

Quisqualis Fructus



Figure 1 A photograph of Quisqualis Fructus

A. Quisqualis Fructus B. Magnified bottom view of the fruit
C. Magnified transverse section of the fruit D. Magnified seeds

Quisqualis Fructus**1. NAMES**

Official Name: *Quisqualis Fructus*

Chinese Name: 使君子

Chinese Phonetic Name: Shijunzi

2. SOURCE

Quisqualis Fructus is the dried ripe fruit of *Quisqualis indica* L. (Combretaceae). The fruit is collected in autumn when ripe, foreign matter removed, then dried under the sun or baked to obtain *Quisqualis Fructus*.

3. DESCRIPTION

Ellipsoid or ovoid, with 5 longitudinal ridges (occasionally 4-9 ridges), 1.8-4.9 cm long, 9-26 mm in diameter. Externally brown to blackish-brown, smooth, slightly lustrous; top end acute, base obtuse, with a protuberant rounded fruit stalk scar. Texture hard and light in weight. Transverse section mostly in the shape of a 5-pointed star, with a subrounded cavity in the centre, containing 1 seed. Seed oblong to fusiform, externally brown to blackish-brown, with numerous longitudinal wrinkles; testa thin, easily stripped off; cotyledons 2, yellowish-white, oily. Odour slightly aromatic; taste slightly sweet (Fig. 1).

4. IDENTIFICATION**4.1 Microscopic Identification** (*Appendix III*)**Transverse section**

Epidermis of pericarp consists of 1 layer of cells, with relatively thickened walls; several layers of parenchymatous cells underneath, sometimes scattered with clusters of calcium oxalate. Fibres layer of pericarp consists of over 10 layers of vertically arranged fibres, with vascular bundles on the inner side. Lignified cells of pericarp abundant at angular regions, the cells on the outer side with relatively thicker walls. Epidermis of testa consists of 1 layer of subrectangular cells. Reticulate cells layer consists of several layers of tangentially elongated reticulate cells. Cotyledon cells contain aleurone grains and clusters of calcium oxalate (Fig. 2).

Powder

Colour brown. Reticulate cells numerous, elliptical, subrounded or irregular, walls slightly thickened, with dense reticulate pits. Epidermal cells of testa yellow to yellowish-brown, subrectangular or polygonal in surface view, some filled with yellowish-brown contents. Lignified cells of pericarp numerous, fusiform, subelliptical or irregular, mostly broken, walls slightly thickened, with dense pits. Fibres mainly in bundles, 7-34 μm in diameter, with distinct pits and pit canals; white to yellowish-white under the polarized microscope. Clusters of calcium oxalate scattered or present in cotyledon cells, 5-41 μm in diameter; polychromatic under the polarized microscope. Epidermal cells of pericarp yellowish-brown, polygonal in surface view. Vessels mainly spiral, 7-34 μm in diameter (Fig. 3).

Quisqualis Fructus

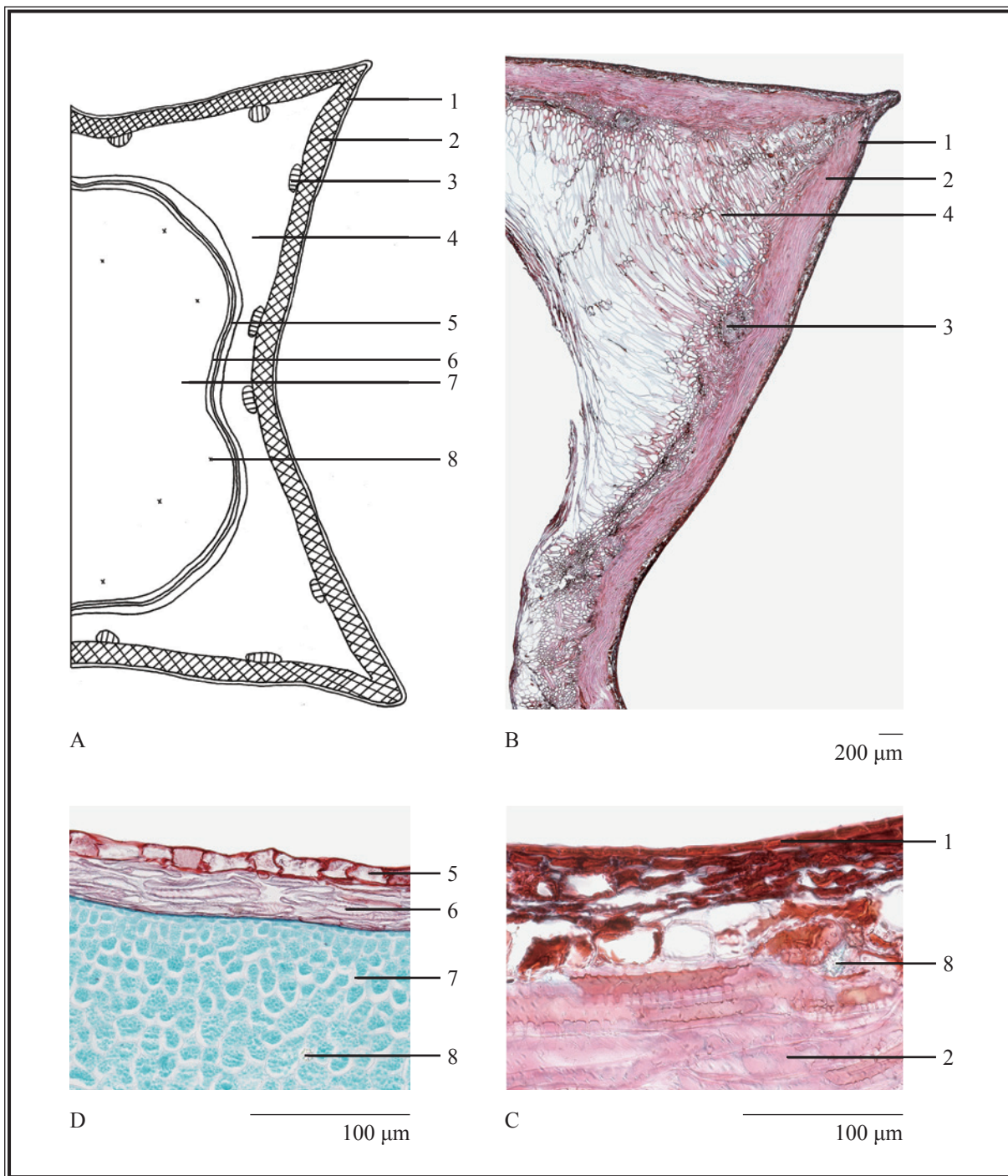


Figure 2 Microscopic features of transverse section of Quisqualis Fructus

A. Sketch B. Section illustration of pericarp C. Section magnified D. Section illustration of seed

- 1. Epidermis of pericarp
- 2. Fibres layer of pericarp
- 3. Vascular bundle
- 4. Lignified cells of pericarp
- 5. Epidermis of testa
- 6. Reticulate cell layer
- 7. Cotyledon
- 8. Clusters of calcium oxalate

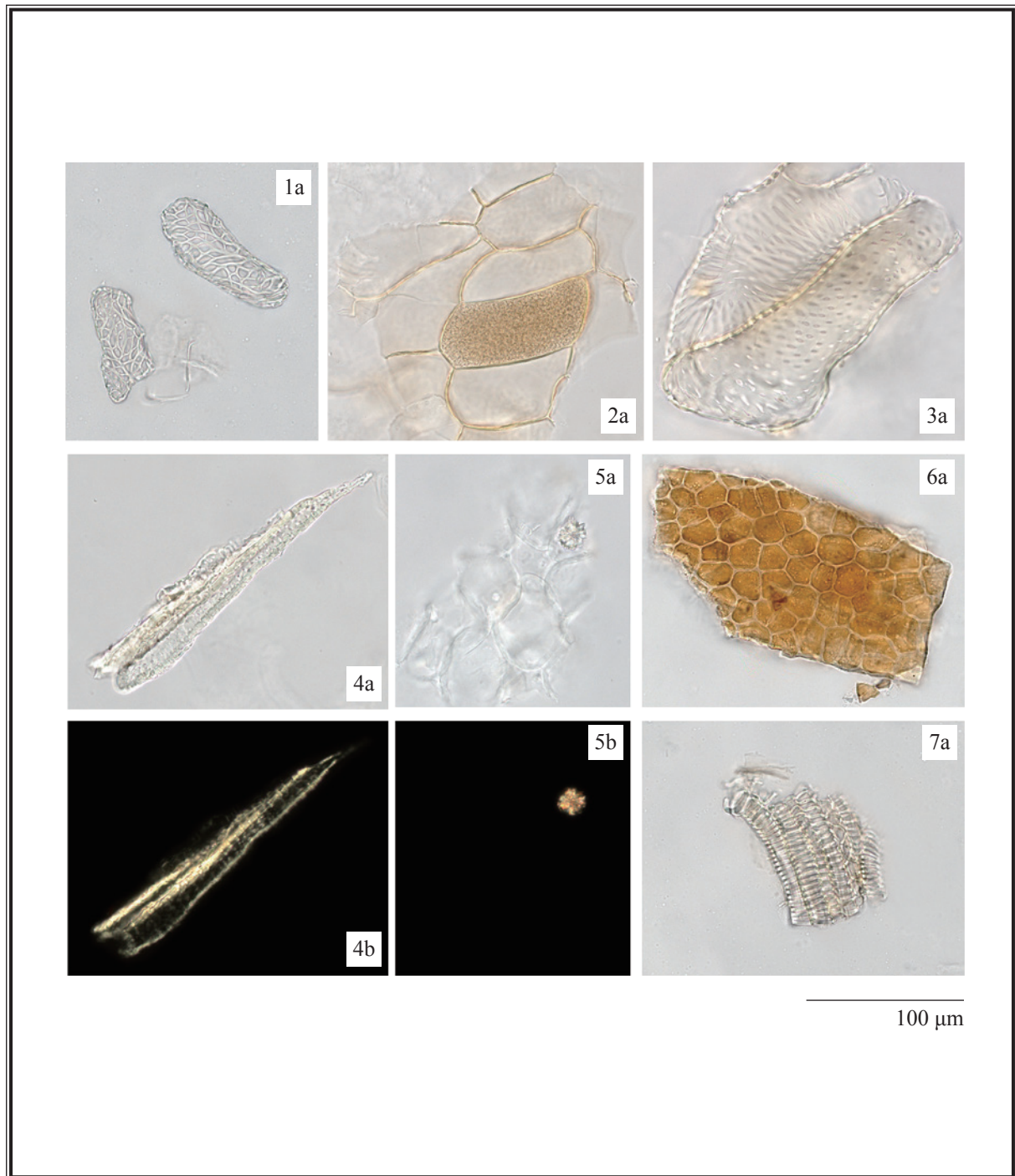


Figure 3 Microscopic features of powder of *Quisqualis Fructus*

1. Reticulate cells 2. Epidermal cells of testa 3. Lignified cells of pericarp 4. Fibres
 5. Cluster of calcium oxalate 6. Epidermal cells of pericarp 7. Spiral vessels
- a. Features under the light microscope b. Features under the polarized microscope

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

Standard solution

Quisqualic acid standard solution

Weigh 0.5 mg of quisqualic acid CRS (Fig. 4) and dissolve in 1 mL of methanol (50%).

Developing solvent system

Prepare a mixture of *n*-butanol, glacial acetic acid and water (3:1:1, v/v).

Spray reagent

Weigh 2 g of ninhydrin and dissolve in 100 mL of acetone.

Test solution

Weigh 0.4 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 5 mL of methanol (50%). Sonicate (140 W) the mixture for 15 min. Centrifuge at about $2800 \times g$ for 10 min. Filter through a 0.45- μm PTFE filter.

Procedure

Carry out the method by using a HPTLC silica gel GHLF₂₅₄ plate (150 μm thickness), a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately quisqualic acid standard solution (1.5 μL) and the test solution (1.5 - 4.5 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. Spray the plate evenly with the spray reagent and heat at about 105°C until the spots or bands become visible (about 3-5 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

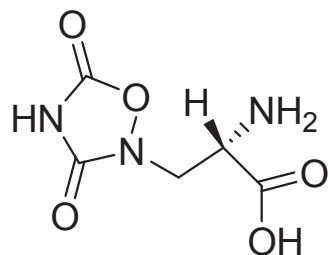


Figure 4 Chemical structure of quisqualic acid

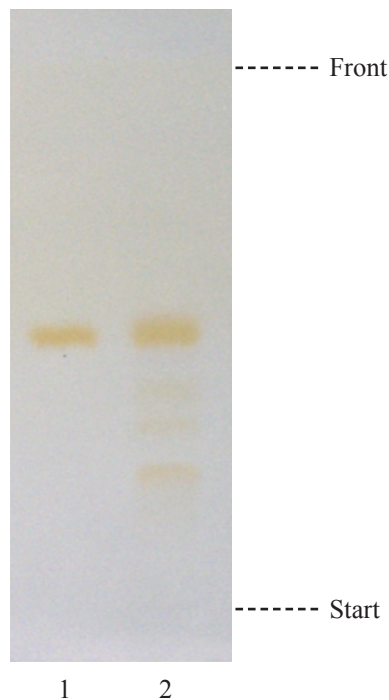


Figure 5 A reference HPTLC chromatogram of *Quisqualis Fructus* extract observed under visible light after staining

1. Quisqualic acid 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 quisqualic acid (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting (*Appendix XII*)

Reagents

0.2 M Acetic acid solution

Pipette 11.4 mL of acetic acid and add 1000 mL of water.

0.01 M Sodium acetate buffer solution (pH 4.6)

Weigh 16.4 g of sodium acetate and dissolve in 1000 mL of water. Pipette 24.5 mL of the solution and 25.5 mL of 0.2 M acetic acid solution to a 1000-mL volumetric flask. Make up to the mark with water.

10 M Sodium hydroxide solution

Weigh 40.0 g of sodium hydroxide and dissolve in 100 mL of water.

0.04 M Boric acid buffer solution (pH 10.5)

Weigh 2.5 g of boric acid and dissolve in 1000 mL of water. Adjust the pH value to 10.5 with 10 M sodium hydroxide solution.

Quisqualis Fructus*o*-Phthalaldehyde solution

Weigh 70.0 mg of *o*-phthalaldehyde and dissolve it in a mixture of 1 mL of methanol, 95 mL of 0.04 M boric acid buffer solution (pH 10.5) and 0.2 mL of 2-mercaptoethanol. Freshly prepare the solution.

Standard solution

Quisqualic acid standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of quisqualic acid CRS and dissolve in 10 mL of methanol (50%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $1800 \times g$ for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Centrifuge at about $1800 \times g$ for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45- μm PTFE filter.

Derivatization

Pipette 200 μL of the standard solution or the test solution to a 2-mL vial, then pipette 400 μL of *o*-phthalaldehyde solution. Freshly prepare the solution and should be analyzed within 10 min.

Chromatographic system

The liquid chromatograph is equipped with a fluorescence detector ($\lambda_{\text{ext}} = 338 \text{ nm}$ and $\lambda_{\text{em}} = 450 \text{ nm}$) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.01M Sodium acetate buffer solution (pH 4.6) (% , v/v)	Methanol (% , v/v)	Elution
0 – 5	80	20	isocratic
5 – 60	80 \rightarrow 20	20 \rightarrow 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μ L of quisqualic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of quisqualic acid should not be more than 5.0%; the RSD of the retention time of quisqualic acid peak should not be more than 2.0%; the column efficiency determined from quisqualic acid peak should not be less than 20000 theoretical plates.

The *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject quisqualic acid Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of quisqualic acid peak in the chromatogram of quisqualic acid Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify quisqualic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of quisqualic acid Std-FP. The retention times of quisqualic acid 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 four characteristic peaks of Quisqualis Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Quisqualis Fructus extract

Peak No.	RRT	Acceptable Range
1 (asparagine)	0.95	± 0.03
2 (marker, quisqualic acid)	1.00	-
3 (glutamic acid)	1.47	± 0.03
4 (arginine)	1.55	± 0.05

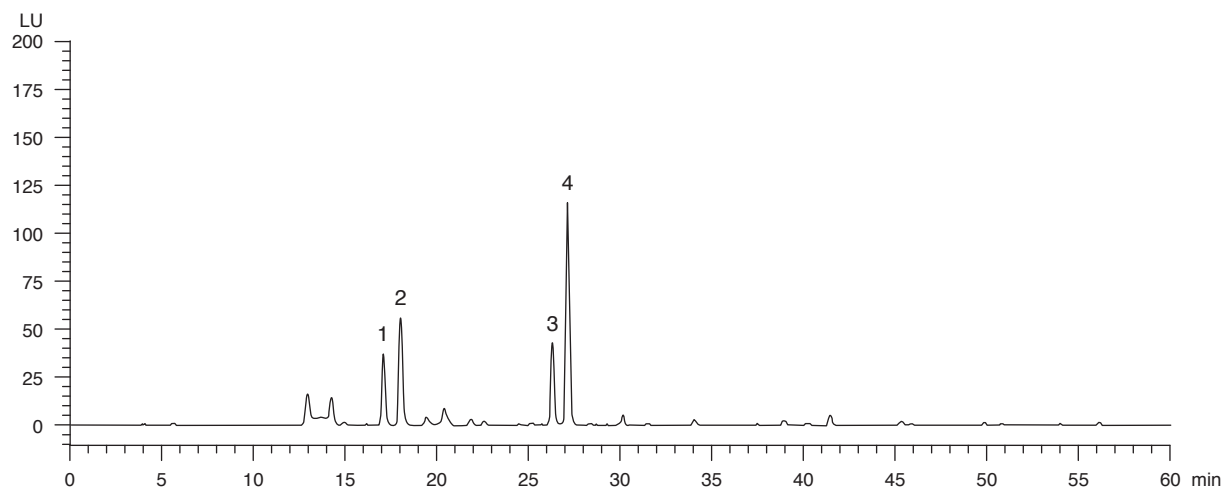
Quisqualis Fructus

Figure 6 A reference fingerprint chromatogram of *Quisqualis Fructus* extract

For positive identification, the sample must give the above four 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 (*Appendix VII*): meet the requirements.

5.4 Sulphur Dioxide Residues (*Appendix XVII*): meet the requirements.

5.5 Foreign Matter (*Appendix VIII*): not more than 1.0%.

5.6 Ash (*Appendix IX*)

Total ash: not more than 4.5%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content (*Appendix X*)

Oven dried method: not more than 16.0%.

6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (cold extraction method): not less than 12.0%.

Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Reagents

0.2 M Acetic acid solution

Pipette 11.4 mL of acetic acid and add 1000 mL of water.

0.01 M Sodium acetate buffer solution (pH 4.6)

Weigh 16.4 g of sodium acetate and dissolve in 1000 mL of water. Pipette 24.5 mL of the solution and 25.5 mL of 0.2 M acetic acid solution to a 1000-mL volumetric flask. Make up to the mark with water.

10 M Sodium hydroxide solution

Weigh 40.0 g of sodium hydroxide and dissolve in 100 mL of water.

0.04 M Boric acid buffer solution (pH 10.5)

Weigh 2.5 g of boric acid and dissolve in 1000 mL of water. Adjust the pH value to 10.5 with 10 M sodium hydroxide solution.

o-Phthalaldehyde solution

Weigh 70.0 mg of *o*-phthalaldehyde and dissolve it in a mixture of 1 mL of methanol, 95 mL of 0.04 M boric acid buffer solution (pH 10.5) and 0.2 mL of 2-mercaptoethanol. Freshly prepare the solution.

Standard solution

Quisqualic acid standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 1.0 mg of quisqualic acid CRS and dissolve in 5 mL of methanol (50%).

Quisqualic acid standard solution for assay, Std-AS

Measure accurately the volume of the quisqualic acid Std-Stock, dilute with methanol (50%) to produce a series of solutions of 10, 20, 50, 100, 200 mg/L for quisqualic acid.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $1800 \times g$ for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Centrifuge at about $1800 \times g$ for 10 min. Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45- μm PTFE filter.

Derivatization

Pipette 200 μL of the standard solution or the test solution to a 2-mL vial, then pipette 400 μL of *o*-phthalaldehyde solution. Freshly prepare the solution and should be analyzed within 10 min.

Chromatographic system

The liquid chromatograph is equipped with a fluorescence detector ($\lambda_{\text{ext}} = 338 \text{ nm}$ and $\lambda_{\text{em}} = 450 \text{ nm}$) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.01M Sodium acetate buffer solution (pH 4.6) (% v/v)	Methanol (% v/v)	Elution
0 – 5	80	20	isocratic
5 – 60	80 \rightarrow 20	20 \rightarrow 80	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quisqualic acid Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of quisqualic acid should not be more than 5.0%; the RSD of the retention time of quisqualic acid peak should not be more than 2.0%; the column efficiency determined from quisqualic acid peak should not be less than 20000 theoretical plates.

The *R* value between quisqualic acid 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 quisqualic acid Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of quisqualic acid against the corresponding concentrations of quisqualic acid Std-AS. Obtain the slope, *y*-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify quisqualic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of quisqualic acid Std-AS. The retention times of quisqualic acid 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 quisqualic acid in the test solution, and calculate the percentage content of quisqualic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.63% of quisqualic acid (C₅H₇N₃O₅), calculated with reference to the dried substance.