is present within a specified Rf value range on the TLC plate as an identification spot, Bixie formula granules are identified rapidly and effectively.

[0039] In the TLC identification method of the present disclosure, the TLC plate of a sample to be identified is compared with that of a *Smilax ferox* Wall. ex Kunth reference medicine material. The presence of the fluorescent spots of the sample to be identified being same with the reference medicine material on the TLC plate indicates the sample is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules; the absence of a fluorescent spot present at the same position within an Rf value range of 0.747-0.885 as that of the reference medicine material indicates the sample is Bixie (*Smilax glauco-china* Warb) formula granules.

[0040] The present disclosure discloses the establishment and application of a method for identifying Bixie (*Smilax ferox* Wall. ex Kunth) formula granules and Bixie (*Smilax glauco-china* Warb) formula granules by thin layer chromatography. The method exhibits good stability, high precision, good repeatability, and is convenient and easy to perform.

BRIEF DESCRIPTION OF DRAWINGS

[0041] FIG. 1 is the investigation of sample loading amount;

[0042] FIG. 2 is the investigation of specificity;

- [0043] FIG. 3 is the investigation of the TLC plate of Tianjin Silida;
- [0044] FIG. 4 is the investigation of the TLC plate of Merck;
- [0045] FIG. 5 is the investigation of TLC plate of Qingdao Ocean;
- [0046] FIG. 6 is the investigation of the temperature of 4°C;
- 5 [0047] FIG. 7 is the investigation of the temperature of 25°C;
- [0048] FIG. 8 is the investigation of the humidity of 32%;
- [0049] FIG. 9 is the investigation of the humidity of 75%;
- [0050] FIG. 10 is the verification of multi-batches;
- [0051] FIG. 11 is the result of the method of comparative example 1;
- 10 [0052] FIG. 12 is the result of the method of comparative example 2;
- [0053] FIG. 13 is the result of the method of comparative example 3 of the present disclosure;
- [0054] FIG. 14 is the result of the method of comparative example 4 of the present disclosure.

DETAILED DESCRIPTION

- 15 [0055] In order to further illustrate the present disclosure, the following describes in detail, in conjunction with embodiments, a method for establishing UPLC characteristic chromatograms of *Cyrtomium fortunei* medicine material, decoction pieces, standard decoction, and formula granules provided by the present disclosure.
- [0056] Heating plate, mortar, thin layer chromatography imaging system: CAMAG TLC
20 Visualizer, silica gel G thin layer chromatography plates (Qingdao Ocean Chemical Factory; Tianjin Silida Technology Co., Ltd.; Merck).
- [0057] Methanol, toluene, ethyl acetate, and formic acid are of analytical grade, and water is ultrapure water (prepared in the laboratory).
- [0058] Resveratrol (National Institutes for Food and Drug Control, China, batch No. 111535-
25 201703, content 99.4%), *Smilax ferox* Wall. ex Kunth as reference medicine material (Sichuan Weikeyi Biotechnology Co., Ltd., batch No. ycwqk22022805), Bixie (*Smilax ferox* Wall. ex Kunth)

formula granules, batch Nos. 2211074, 2211075, 2211076, and 2211077, and Bixie (*Smilax glauco-china* Warb) formula granules, batch Nos. 2212082, 2212083, 2212084, and 2212085.

[0059] Example 1

[0060] 1 g of the formula granules was taken, finely ground, added with 10 ml of methanol, ultrasonicated for 20 minutes, and filtered. The filtrate was taken as the sample solution. 1 g of the reference medicine material was taken, added with 20 ml of water, decocted for half an hour, filtered, evaporated to dryness, added with 10 ml of methanol, ultrasonicated for 20 minutes, and filtered. The filtrate was taken as the reference medicine material solution. Separately, resveratrol was taken as reference substance and added with methanol to prepare a reference substance solution with a content of 0.2 mg per 1 ml.

[0061] According to the method for thin layer chromatography (General Rule 0502), 15 μ l of each of the above two solutions were taken and were respectively loaded on the same silica gel G TLC plate, which was then developed using toluene-ethyl acetate-formic acid (4:3:0.2) as the developing solvent, taken out, dried, and detected under ultraviolet light (365 nm). In the chromatogram of the sample to be identified, at the position corresponding to that of the chromatogram of the resveratrol reference substance, fluorescent spots of the same color were present.

[0062] Example 2 Methodological investigation

[0063] 2.1 Sample loading amount investigation

[0064] Under the designed experimental conditions above, 5 μ l, 10 μ l and 15 μ l of each of the resveratrol reference solution and the sample solution were loaded respectively on the same silica gel G TLC plate, and the results are shown in FIG. 1. In the case that the reference medicine material solution was loaded at 5-15 μ l, and the sample solution was loaded at 5-15 μ l, both could achieve ideal effects. Moreover, at the retention factor (Rf) of 0.747, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibited fluorescent spots, while Bixie (*Smilax glauco-china* Warb) formula granules exhibited no fluorescent spot, according to which Bixie (*Smilax ferox* Wall. ex Kunth) formula granules and Bixie (*Smilax glauco-china* Warb) formula granules could be identified.

[0065] FIG. 1 shows the investigation of sample loading amount. Note: 1-3 are resveratrol, with

sample loading amounts of 5 μ l, 10 μ l, and 15 μ l in sequence; 4-6 are Bixie (*Smilax ferox* Wall. ex Kunth) reference medicine material, with sample loading amounts of 5 μ l, 10 μ l, and 15 μ l in sequence; 7-9 are Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074), with sample loading amounts of 5 μ l, 10 μ l, and 15 μ l in sequence; 10-12 are Bixie (*Smilax glauco-china* Warb) formula granules (2212082), with sample loading amounts of 5 μ l, 10 μ l, and 15 μ l in sequence.

[0066] 2.2 Investigation of specificity

[0067] According to the method described above for preparing the sample solution, a resveratrol reference solution, a reference medicine material solution, a Bixie (*Smilax ferox* Wall. ex Kunth) formula granules sample solution, a Bixie (*Smilax glauco-china* Warb) formula granules sample solution, and a negative control solution were respectively prepared, and thin layer chromatography identification was performed. The results are shown in FIG. 2. No interference from the negative control sample was observed for the resveratrol reference solution, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules sample solution, and Bixie (*Smilax glauco-china* Warb) formula granules sample solution, indicating good specificity of the method. Moreover, at a retention factor (Rf) of 0.788, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibited fluorescent spots, while Bixie (*Smilax glauco-china* Warb) formula granules exhibited no fluorescent spot, according to which Bixie (*Smilax ferox* Wall. ex Kunth) formula granules were distinguished from Bixie (*Smilax glauco-china* Warb) formula granules.

[0068] FIG. 2 shows investigation of specificity. Note: 1 is negative control; 2 is resveratrol; 3 is *Smilax ferox* Wall. ex Kunth reference medicine material; 4 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074); 5 is Bixie (*Smilax glauco-china* Warb) formula granules (2212082).

[0069] 2.3 Investigation of durability

[0070] 2.3.1 Comparison of different TLC plates

[0071] Cuttable thin layer chromatography plates from Tianjin Silida Technology Co., Ltd., Merck, and precast silica gel G plates from the branch factory of Qingdao Ocean Chemical Factory were selected, and tests were carried out respectively according to the designed experimental method. The results are shown in FIGs. 3-5. The results indicate that this method had good durability and all TLC plates met the identification requirements. Moreover, at the retention factors (Rf) of 0.885, 0.800, and 0.833, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibited

fluorescent spots, while Bixie (*Smilax glauco-china* Warb) formula granules exhibited no fluorescent spot, according to which Bixie (*Smilax ferox* Wall. ex Kunth) formula granules and Bixie (*Smilax glauco-china* Warb) formula granules were distinguished.

[0072] Results are shown in FIG. 3. FIG. 3 shows the investigation of the TLC plate of Tianjin Silida. Note: 1 is resveratrol; 2 is *Smilax ferox* Wall. ex Kunth reference medicine material; 3 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074); 4 is Bixie (*Smilax glauco-china* Warb) formula granules (2212082). FIG. 4 shows the investigation of the TLC plate of Merck. Note: 1 is resveratrol; 2 is *Smilax ferox* Wall. ex Kunth reference medicine material; 3 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074); 4 is Bixie (*Smilax glauco-china* Warb) formula granules (2212082). FIG. 5 shows the investigation of the TLC plate of Qingdao Ocean. Note: 1 is resveratrol; 2 is *Smilax ferox* Wall. ex Kunth reference medicine material; 3 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074); 4 is Bixie (*Smilax glauco-china* Warb) formula granules (2212082).

[0073] 2.3.2 Comparison of different temperatures

[0074] The TLC plates loaded with samples were developed under temperature conditions of a low temperature at 4°C and a normal temperature at 25°C, respectively. From FIGs. 6-7, it can be seen that this method had good durability at different temperatures. Moreover, at the retention factors of 0.856 and 0.875, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibited fluorescent spots, while Bixie (*Smilax glauco-china* Warb) formula granules exhibited no fluorescent spot, according to which Bixie (*Smilax ferox* Wall. ex Kunth) formula granules were distinguished from Bixie (*Smilax glauco-china* Warb) formula granules. FIG. 6 shows the investigation of the temperature of 4°C. Note: 1 is resveratrol; 2 is *Smilax ferox* Wall. ex Kunth reference medicine material; 3 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074); 4 is Bixie (*Smilax glauco-china* Warb) formula granules (2212082). FIG. 7 shows the investigation of the temperature of 25°C. Note: 1 is resveratrol; 2 is *Smilax ferox* Wall. ex Kunth reference medicine material; 3 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074); 4 is Bixie (*Smilax glauco-china* Warb) formula granules (2212082).

[0075] 2.3.3 Comparison of different humidity

[0076] The TLC plates loaded with samples were developed under humidity of 32% and 75%,

respectively. From FIGs. 8-9, it can be seen that this method had good durability at different humidity. Moreover, at the retention factors (Rf) of 0.883 and 0.862, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibited fluorescent spots, while Bixie (*Smilax glauco-china* Warb) formula granules exhibited no fluorescent spot, according to which Bixie (*Smilax ferox* Wall. ex Kunth) formula granules were distinguished from Bixie (*Smilax glauco-china* Warb) formula granules.

[0077] FIG. 8 shows the investigation of the humidity of 32%. Note: 1 is resveratrol; 2 is *Smilax ferox* Wall. ex Kunth reference medicine material; 3 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074); 4 is Bixie (*Smilax glauco-china* Warb) formula granules (2212082).

[0078] FIG. 9 shows the investigation of the humidity of 75%. Note: 1 is resveratrol; 2 is *Smilax ferox* Wall. ex Kunth reference medicine material; 3 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074); 4 is Bixie (*Smilax glauco-china* Warb) formula granules.

[0079] 2.3.4 Verification

[0080] Four batches of *Smilax ferox* Wall. ex Kunth formula granules test samples and four batches of *Smilax glauco-china* Warb formula granules test samples were subjected to thin layer chromatography identification for verification, and the results are shown in FIG. 10. The results indicate that the method established by the present disclosure is able to accurately distinguish Bixie (*Smilax ferox* Wall. ex Kunth) formula granules from Bixie (*Smilax glauco-china* Warb) formula granules. At the retention factor (Rf) of 0.776, Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibited an obvious blue spot, which could be used to distinguish between the two.

[0081] FIG. 10 shows the verification of multi-batches. Note: 1 is resveratrol; 2 is *Smilax ferox* Wall. ex Kunth reference medicine material; 3-6 are Bixie (*Smilax ferox* Wall. ex Kunth) formula granules (2211074, 2211075, 2211076, 2211077); and 7-10 are Bixie (*Smilax glauco-china* Warb) formula granules (2212082, 2212083, 2212084, 2212085).

[0082] 2.4 Determination of identification spots Rf for Bixie (*Smilax ferox* Wall. ex Kunth) formula granules and Bixie (*Smilax glauco-china* Warb) formula granules.

[0083] The retention factor data from the methodological investigation of thin layer chromatography were summarized, and the results are shown in Table 1. Based on the summarized results, the retention factor (Rf) of the identification spot of Bixie (*Smilax ferox* Wall. ex Kunth)

formula granules and Bixie (*Smilax glauco-china* Warb) formula granules fell within a 10% range, and the specified value was 0.829.

Table 1 Summary of retention factor data from methodological investigation

Item	Retention factor	Mean	RSD%
Sample loading amount	0.747		
Specificity	0.778		
Silida TLC plate	0.885		
Merck TLC plate	0.800		
Qingdao Ocean TLC plate	0.833	0.829	6.25
Temperature of 4°C	0.856		
Temperature of 25°C	0.875		
Humidity of 32%RH	0.883		
Humidity of 51%RH	0.862		
Verification of multi-batches	0.766		

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[0084] Comparative Example 1

[0085] 2 g of the formula granules was taken, added with 50 ml of methanol, heated under reflux for 1 hour, and filtered. The filtrate was evaporated to dryness. The residue was dissolved with 25 ml of water and washed with 25 ml of ether, and the ether layer was discarded. The aqueous layer was added with 2 ml of hydrochloric acid, heated under reflux for 1.5 hours, cooled, and extracted under shaking with ether twice, each time 25 ml. The ether extracts were combined and evaporated to dryness. The residue was dissolved with 1 ml of chloroform and used as the sample solution. 5 g of Bixie medicine material was taken, added with 50 ml of water, decocted for 30 minutes, and filtered, and the filtrate was evaporated to dryness. The residue was added with 50 ml of methanol and prepared into the reference medicine material solution according to the same method. According to the “Operating Procedure for Thin Layer Chromatography” (XLS-09-OP-2897), 5 µl of each of the above two solutions were taken and respectively loaded on the same silica gel G TLC plate, chloroform-acetone (9:1) was used as the developing solvent, pre-equilibration was performed for 30 minutes, and the TLC plate was developed, taken out, dried, sprayed with 10%

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phosphomolybdic acid ethanol solution and heated at 105°C until the spots became clearly colored, and the TLC plate was observed under daylight. In the chromatogram of the sample solution, at the position corresponding to that of the reference medicine material chromatogram, fluorescent spots of the same color were present.

5 **[0086]** FIG. 11 shows the result of the method of comparative example 1. Note: 1 and 3 are the *Smilax ferox* Wall. ex Kunth reference medicine material solution; 2 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules solution; 4 is Bixie (*Smilax glauco-china* Warb) formula granules solution. The results indicate that, by using the above thin layer chromatography identification method, both Bixie (*Smilax glauco-china* Warb) formula granules and Bixie (*Smilax ferox* Wall. ex
10 Kunth) formula granules exhibited no obvious fluorescent spot, and the two cannot be identified.

[0087] Comparative example 2

[0088] 1 g of the formula granules was taken, added with 10 ml of n-butanol, ultrasonicated for 20 minutes, and filtered. The filtrate was taken as the sample solution. According to the method for thin layer chromatography (General Rule 0502), 15 µl of each of the above two solutions were
15 taken and respectively loaded on the same silica gel G TLC plate. Toluene-ethyl acetate-formic acid (4:3:0.2) was used as the developing solvent. The TLC plate was developed, taken out, dried, and detected under ultraviolet light (365 nm). In the chromatogram of the sample solution, at the position corresponding to that of the reference medicine material chromatogram, fluorescent spots of the same color were present.

20 **[0089]** FIG. 12 shows the result of the method of comparative example 2. Note: 1 and 3 are the *Smilax ferox* Wall. ex Kunth reference medicine material solution; 2 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules solution; 4 is Bixie (*Smilax glauco-china* Warb) formula granules solution.

[0090] The results indicate that, by using the above thin layer chromatography identification
25 method, both Bixie (*Smilax glauco-china* Warb) formula granules and Bixie (*Smilax ferox* Wall. ex Kunth) formula granules exhibited no obvious fluorescent spot, and the two cannot be distinguished.

[0091] Comparative example 3

[0092] 1 g of formula granules was taken, finely ground, added with 10 ml of methanol, ultrasonicated for 20 minutes, and filtered. The filtrate was taken as the sample solution. 1 g of the

reference medicine material was taken, added with 20 ml of water, decocted for half an hour, filtered, evaporated to dryness, added with 10 ml of methanol, ultrasonicated for 20 minutes, and filtered. The filtrate was taken as the reference medicine material solution.

[0093] According to the method for thin layer chromatography (General Rule 0502), 15 µl of each of the above two solutions were taken and respectively loaded on the same silica gel G TLC plate. Petroleum ether-ethyl acetate (17:3) was used as the developing solvent. The TLC plate was developed, taken out, dried, and detected under ultraviolet light (365 nm). In the chromatogram of the sample solution, at the position corresponding to that of the reference medicine material chromatogram, fluorescent spots of the same color were present.

[0094] FIG. 13 shows the result of the method of comparative example 3. Note: 1 and 3 are the *Smilax ferox* Wall. ex Kunth reference medicine material solution; 2 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules solution; 4 is Bixie (*Smilax glauco-china* Warb) formula granules solution.

[0095] The results indicate that, by using the above thin layer chromatography identification method, both Bixie (*Smilax ferox* Wall. ex Kunth) formula granules and Bixie (*Smilax glauco-china* Warb) formula granules exhibited no obvious fluorescent spot, and the two cannot be identified.

[0096] Comparative example 4

[0097] 1 g of the formula granules was taken, finely ground, added with 10 ml of methanol, ultrasonicated for 20 minutes, and filtered. The filtrate was taken as the sample solution. 1 g of the reference medicine material was taken, added with 20 ml of water, decocted for half an hour, filtered, evaporated to dryness, added with 10 ml of methanol, ultrasonicated for 20 minutes, and filtered. The filtrate was taken as the reference medicine material solution.

[0098] According to the method for thin layer chromatography (General Rule 0502), 15 µl of each of the above two solutions were taken and respectively loaded on the same silica gel G TLC plate. Chloroform-acetone (10:0.3) was used as the developing solvent. The TLC plate was developed, taken out, dried, and detected under ultraviolet light (365 nm). In the chromatogram of the sample solution, at the position corresponding to that of the reference medicine material chromatogram, fluorescent spots of the same color were present.

[0099] FIG. 14 shows the result of the method of comparative example 4. Note: 1 and 3 are the

Smilax ferox Wall. ex Kunth reference medicine material solution; 2 is Bixie (*Smilax ferox* Wall. ex Kunth) formula granules solution; 4 is Bixie (*Smilax glauco-china* Warb) formula granules solution.

5 **[00100]** The above description is merely preferred embodiments of the present disclosure. It should be noted that for those skilled in the art, several variations and modifications may be made without departing from the principle of the present disclosure, and such variations and modifications shall also be construed as falling within the scope of protection of the present disclosure.

CLAIMS

1. A method for identifying Bixie formula granules by thin layer chromatography (TLC), comprising:

5 A) taking a sample to be identified, dissolving with a solvent to obtain a sample solution; wherein the sample to be identified is *Smilax glauco*-china Warb formula granules or *Smilax ferox* Wall. ex Kunth formula granules;

 B) taking resveratrol as reference substance, and dissolving with a solvent to obtain a reference substance solution;

10 C) taking *Smilax ferox* Wall. ex Kunth as reference medicine material, and dissolving with a solvent to obtain a reference medicine material solution;

 D) performing thin layer chromatographic detection on the sample solution, the reference substance solution and the reference medicine material solution, wherein a TLC plate is a silica gel G TLC plate, and a developing solvent is toluene-ethyl acetate-formic acid; and

15 E) observing the TLC plate under ultraviolet light, wherein the presence of a blue fluorescent spot in the TLC plate of the sample solution at an R_f value of $0.829 \pm 10\%$ indicates the sample is *Smilax ferox* Wall. ex Kunth; and the absence of a blue fluorescent spot in the TLC plate of the sample solution at an R_f value of $0.829 \pm 10\%$ indicates the sample is *Smilax glauco*-china Warb.

20 2. The method according to claim 1, wherein step A) specifically comprises: taking the sample to be identified, adding methanol, and ultrasonicing to obtain the sample solution; wherein the ultrasonic treatment is performed for 10 to 20 minutes; and a mass-to-volume ratio of the sample to be identified to methanol is 1g : 5 to 15 mL.

 3. The method according to claim 1, wherein in step B), the solvent is methanol; and a mass concentration of resveratrol as reference substance is 0.1 to 2.0 mg/mL.

25 4. The method according to claim 1, wherein step C) specifically comprises: taking *Smilax ferox* Wall. ex Kunth as reference medicine material, decocting with water, then adding methanol for extraction, ultrasonicing, and filtering to obtain the reference medicine material solution; wherein a mass ratio of the *Smilax ferox* Wall. ex Kunth as reference medicine material, water and

methanol is 1g : 10 to 20 mL : 5 to 10 mL.

5. The method according to claim 1, wherein a sample loading amount for thin layer chromatography is 5 to 15 μ L.

6. The method according to claim 1, wherein in step D), a mass ratio of toluene, ethyl acetate and formic acid is 4:3:0.2.

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

8. The method according to claim 1, wherein in step E), the observation is performed at a temperature of 4°C to 25°C.

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

10. The method according to claim 1, wherein the R_f value is 0.747 to 0.885.