Cassiae Occidentalis Semen



- A. Cassiae Occidentalis Semen B. Magnified seed (lateral view)
- C. Magnified seeds (surface view)

Cassiae Occidentalis Semen

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1. NAMES

Official Name: Cassiae Occidentalis Semen

Chinese Name: 望江南

Chinese Phonetic Name: Wangjiangnan

2. SOURCE

Cassiae Occidentalis Semen is the dried seed of *Cassia occidentalis* L. (Fabaceae). The ripe legume is collected in autumn and dried under the sun, the seed is tapped out, gathered and then dried again under the sun to obtain Cassiae Occidentalis Semen.

3. DESCRIPTION

Flattened-ovoid, 2-5.5 mm long, 2-5 mm wide, 1.0-1.5 mm thick. Externally greyish-green to greyish-brown, with elliptical and dented striations. Hilum located at the apex. Texture hard, uneasily broken. Odour slight; taste bland (Fig. 1).

4. **IDENTIFICATION**

4.1 Microscopic Identification (Appendix III)

Transverse section

Epidermis of testa consists of 1 layer of extremely narrow-rectangular palisade cells, covered with smooth and transparent cuticle, cuticle gradually thickened; 1/3 part of the layer on the lower part comprises a light line. Brace cells lined up in 1 layer, the cells dumbbell-shaped, wall thick. Nutritive layer consists of 3-7 layers of parenchymatous cells, the innermost layer degenerated and obliterated; vascular bundles visible in nutritive layer at both sides of the outer edge of the seed. Endosperm consists of several layers of irregular cells, containing aleurone grains. Cotyledons 2, consisting of oval and elongated cells, primary vascular bundles scattered among (Fig. 2).

Sophorae Honkinensis Radix et Rinzolma Polygoni Chinensis Herba 火灰母 壮荊葉 車前草 蓮鬚 aussureae Involucratae Herba 天山雪蓮 白花丹 Polygoni Perfoliati Herba 北豆根 Lonicerae Flos Plumbaginis Zeylanicae Radix 杠板歸 Menispermi Rhizoma 山銀花 Cassiae Occidentalis Semen

Powder

Colour pale yellowish-brown to reddish-brown. Palisade cells colourless, narrow-rectangular in lateral view, polygonal in surface view, 31-78 μ m long, walls thickened, lumens small, with a light line. Cuticle transparent. Spiral vessels sometimes visible. Brace cells dumbbell-shaped, suborbicular in surface view, 2 concentric circles visible, 6-34 μ m in diameter. Endosperm cells irregular in shape, containing aleurone grains and oil droplets. Cotyledon cells oval or elongated, containing oil droplets (Fig. 3).





Figure 2 Microscopic features of transverse section of Cassiae Occidentalis Semen

A. Sketch B. Section illustration C. Section magnified D. Vascular bundle

1. Epidermis of testa 2. Brace cells 3. Nutritive layer 4. Endosperm

5. Cotyledon 6. Primary vascular bundles in cotyledon 7. Cuticle

8. Vascular bundle in nutritive layer





Figure 3 Microscopic features of powder of Cassiae Occidentalis Semen (under the light microscope)

- 1. Palisade cells (1-1 in lateral view, 1-2 in surface view) 2. Cuticle 3. Spiral vessels
- 4. Brace cells (4-1 in lateral view, 4-2 in surface view) 5. Endosperm cells
- 6. Cotyledon cells (oil droplets \rightarrow)

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4.2 Thin-Layer Chromatographic Identification [Appendix IV(A)]

Standard solution

Physcion standard solution

Weigh 0.5 mg of physcion CRS (Fig. 4) and dissolve in 1 mL of ethanol (95%).

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C), ethyl acetate and formic acid (15:5:1, v/v).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (95%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (95%). Filter through a 0.45-µm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately physcion standard solution (2 µL) and the test solution (5 µL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).



Figure 4 Chemical structure of physcion

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1. Physcion standard solution 2. Test solution

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the R_f value, corresponding to that of physicon (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Physcion standard solution for fingerprinting, Std-FP (2 mg/L) Weigh 0.2 mg of physcion CRS and dissolve in 100 mL of ethanol (95%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (75%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 10 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (75%). Combine the solutions and make up to the mark with ethanol (75%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (285 nm) and a column (4.6×250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 35°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Time (min)	Acetonitrile (%, v/v)	0.5% Formic acid (%, v/v)	Elution
0-5	47	53	isocratic
5 - 25	$47 \rightarrow 75$	$53 \rightarrow 25$	linear gradient
25 - 50	$75 \rightarrow 95$	$25 \rightarrow 5$	linear gradient
50 - 60	95	5	isocratic

Table 1 Chromatogra	ohic	system	conditions
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System suitability requirements

Perform at least five replicate injections, each using 20 μ L of physcion Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of physcion should not be more than 5.0%; the RSD of the retention time of physcion peak should not be more than 2.0%; the column efficiency determined from physcion peak should not be less than 60000 theoretical plates.

The R value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject physicon Std-FP and the test solution (20 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of physicon peak in the chromatogram of physicon Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify physicon peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of physicon Std-FP. The retention times of physicon peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the five characteristic peaks of Cassiae Occidentalis Semen extract are listed in Table 2.



Table 2	The RRTs and	acceptable	ranges	of the	five	characteristic	peaks	of	Cassiae	Occide	ntalis
	Semen extract										

Peak No.	RRT	Acceptable Range
1	0.34	± 0.03
2	0.54	± 0.03
3	0.98	± 0.03
4 (marker, physcion)	1.00	-
5	1.26	± 0.03



Figure 6 A reference fingerprint chromatogram of Cassiae Occidentalis Semen extract

For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 6).

5. TESTS

- **5.1 Heavy Metals** (*Appendix V*): meet the requirements.
- **5.2** Pesticide Residues (Appendix VI): meet the requirements.
- **5.3** Mycotoxins Aflatoxins (Appendix VII): meet the requirements.
- **5.4** Sulphur Dioxide Residues (Appendix XVI): meet the requirements.
- 5.5 Foreign Matter (Appendix VIII): not more than 6.0%.
- 5.6 Ash (Appendix IX)

Total ash: not more than 5.0%. Acid-insoluble ash: not more than 0.5%. 望江南 陳皮 Melicopes Pteleifoliae Caulis 三叉苦 Rhapontici Rad Smilacis Chinae Rhizoma 豆蔻 漏蘆 Smilacis Chinae Rhizoma 豆蔻 漏蘆 野莉花 Panacis Japonici Rhizoma 安陵菜 石蒜 洋金花 Daturae Flos 全果欖

5.7 Water Content (Appendix X)

Oven dried method: not more than 13.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (hot extraction method): not less than 21.0%. Ethanol-soluble extractives (hot extraction method): not less than 12.0%.

7. ASSAY

Carry out the method as directed in Appendix IV (B).

Standard solution

Physcion standard stock solution, Std-Stock (30 mg/L)

Weigh accurately 0.3 mg of physcion CRS and dissolve in 10 mL of ethanol (95%).

Physcion standard solution for assay, Std-AS

Measure accurately the volume of the physcion Std-Stock, dilute with ethanol (95%) to produce a series of solutions of 0.2, 0.5, 1, 2, 4 mg/L for physcion.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of ethanol (75%). Sonicate (400 W) the mixture for 30 min. Centrifuge at about $3000 \times g$ for 10 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with ethanol (75%). Combine the solutions and make up to the mark with ethanol (75%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (285 nm) and a column (4.6×250 mm) packed with ODS bonded silica gel (5 µm particle size). The column temperature is maintained at 35°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

	Cassiae Occ	identalis Se	men

Time (min)	Acetonitrile (%, v/v)	0.5% Formic acid (%, v/v)	Elution
0 – 5	47	53	isocratic
5-25	$47 \rightarrow 75$	$53 \rightarrow 25$	linear gradient
25 - 50	$75 \rightarrow 95$	$25 \rightarrow 5$	linear gradient
50 - 60	95	5	isocratic

 Table 3
 Chromatographic system conditions

System suitability requirements

Perform at least five replicate injections, each using 20 μ L of physcion Std-AS (1 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of physcion should not be more than 5.0%; the RSD of the retention time of physcion peak should not be more than 2.0%; the column efficiency determined from physcion peak should not be less than 60000 theoretical plates.

The R value between physicon peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of physicon Std-AS (20 μ L each) into the HPLC system and record the chromatograms. Plot the peak areas of physicon against the corresponding concentrations of physicon Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 μ L of the test solution into the HPLC system and record the chromatogram. Identify physcion peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of physcion Std-AS. The retention times of physcion peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of physcion in the test solution, and calculate the percentage content of physcion in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.025% of physcion ($C_{16}H_{12}O_5$), calculated with reference to the dried substance.

Cassiae Occidentalis Semen (望江南)



Figure 1 A reference assay chromatogram of Cassiae Occidentalis Semen extract