

SPECIFICATION

TITLE OF INVENTION: THIN-LAYER IDENTIFICATION METHOD FOR FOLIUM ARTEMISIA ARGYI AND VINEGAR FOLIUM ARTEMISIA ARGYI FORMULA 5 GRANULES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority of Chinese Patent Application No. 202311301309.X, filed with the China National Intellectual Property Administration on October 9, 2023, and titled
10 with “THIN-LAYER IDENTIFICATION METHOD FOR FOLIUM ARTEMISIA ARGYI AND VINEGAR FOLIUM ARTEMISIA ARGYI FORMULA GRANULES”, which is hereby incorporated by reference in its entirety.

FIELD

15 [0002] The present disclosure relates to the field of analytical detection, especially relates to a method for identifying Artemisiae argyi folium formula granules and Artemisiae argyi folium praeparata cum aceto formula granules by thin-layer chromatography.

BACKGROUND

20 [0003] Artemisiae argyi folium is the dried leaf of *Artemisia argyi* Lévl. et Vant., a plant in the Asteraceae family [1]. It has the effects of warming the meridians, stopping bleeding, dispersing cold, relieving pain, and when used externally, eliminating dampness and relieving itching. Artemisiae argyi folium praeparata cum aceto is warm but not dry, mainly focusing on warming the meridians and stopping bleeding, and can enhance the effect of dispelling cold and relieving
25 pain. The physicochemical properties and the effects of eliminating dampness and relieving itching are different before and after the processing of Artemisiae argyi folium. The effects of stopping bleeding and warming the interior are better after vinegar processing. It can thus be seen that the chemical ingredients of Artemisiae argyi folium and Artemisiae argyi folium praeparata

cum aceto have already changed during the processing.

[0004] The chemical ingredients of *Artemisiae argyi folium* mainly comprise volatile oils, flavonoids, terpenoids, phenylpropanoids, organic acids, steroids, polysaccharides and trace elements. At present, studies on *Artemisiae argyi folium* mainly focus on the ingredient detection and pharmacological effects of different processed products. For example, Tiantian Zhang, Lili Sun, Qian Zhou, et al. conducted a GC-MS study on the volatile oil ingredients of *Artemisiae argyi folium* and its processed products, which showed that after vinegar processing of *Artemisiae argyi folium*, the chemical ingredients change significantly. *Artemisiae argyi folium* and vinegar-processed *Artemisiae argyi folium* decoction pieces can be distinguished by means of character identification, but the identification method for the formula granules of the two has not yet been reported.

[0005] Because *Artemisiae argyi folium* formula granules and *Artemisiae argyi folium praeparata cum aceto* formula granules are prepared from the *Artemisiae argyi folium* medicinal material through a series of processes, some of the processes may destroy the original character features, and the character identification method is not suitable for the identification of the formula granules. At present, there is no effective method for identifying *Artemisiae argyi folium* formula granules from *Artemisiae argyi folium praeparata cum aceto* formula granules. Therefore, it is urgent to establish a method for identifying *Artemisiae argyi folium* formula granules and *Artemisiae argyi folium praeparata cum aceto* formula granules.

[0006] SUMMARY

[0007] In view of this, the technical problem to be solved by the present disclosure is to provide a method for identifying *Artemisiae argyi folium* formula granules and *Artemisiae argyi folium praeparata cum aceto* formula granules by thin-layer chromatography.

[0008] 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”.

[0009] 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.

5 [0010] 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 items, including any combination of a single item or a plurality of items.

[0011] 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
10 order of each process should be determined according to its functions and internal logic and shall not constitute any limitation to the implementation process of the embodiments of the present disclosure.

[0012] The present disclosure provides a construction and application method for identifying
15 Artemisiae argyi folium formula granules and Artemisiae argyi folium praeparata cum aceto formula granules by thin-layer chromatography. The method uses the appearance of a red fluorescent spot at an retention factor of 0.556-0.752 in the thin-layer chromatogram of a sample as the identification spot for Artemisiae argyi folium formula granules, whereas the absence of a red fluorescent spot at the same position is used as the identification spot for Artemisiae argyi
20 folium praeparata cum aceto formula granules. The established thin-layer chromatography method can rapidly and effectively identify Artemisiae argyi folium formula granules and Artemisiae argyi folium praeparata cum aceto formula granules. The method has strong specificity, good stability, high precision, and is convenient and easy to perform. The method enables more standardized quality control of Artemisiae argyi folium formula granules and
25 Artemisiae argyi folium praeparata cum aceto formula granules, thereby ensuring the therapeutic efficacy of the medicament.

[0013] A method for identifying Artemisiae argyi folium formula granules and Artemisiae argyi folium praeparata cum aceto formula granules by thin-layer chromatography, comprising:

[0014] A) preprocessing Artemisiae argyi folium formula granules to obtain a test sample

solution;

[0015] preprocessing *Artemisiae argyi folium praeparata cum aceto formula granules* to obtain a *Artemisiae argyi folium praeparata cum aceto formula granule solution*;

[0016] B) dissolving a scopoletin reference substance in methanol to obtain a reference solution;

[0017] C) performing thin-layer chromatographic detection on the sample solution, the *Artemisiae argyi folium praeparata cum aceto formula granule solution*, and the reference solution, wherein a thin-layer plate is a silica gel G thin-layer plate, and a developing solvent is n-hexane - ethyl acetate - toluene;

[0018] D) observing under an ultraviolet light, wherein in the case that a fluorescent spot of the same color is present at equal R_f values of the test sample solution and the reference solution, and a fluorescent spot is simultaneously present at $R_f = 0.625$ to 0.741 , the test sample is identified as *Artemisiae argyi folium formula granules*; and in the case that no fluorescent spot is present at $R_f = 0.625$ to 0.741 , the test sample is identified as *Artemisiae argyi folium praeparata cum aceto formula granules*.

[0019] The present disclosure provides *Artemisiae argyi folium formula granules* and *Artemisiae argyi folium praeparata cum aceto formula granules*. Firstly, *Artemisiae argyi folium formula granules* are preprocessed to obtain a test sample solution.

[0020] In the present disclosure, the preprocessing of the *Artemisiae argyi folium formula granules* or the *Artemisiae argyi folium praeparata cum aceto formula granules* specifically are:

[0021] a) adding methanol to the *Artemisiae argyi folium formula granules* or the *Artemisiae argyi folium praeparata cum aceto formula granules*, ultrasonically, filtering, evaporating to dryness, dissolving residue in water, passing through a D101 macroporous adsorption resin column, eluting with 30% methanol and discarding eluate, and then eluting with ethyl acetate and collecting eluate; and

[0022] b) subjecting the eluate to solvent recovery to dryness and dissolving residue in ethyl acetate.

[0023] The present disclosure provides a processing method for *Artemisiae argyi folium*

formula granules and *Artemisiae argyi folium praeparata cum aceto* formula granules. The *Artemisiae argyi folium* formula granules or *Artemisiae argyi folium praeparata cum aceto* formula granules are firstly subject to ultrasonic treatment with methanol, filtering, evaporating to dryness, and the residue is dissolved in water.

5 **[0024]** In some embodiments of the present disclosure, the ultrasonic treatment is performed for 5 min to 10 min.

[0025] In some embodiments of the present disclosure, the ultrasonic treatment is performed for 10 min.

10 **[0026]** Then, the solution is passed through a D101 macroporous adsorption resin column, eluted with 30% methanol and the eluate is discarded, followed by elution with ethyl acetate, and the eluate is collected. The D101 macroporous adsorption resin column has an inner diameter of 1 cm and a column height of 14 cm.

15 **[0027]** In some embodiments of the present disclosure, a mass-to-volume ratio of the *Artemisiae argyi folium* formula granules or *Artemisiae argyi folium praeparata cum aceto* formula granules, methanol, water, 30% methanol, and ethyl acetate is 0.5 g : 10 mL : 5 mL : 20 mL : 20 mL;

[0028] The present disclosure does not limit the specific preprocessing steps for the *Artemisiae argyi folium praeparata cum aceto* formula granules, and the same procedure as described above may be adopted.

20 **[0029]** A scopoletin reference substance is dissolved in methanol to obtain a reference solution.

[0030] In the present disclosure, a mass-to-volume ratio of the scopoletin reference substance and methanol is 40 µg : 1 mL.

[0031] The test sample solution, the *Artemisiae argyi folium praeparata cum aceto* formula granule solution, and the reference solution are subjected to thin-layer chromatography detection.

25 **[0032]** Preferably, the test sample solution, the *Artemisiae argyi folium praeparata cum aceto* formula granule solution, and the reference solution are separately loaded onto the same silica gel G thin-layer plate, developed with a developing solvent, removed, air-dried, and observed under ultraviolet light. The ultraviolet light has a wavelength of 365 nm.

[0033] According to the present disclosure, the develop solvent is n-hexane - ethyl acetate - toluene.

[0034] In some preferred embodiments of the present disclosure, a mass ratio of n-hexane, ethyl acetate and toluene is 1 : 3 : 2.

5 [0035] The thin-layer plate used in the present disclosure is a silica gel G thin-layer plate; preferably a GF254 thin-layer plate. The thin-layer plates may be selected from the group consisting of Tianjin Silida GF254 silica gel plates, Merck GF254 silica gel plates, and Qingdao Haiyang GF254 silica gel plates. The results show that the method has good durability and can meet the identification requirements. Compared with the chromatogram of the reference
10 substance, a spot of the same color is present at the corresponding position in the chromatogram of the test sample.

[0036] The plate is observed under ultraviolet light, and in the case that a fluorescent spot of the same color is present at equal R_f values of the test sample solution and the reference solution, and a red fluorescent spot is simultaneously present at R_f = 0.625 to 0.741, the test sample is
15 identified as Artemisiae argyi folium formula granules;

[0037] in the case that no fluorescent spot is present at R_f = 0.625 to 0.741, the test sample is identified as Artemisiae argyi folium praeparata cum aceto formula granules.

[0038] In a preferred embodiment of the present disclosure, in the case that a fluorescent spot of the same color is present at equal R_f values of the test sample solution and the reference
20 substance solution, and a fluorescent spot is simultaneously present at R_f = 0.654, the test sample is identified as Artemisiae argyi folium formula granules;

[0039] in the case that no fluorescent spot is present at R_f = 0.654, the test sample is identified as Artemisiae argyi folium praeparata cum aceto formula granules.

[0040] In the present disclosure, a loading amount of the test sample solution, the Artemisiae argyi folium praeparata cum aceto formula granule solution, and the reference solution for
25 thin-layer chromatography is 5 μL to 15 μL.

[0041] When 5 μL to 15 μL of the reference solution and the test sample solution are loaded onto the thin-layer plate, the spots show clear coloration and good separation. Accordingly, the

loading amount is determined to be 5 µL to 15 µL.

[0042] In the present disclosure, the observation is performed at a temperature of 4°C to 35°C.

[0043] The method shows good durability at different temperatures. Compared with the chromatogram of the reference substance, a spot of the same color is present at the corresponding position in the chromatogram of the test sample.

[0044] In some specific embodiments, a red fluorescent spot is present at retention factors of 0.634, 0.633, and 0.648 for *Artemisiae argyi folium formula granules*, whereas no spot is present for *Artemisiae argyi folium praeparata cum aceto formula granules*.

[0045] The method also shows good durability at different humidities. Compared with the chromatogram of the reference substance, a spot of the same color is present at the corresponding position in the chromatogram of the test sample.

[0046] In some specific embodiments, a red fluorescent spot is present at retention factors of 0.638, 0.610, and 0.667 for *Artemisiae argyi folium formula granules*, whereas no spot is present for *Artemisiae argyi folium praeparata cum aceto formula granules*.

[0047] A red fluorescent spot is present at the above retention factors (Rf) for *Artemisiae argyi folium formula granules*, whereas no spot is present for *Artemisiae argyi folium praeparata cum aceto formula granules*, thereby the two can be distinguished based on the presence or absence of the fluorescent spot.

[0048] The present disclosure provides a method for identifying *Artemisiae argyi folium formula granules* and *Artemisiae argyi folium praeparata cum aceto formula granules* by thin-layer chromatography, comprising: A) preprocessing *Artemisiae argyi folium formula granules* to obtain a test sample solution; preprocessing *Artemisiae argyi folium praeparata cum aceto formula granules* to obtain a *Artemisiae argyi folium praeparata cum aceto formula granule solution*; B) dissolving a scopoletin reference substance in methanol to obtain a reference solution;

[0049] C) performing thin-layer chromatographic detection on the sample solution, the *Artemisiae argyi folium praeparata cum aceto formula granule solution*, and the reference solution, wherein a thin-layer plate is a silica gel G thin-layer plate, and a developing solvent is

n-hexane - ethyl acetate - toluene; D) observing under ultraviolet light, wherein in the case that a fluorescent spot of the same color is present at equal Rf values of the test sample solution and the reference solution, and a fluorescent spot is simultaneously present at Rf = 0.625 to 0.741, the test sample is identified as *Artemisiae argyi folium formula granules*; and in the case that no fluorescent spot is present at Rf = 0.625 to 0.741, the test sample is identified as *Artemisiae argyi folium praeparata cum aceto formula granules*. The present disclosure provides a construction and application method for identifying *Artemisiae argyi folium formula granules* and *Artemisiae argyi folium praeparata cum aceto formula granules* by thin-layer chromatography. The method uses the appearance of a red fluorescent spot at a retention factor of 0.625-0.741 in the thin-layer chromatogram of a test sample as the identification spot for *Artemisiae argyi folium formula granules*, whereas the absence of a red fluorescent spot at the same position is used as the identification spot for *Artemisiae argyi folium praeparata cum aceto formula granules*. The established thin-layer chromatography method can rapidly and effectively identify *Artemisiae argyi folium formula granules* and *Artemisiae argyi folium praeparata cum aceto formula granules*. The method has strong specificity, good stability, high precision, and is convenient and easy to perform. The method enables more standardized quality control of *Artemisiae argyi folium formula granules* and *Artemisiae argyi folium praeparata cum aceto formula granules*, thereby ensuring the therapeutic efficacy of the medicament.

BRIEF DESCRIPTION OF DRAWINGS

- [0050]** FIG. 1 is the investigation of sample loading amount;
- [0051]** FIG. 2 is the investigation of specificity;
- [0052]** FIG. 3 is the investigation of the thin layer plate from Tianjin Silida;
- [0053]** FIG. 4 is the investigation of the thin layer plate from Merck;
- [0054]** FIG. 5 is the investigation of thin layer plate from Qingdao Haiyang;
- [0055]** FIG. 6 is the investigation of the temperature of 4°C;
- [0056]** FIG. 7 is the investigation of the temperature of 25°C;
- [0057]** FIG. 8 is the investigation of the temperature of 35°C;

[0058] FIG. 9 is the investigation of the humidity of 32%;

[0059] FIG. 10 is the investigation of the humidity of 51%;

[0060] FIG. 11 is the investigation of the humidity of 75%;

[0061] FIG. 12 is the verification of multi-batches;

5 [0062] FIG. 13 is the result of the method of comparative example 1;

[0063] FIG. 14 is the result of the method of comparative example 2;

[0064] FIG. 15 is the result of the method of comparative example 3 using a develop solvent of n-hexane - ethyl acetate at a mass ratio of 9:1.

10 DETAILED DESCRIPTION

[0065] In order to further illustrate the present disclosure, the thin-layer chromatography identification method for *Artemisiae argyi* folium formula granules and *Artemisiae argyi* folium praeparata cum aceto formula granules provided by the present disclosure is described in detail below with reference to the following examples.

15 [0066] Mortar; thin-layer imaging system: CAMAG TLC Visualizer; silica gel G thin-layer plates (Qingdao Haiyang Chemical Factory, batch No. 20180527; Tianjin Silida Technology Co., Ltd., batch No. 191016; Merck, Germany, batch No. HX87183353).

[0067] n-Hexane, toluene, ethyl acetate, and methanol were all of analytical grade.

20 [0068] Ethanol, n-butanol, isopropanol, and glacial acetic acid were all of analytical grade, and water was ultrapure water (prepared in the laboratory).

[0069] Scopoletin (National Institutes for Food and Drug Control, batch No. 110768-202105, purity 99.7%); *Artemisiae argyi* folium formula granules (Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd., batch Nos. 2208001, 2208002, and 2208003); *Artemisiae argyi* folium praeparata cum aceto formula granules (Sichuan Neo-Green Pharmaceutical
25 Technology Development Co., Ltd., batch Nos. 2302081, 2302082, and 2302083).

[0070] Example 1

[0071] 0.5 g of the formula granules was taken and finely ground, 10 mL of methanol was added, and the mixture was ultrasonically treated for 10 min. The mixture was filtered, the filtrate was evaporated to dryness, and the residue was dissolved in 5 mL of water. The solution was passed through a D101 macroporous adsorption resin column (inner diameter: 1 cm; column height: 14 cm), eluted with 20 mL of 30% methanol, and the eluate was discarded. The column was then eluted with 20 mL of ethyl acetate, the eluate was collected and evaporated to dryness, and the residue was dissolved in 1 mL of ethyl acetate to obtain a test sample solution. A scopoletin reference substance was dissolved in methanol to obtain a solution containing 40 µg per mL, which was used as a reference solution.

[0072] The test was carried out according to the thin-layer chromatography method (General Chapter 0502 of the Chinese Pharmacopoeia, 2020 edition). 10 µL of each of the test sample solution and the reference solution was loaded onto the same silica gel G thin-layer plate, respectively. n-Hexane - ethyl acetate - toluene (1:3:2) was used as the developing solvent. The plate was developed, removed, air-dried, and observed under ultraviolet light (365 nm). In the chromatogram of the test sample, a principal spot of the same color was present at the corresponding position as that in the chromatogram of the reference solution.

[0073] Example 2: Methodological Investigation

[0074] 2.1 Investigation of Loading Amount

[0075] Under the above experimental conditions, 5 µL, 10 µL, and 15 µL of the scopoletin reference solution, the *Artemisiae argyi* folium formula granule solution, and the *Artemisiae argyi* folium praeparata cum aceto formula granule solution were respectively loaded onto the same silica gel G thin-layer plate. The results are shown in FIG. 1. The results showed that when 5-15 µL of the reference solution and the test sample solution were loaded, the spots showed clear coloration and good separation. Therefore, the loading amount was determined to be 5-15 µL. In addition, at an R_f value of 0.674, a red fluorescent spot was present for *Artemisiae argyi* folium formula granules, whereas no spot was present for *Artemisiae argyi* folium praeparata cum aceto formula granules, which could be used to distinguish *Artemisiae argyi* folium formula granules from *Artemisiae argyi* folium praeparata cum aceto formula granules. Accordingly, whether a red fluorescent spot is present at an R_f value of 0.674 may be used as an identification spot.

[0076] FIG. 1 is the investigation of loading amount. Note: 1-3, scopoletin reference solution; 4-6, *Artemisiae argyi* folium formula granule solution; 7-9, *Artemisiae argyi* folium praeparata cum aceto formula granule solution.

[0077] 2.2 Investigation of Specificity

5 [0078] According to the above preparation method for the test sample, a negative solution, a scopoletin reference solution, a *Artemisiae argyi* folium formula granule solution, and a *Artemisiae argyi* folium praeparata cum aceto formula granule solution were respectively prepared and identified by the thin-layer chromatography. The results are shown in FIG. 2. As can be seen from the figure, the negative sample produced no interference with the test samples of
10 *Artemisiae argyi* folium formula granules and *Artemisiae argyi* folium praeparata cum aceto formula granules, indicating that the method had good specificity. In addition, at retention factor of 0.741, a red fluorescent spot was present for *Artemisiae argyi* folium formula granules, whereas no spot was present for *Artemisiae argyi* folium praeparata cum aceto formula granules. FIG. 2 is the investigation of specificity. Note: 1, negative solution; 2, scopoletin reference
15 solution; 3, *Artemisiae argyi* folium formula granule solution; 4, *Artemisiae argyi* folium praeparata cum aceto formula granule solution.

[0079] 2.3 Investigation of Durability

[0080] 2.3.1 Comparison of Different Thin-layer Plates

[0081] A cuttable thin-layer chromatography plate from Tianjin Silida Technology Co., Ltd., a
20 pre-coated silica gel GF254 plate from Merck and Qingdao Haiyang Chemical Factory Branch were selected and tested according to the proposed experimental method. The results are shown in FIGs. 3-5. The results showed that the retention factor (Rf) of the red fluorescent spots located 8.5-11 cm from the origin on the three types of thin-layer plates were 0.662, 0.659, and 0.655, respectively, all of which met the identification requirements. In addition, at retention factors of
25 0.613, 0.623, and 0.504, red fluorescent spots were present for *Artemisiae argyi* folium formula granules, whereas no spot was present for *Artemisiae argyi* folium praeparata cum aceto formula granules.

[0082] The results are shown in FIG. 3. FIG. 3 is the investigation of the thin layer plate from Tianjin Silida. Note: 1, scopoletin reference solution; 2, *Artemisiae argyi* folium formula granule

solution; 3, *Artemisiae argyi* folium praeparata cum aceto formula granule solution. FIG. 4 is the investigation of the thin layer plate from Merck. Note: 1, scopoletin reference solution; 2, *Artemisiae argyi* folium formula granule solution; 3, *Artemisiae argyi* folium praeparata cum aceto formula granule solution. FIG. 5 is the investigation of thin layer plate from Qingdao Haiyang. Note: 1, scopoletin reference solution; 2, *Artemisiae argyi* folium formula granule solution; 3, *Artemisiae argyi* folium praeparata cum aceto formula granule solution.

[0083] 2.3.2 Comparison at Different Temperatures

[0084] After loading, the thin-layer plates were developed at a low temperature of 4°C, a room temperature of 25°C, and a high temperature of 35°C, respectively. As shown in FIGs. 6-8, the method showed good adaptability to different temperatures. In addition, at retention factors of 0.634, 0.633, and 0.648, red fluorescent spots were present for *Artemisiae argyi* folium formula granules, whereas no spot was present for *Artemisiae argyi* folium praeparata cum aceto formula granules. FIG. 6 is the investigation of the temperature of 4°C. Note: 1, scopoletin reference solution; 2, *Artemisiae argyi* folium formula granule solution; 3, *Artemisiae argyi* folium praeparata cum aceto formula granule solution. FIG. 7 is the investigation of the temperature of 25°C. Note: 1, scopoletin reference solution; 2, *Artemisiae argyi* folium formula granule solution; 3, *Artemisiae argyi* folium praeparata cum aceto formula granule solution. FIG. 8 is the investigation of the temperature of 35°C. Note: 1, scopoletin reference solution; 2, *Artemisiae argyi* folium formula granule solution; 3, *Artemisiae argyi* folium praeparata cum aceto formula granule solution.

[0085] 2.3.3 Comparison at Different Humidities

[0086] After loading, the thin-layer plates were developed under humidity conditions of 32% RH, 51% RH, and 75% RH, respectively. The results are shown in FIGs. 9-11. As can be seen from the figures, the method showed good adaptability to different humidities. In addition, at retention factors of 0.638, 0.610, and 0.667, red fluorescent spots were present for *Artemisiae argyi* folium formula granules, whereas no spot was present for *Artemisiae argyi* folium praeparata cum aceto formula granules. FIG. 9 is the investigation of the humidity of 32%. Note: 1, scopoletin reference solution; 2, *Artemisiae argyi* folium formula granule solution; 3, *Artemisiae argyi* folium praeparata cum aceto formula granule solution. FIG. 10 is the

investigation of the humidity of 51%. Note: 1, scopoletin reference solution; 2, Artemisiae argyi folium formula granule solution; 3, Artemisiae argyi folium praeparata cum aceto formula granule solution. FIG. 11 is the investigation of the humidity of 75%. Note: 1, scopoletin reference solution; 2, Artemisiae argyi folium formula granule solution; 3, Artemisiae argyi folium praeparata cum aceto formula granule solution.

[0087] 2.3.4 Verification

[0088] The scopoletin reference solution, the Artemisiae argyi folium formula granule solution, and the Artemisiae argyi folium praeparata cum aceto formula granule solution were respectively loaded onto the same silica gel GF254 thin-layer plate, and analyzed according to the determined method. The experimental results are shown in FIG. 12. The results showed that at retention factor (Rf) of 0.625, a red fluorescent spot was present for Artemisiae argyi folium formula granules, whereas no spot was present for Artemisiae argyi folium praeparata cum aceto formula granules. The two could therefore be distinguished based on the presence or absence of this fluorescent spot. FIG. 12 is the verification of multi-batches. Note: 1, scopoletin reference solution; 2-4, Artemisiae argyi folium formula granule solution; 5-7, Artemisiae argyi folium praeparata cum aceto formula granule solution.

[0089] 2.4 Determination of identification spots Rf value for *Artemisiae argyi* formula granules and *Artemisiae argyi* folium praeparata cum aceto formula granules

[0090] 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 *Artemisiae argyi* folium formula granules was determined to fall within a 15% range, and the specified value was 0.654.

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

Items	Artemisiae argyi folium formula granules		
	Retention factors	Average value	RSD%
Loading amount	0.674	0.654	5.08
Specificity	0.741		
Silica thin-layer plate	0.662		
Merck thin-layer plate	0.6593		
Qingdao Haiyang thin-layer plate	0.655		

Temperature of 4°C	0.634		
Temperature of 25°C	0.633		
Temperature of 35°C	0.648		
Humidity of 32%RH	0.638		
Humidity of 51%RH	0.610		
Humidity of 75%RH	0.667		
Verification of multi-batches	0.625		

[0092] The results showed that at retention factor of 0.654, no fluorescent spot was present for Artemisiae argyi folium praeparata cum aceto formula granules, whereas a distinct red fluorescent spot was present for Artemisiae argyi folium formula granules. Therefore, this spot could be used as an identification point to distinguish dolium Artemisiae argyi formula granules from Artemisiae argyi folium praeparata cum aceto formula granules.

[0093] Comparative Example 1

[0094] 4 g of the formula granules was taken, 10 mL of chloroform was added, and the mixture was ultrasonically treated for 15 min, followed by standing for 1 h. The mixture was filtered, the filtrate was evaporated to dryness, and the residue was dissolved in 1 mL of chloroform to obtain a test sample solution. A scopoletin reference substance was dissolved in methanol to obtain a solution containing 40 µg per mL, which was used as a reference solution. 4 µL of the above solutions were loaded onto the same silica gel G thin-layer plate, respectively. Cyclohexane - diethyl ether - ethyl acetate (20:5.5:2.5) was used as the developing solvent. The plate was developed, removed, and air-dried, and then sprayed with 10% sulfuric acid ethanol solution, followed by heating at 100 °C for about 10 min to develop the color. In the chromatogram of the test sample, a fluorescent spot of the same color was present at the corresponding position as that in the chromatogram of the reference solution. The experimental results are shown in FIG. 13. FIG. 13 is the result of the method of comparative example 1. Note: 1, scopoletin reference solution; 2-4, Artemisiae argyi folium formula granule solution; 5-8, Artemisiae argyi folium praeparata cum aceto formula granule solution.

[0095] The results showed that when the above thin-layer chromatography identification method was used, no obvious spot was observed for Artemisiae argyi folium formula granules and Artemisiae argyi folium praeparata cum aceto formula granules, and therefore the two could

not be distinguished.

[0096] Comparative Example 2

[0097] 1 g of the formula granules was taken and finely ground. 30 mL of 70% ethanol and 2 mL of hydrochloric acid were added, and the mixture was heated under reflux for 1 h. The mixture was filtered, the filtrate was evaporated to dryness, and the residue was dissolved in 20 mL of water. The solution was extracted with ethyl acetate by shaking twice, 20 mL each time. The ethyl acetate extracts were combined and evaporated to dryness, and the residue was dissolved in 1 mL of methanol to obtain a test sample solution. 3 g of *Artemisiae argyi folium* reference medicinal material was taken, 50 mL of water was added, and the mixture was boiled for 30 min. The mixture was filtered, the filtrate was evaporated to dryness, and 30 mL of 70% ethanol and 2 mL of hydrochloric acid were added to the residue. The mixture was processed in the same manner to obtain a reference medicinal material solution. A Scopoletin reference substance was dissolved in methanol to obtain a solution containing 40 µg per mL, which was used as a reference solution. The test was carried out according to the thin-layer chromatography method (General Chapter 0502 of the Chinese Pharmacopoeia, 2020 edition). 5 µL of each of the above solutions were loaded onto the same silica gel G thin-layer plate, respectively. Chloroform - methanol - formic acid (20:3.5:2) was used as the developing solvent. The plate was developed, removed, air-dried, sprayed with aluminum chloride test solution, dried with hot air, and observed under ultraviolet light (365 nm). In the chromatogram of the test sample, fluorescent spots of the same color were present at the corresponding positions as those in the chromatograms of the reference solution and the reference medicinal material solution. The results are shown in FIG. 14. FIG. 14 is the result of the method of comparative example 2. Note: 1, scopoletin reference solution; 2, *Artemisiae argyi folium* reference medicinal material solution; 3, *Artemisiae argyi folium* formula granule solution; 4, *Artemisiae argyi folium praeparata cum aceto* formula granule solution.

[0098] The results showed that when the above thin-layer chromatography identification method was used, no obvious differential spot was observed for *Artemisiae argyi folium* formula granules and *Artemisiae argyi folium praeparata cum aceto* formula granules, and therefore the two could not be distinguished.

[0099] Comparative Example 3

[00100] 1 g of the formula granules was taken and finely ground. 50 mL of diethyl ether was added and the mixture was ultrasonically treated for 30 min. The mixture was filtered, the filtrate was evaporated to dryness, and the residue was dissolved in 1 mL of ethyl acetate to obtain a test sample solution. The test was carried out according to the thin-layer chromatography method (General Chapter 0502). 10 µL of the above solution was spotted onto a silica gel G thin-layer plate. n-Hexane - ethyl acetate (9:1) was used as the developing solvent. The plate was developed, removed, and air-dried. A 10% sulfuric acid ethanol solution was used as the color-developing reagent. After heating at 105 °C, the plate was observed under ultraviolet light (365 nm). The results are shown in FIG. 15. FIG. 15 is the result of the method of comparative example 3 using a develop solvent of n-hexane - ethyl acetate at a mass ratio of 9:1. Note: 1, Scopoletin reference solution; 2, Artemisiae argyi folium formula granule solution; 3, Artemisiae argyi folium praeparata cum aceto formula granule solution.

[00101] The results showed that when the above thin-layer chromatography identification method was used, no obvious differential spot was observed for Artemisiae argyi folium formula granules and Artemisiae argyi folium praeparata cum aceto formula granules, and therefore the two could not be distinguished.

[00102] The above description merely illustrates 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 *Artemisiae argyi* folium formula granules and *Artemisiae argyi* folium praeparata cum aceto formula granules by thin-layer chromatography, comprising:

5 A) preprocessing *Artemisiae argyi* folium formula granules to obtain a test sample solution;
preprocessing *Artemisiae argyi* folium praeparata cum aceto formula granules to obtain a
Artemisiae argyi folium praeparata cum aceto formula granule solution;

B) dissolving a scopoletin reference substance in methanol to obtain a reference solution;

10 C) performing thin-layer chromatographic detection on the sample solution, the *Artemisiae argyi* folium praeparata cum aceto formula granule solution, and the reference solution, wherein a thin-layer plate is a silica gel G thin-layer plate, and a developing solvent is n-hexane - ethyl acetate - toluene;

D) observing under ultraviolet light, wherein in the case that a fluorescent spot of the same color is present at equal R_f values of the test sample solution and the reference solution, and a
15 fluorescent spot is simultaneously present at R_f = 0.625 to 0.741, the test sample is identified as *Artemisiae argyi* folium formula granules; and in the case that no fluorescent spot is present at R_f = 0.625 to 0.741, the test sample is identified as *Artemisiae argyi* folium praeparata cum aceto formula granules.

20 [Claim 2] The method according to claim 1, wherein in step A) the preprocessing of the *Artemisiae argyi* folium formula granules or the *Artemisiae argyi* folium praeparata cum aceto formula granules specifically are:

a) adding methanol to the *Artemisiae argyi* folium formula granules or the *Artemisiae argyi* folium praeparata cum aceto formula granules, ultrasonicing, filtering, evaporating to dryness,
25 dissolving residue in water, passing through a D101 macroporous adsorption resin column, eluting with 30% methanol and discarding eluate, and then eluting with ethyl acetate and collecting eluate; and

b) subjecting the eluate to solvent recovery to dryness and dissolving residue in ethyl

acetate.

[Claim 3] The method according to claim 1, wherein in step a) the ultrasonication is performed for 5 min to 10 min; and a mass-to-volume ratio of the *Artemisiae argyi* folium formula granules or the *Artemisiae argyi* folium praeparata cum aceto formula granules, methanol, water, 30% methanol, and ethyl acetate is 0.5 g : 10 mL : 5 mL : 20 mL : 20 mL;

and the D101 macroporous adsorption resin column has an inner diameter of 1 cm and a column height of 14 cm.

[Claim 4] The method according to claim 1, wherein in step B) a mass-to-volume ratio of the scopoletin reference substance and methanol is 40 µg : 1 mL.

[Claim 5] The method according to claim 1, wherein a loading amount of the test sample solution, the *Artemisiae argyi* folium praeparata cum aceto formula granule solution, and the reference solution for thin-layer chromatography is 5 µL to 15 µL.

[Claim 6] The method according to claim 1, wherein in step C) a mass ratio of n-hexane, ethyl acetate and toluene is 1 : 3 : 2.

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

[Claim 8] The method according to claim 1, wherein in step D) the silica gel G thin-layer plate is a GF254 thin-layer plate.

[Claim 9] The method according to claim 8, wherein in step D) the observation is performed

at a temperature of 4°C to 35°C and a humidity of 32% RH to 75% RH.

[Claim 10] The method according to claim 1, wherein in step D) the Rf value is 0.654.