Arecae Semen

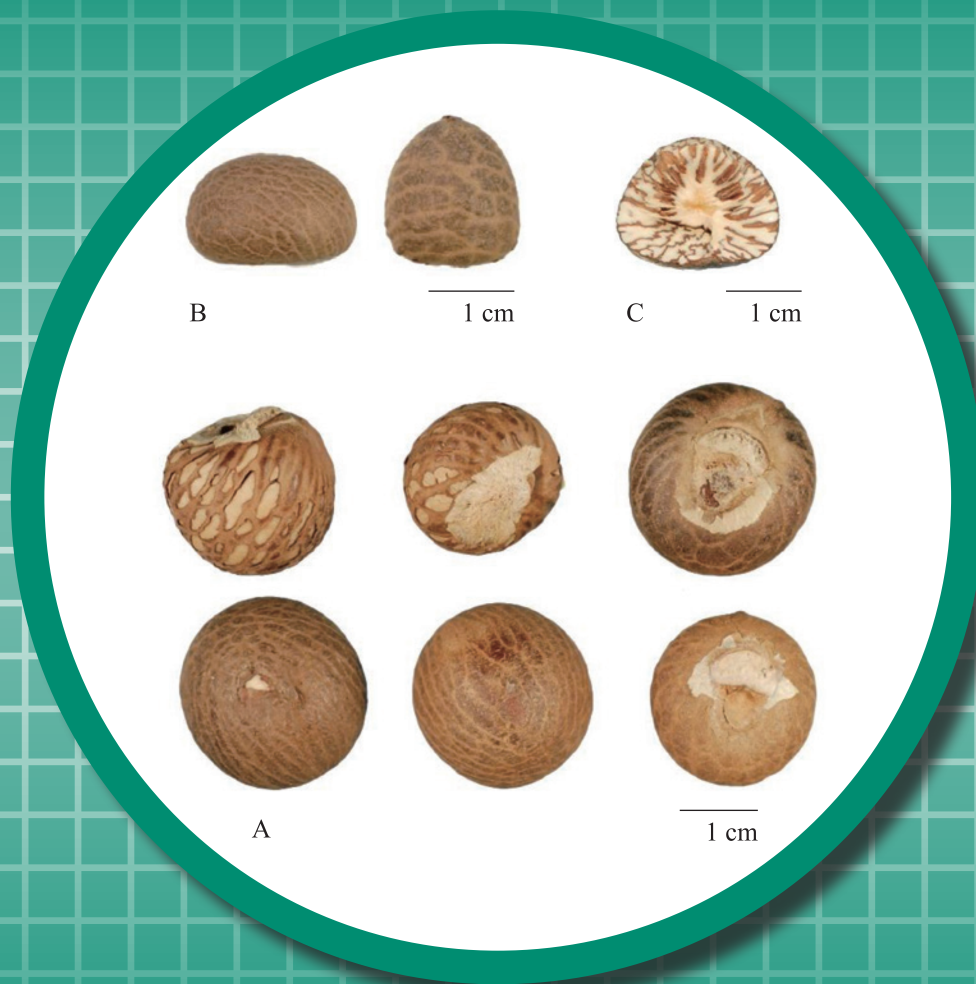

Figure 1 A photograph of Arecae Semen

A. Arecae Semen B. Lateral view of Arecae Semen

C. Longitudinal section of seed with marble-like striations

1. NAMES

Official Name: Arecae Semen

Chinese Name: 檳榔

Chinese Phonetic Name: Binglang

2. SOURCE

Arecae Semen is the dried ripe seed of *Areca catechu* L. (Arecaceae). The ripe fruit is collected from late spring to early autumn; boiled in water, dried, pericarp removed, and the seed is collected, then dried under the sun to obtain Arecae Semen.

3. DESCRIPTION

Oblate or conical, 1.1-2.7 cm high, base 15-30 mm in diameter. Externally pale yellowish-brown or pale reddish-brown, with slightly dented reticulate furrows; a rounded concave micropyle located at the centre of the base and accompanying with an obvious scar-shaped hilum by the side. Texture hard, uneasily broken. Fracture showing marble-like striations alternating with brown testa and white endosperm. Odour slight; taste astringent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Outer layers of testa consist of several layers of flattened stone cells, elongated tangentially, containing reddish-brown contents; stone cells vary in shape and size, usually with intercellular space. Inner layers of testa consist of several layers of parenchymatous cells, containing reddish-brown contents, scattered with few vascular bundles. Perisperm relatively narrow, the inner layers of testa and perisperm usually inserted into the endosperm, forming a crisscross tissue. Endosperm cells polygonal, with thickened walls, pits large, containing aleurone grains (Fig. 2).

Powder

Colour charred yellow. Endosperm cells extremely numerous, colourless, mostly broken, intact cells irregular polygonal or subsquare, 32-136 µm in diameter, intercellular layers indistinct, walls 5-14 µm thick, pits abundant, subrounded or rectangular-rounded, very large, 4-25 µm in diameter. Perisperm cells subsquare, subpolygonal or strip-shaped, 32-75 µm in diameter, pit canals distinct, most lumens filled with reddish-brown or dark brown masses; bright white to reddish-brown under the polarized microscope. Vessels spiral or reticulate, occasionally visible, 5-20 µm in diameter. Stone cells of testa subrounded, polygonal, fusiform or irregular, 11-73 µm in diameter, walls pale yellowish-brown, 4-16 µm thick, pits few, pits slit-shaped, some lumens filled with reddish-brown masses (Fig. 3).

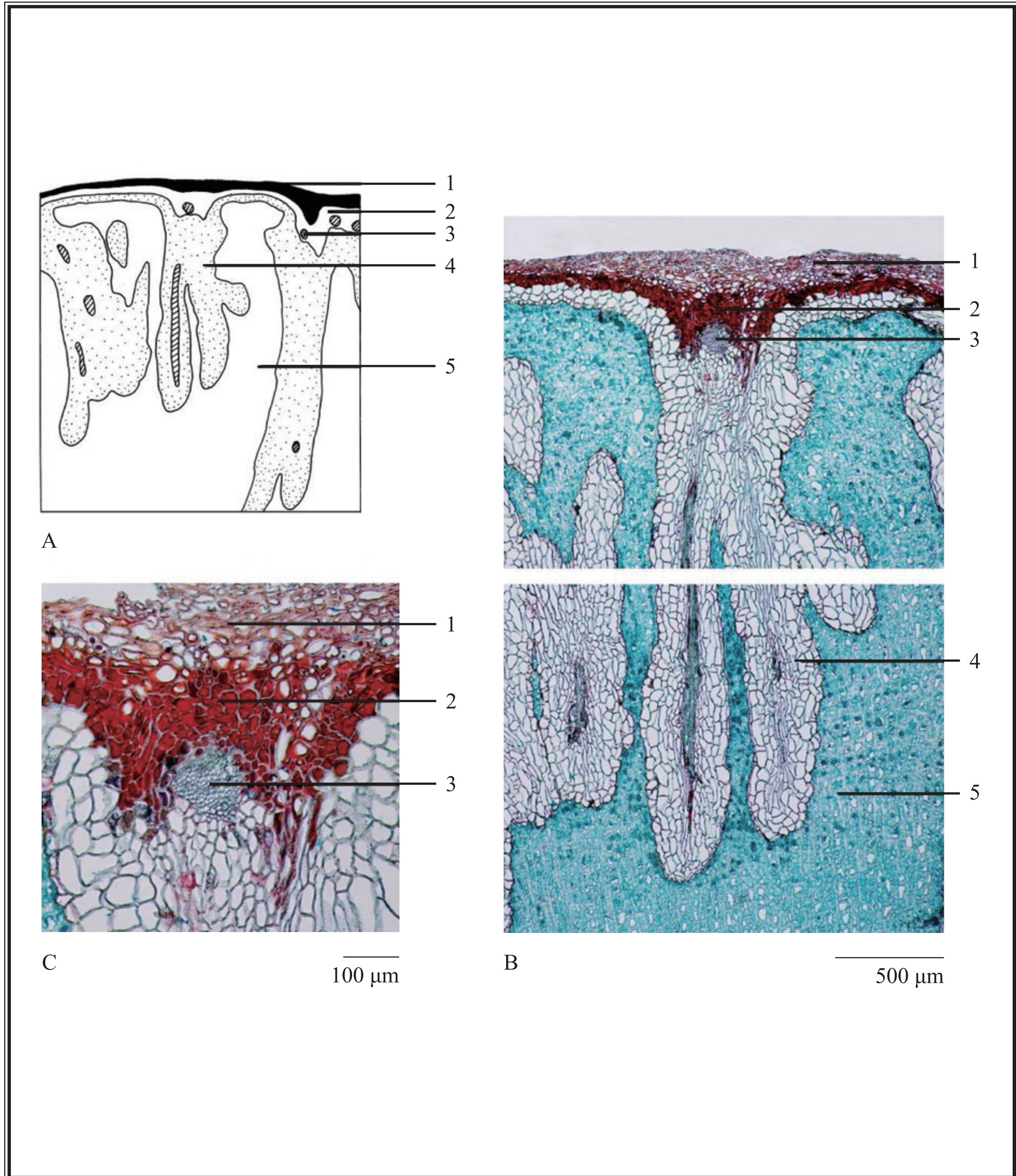

Figure 2 Microscopic features of transverse section of Arecae Semen

A. Sketch B. Section illustration C. Vascular bundle

1. Outer layers of testa 2. Inner layers of testa 3. Vascular bundle
4. Perisperm (crisscross tissue) 5. Endosperm

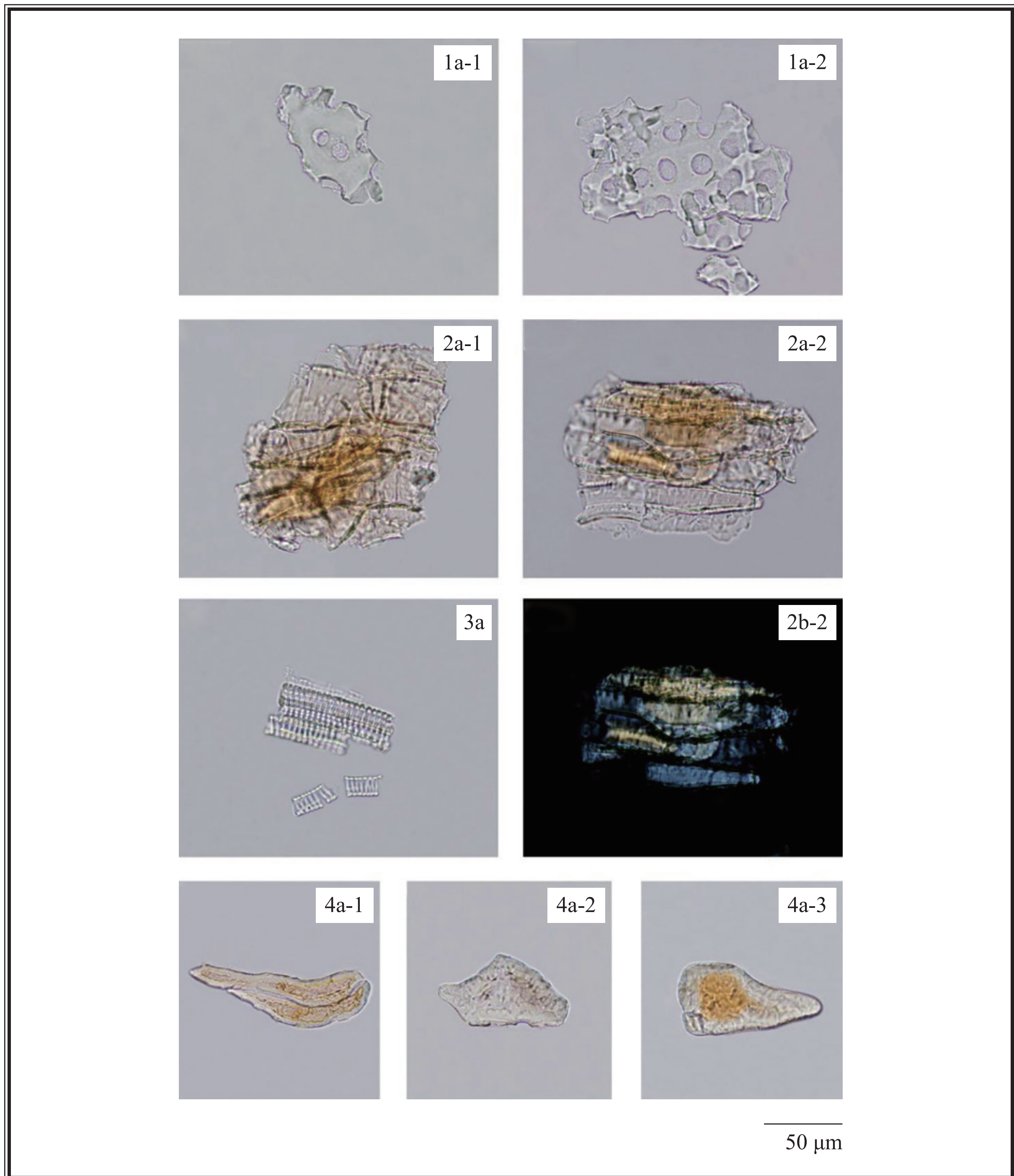

Figure 3 Microscopic features of powder of Arecae Semen

1. Endosperm cells 2. Perisperm cells 3. Vessels 4. Stone cells

a. Features under the light microscope b. Features under the polarized microscope

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

Standard solutions

Arecaidine hydrochloride standard solution

Weigh 1.0 mg of arecaidine hydrochloride CRS (Fig. 4) and dissolve in 1 mL of methanol.

Arecoline hydrobromide standard solution

Weigh 1.0 mg of arecoline hydrobromide CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ammonium hydroxide solution (28%, v/v), methanol and dichloromethane (0.5:2:6, v/v).

Staining reagent

Iodine.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 100-mL conical flask, then add 10 mL of methanol (80%). Sonicate (400 W) the mixture for 1 h. Filter the mixture.

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 arecaidine hydrochloride standard solution (2 μL), arecoline hydrobromide 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. Fumigate the plate with iodine vapor chamber for about 20 min until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

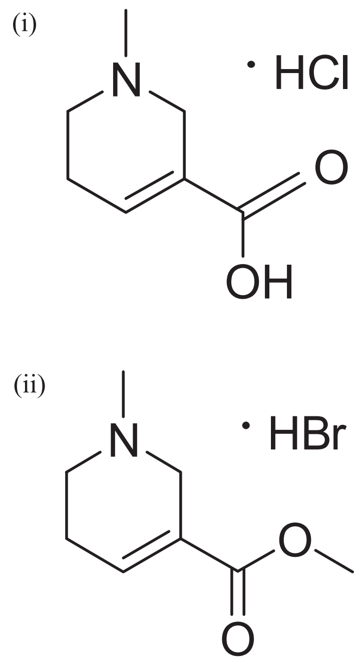

Figure 4 Chemical structures of (i) arecaidine hydrochloride and (ii) arecoline hydrobromide

Figure 5 A reference HPTLC chromatogram of Arecae Semen extract observed under visible light after staining

1. Arecoline hydrobromide standard solution
2. Arecaidine hydrochloride standard solution 3. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of arecaidine and arecoline (Fig. 5).

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

Standard solutions

Arecaidine hydrochloride standard solution for fingerprinting, Std-FP (13 mg/L)

Weigh 0.65 mg of arecaidine hydrochloride CRS and dissolve in 50 mL of methanol (50%).

Arecoline hydrobromide standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 1.5 mg of arecoline hydrobromide CRS and dissolve in 25 mL of methanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (50%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with strong cation exchanger bonded silica gel (5 µm particle size). The column temperature is maintained at 35ºC during the separation. The flow rate is about 1.2 mL/min. The mobile phase is a mixture of 0.2% phosphoric acid* and acetonitrile (35:65, v/v). The elution time is about 35 min.

*Adjust the pH to 3.8 with ammonium hydroxide solution (28%, v/v)

System suitability requirements

Perform at least five replicate injections, each using 10 µL of arecaidine hydrochloride Std-FP and arecoline hydrobromide Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of arecaidine and arecoline should not be more than 5.0%; the RSD of the retention times of arecaidine and arecoline peaks should not be more than 2.0%; the column efficiencies determined from arecaidine and arecoline peaks should not be less than 6000 theoretical plates.

The *R* value between peak 2 and the closest peak; and 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 arecaidine hydrochloride Std-FP, arecoline hydrobromide Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of arecaidine and arecoline peaks in the chromatograms of arecaidine hydrochloride Std-FP, arecoline hydrobromide Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify arecaidine and arecoline peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of arecaidine hydrochloride Std-FP and arecoline hydrobromide Std-FP. The retention times of arecaidine and arecoline peaks in the chromatograms of the test solution and the corresponding Std-FP 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 Arecae Semen extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Arecae Semen extract

Peak No.	RRT	Acceptable Range
1	0.44	± 0.03
2 (arecaidine)	0.50	± 0.03
3	0.80	± 0.03
4 (marker, arecoline)	1.00	-

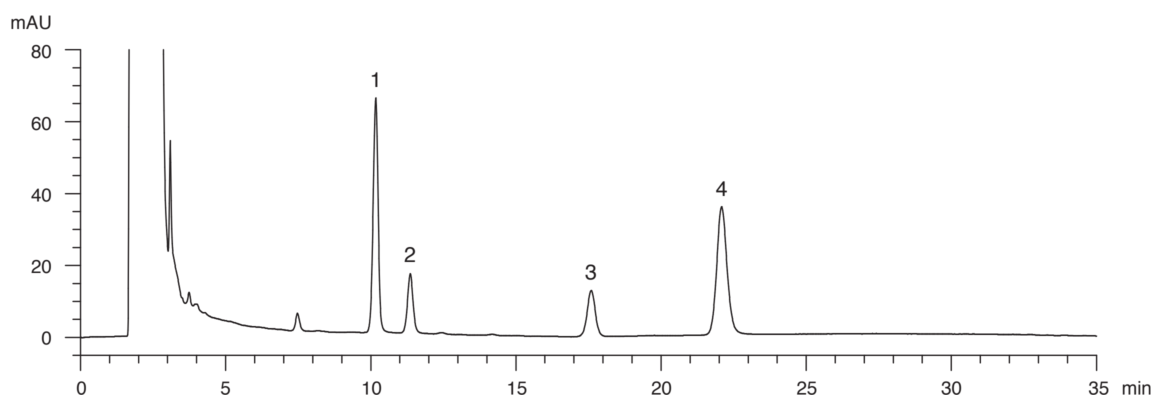

Figure 6 A reference fingerprint chromatogram of Arecae Semen extract

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 2.0%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 12.0%.
Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

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

Standard solution

Mixed arecaidine hydrochloride and arecoline hydrobromide standard stock solution, Std-Stock (63.2 mg/L for arecaidine hydrochloride and 304 mg/L for arecoline hydrobromide)

Weigh accurately 1.58 mg of arecaidine hydrochloride CRS and 7.6 mg of arecoline hydrobromide CRS, and dissolve in 25 mL of methanol (50%).

Mixed arecaidine hydrochloride and arecoline hydrobromide standard solution for assay, Std-AS

Measure accurately the volume of the mixed arecaidine hydrochloride and arecoline hydrobromide Std-Stock, dilute with methanol (50%) to produce a series of solutions of 0.6, 1.3, 3.2, 6.3, 12.6 mg/L for arecaidine hydrochloride and 6.1, 15.2, 30.4, 60.9, 152.2 mg/L for arecoline hydrobromide.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of methanol (50%). Sonicate (400 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Combine the solutions and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (210 nm) and a column (4.6 × 250 mm) packed with strong cation exchanger bonded silica gel (5 μm particle size). The column temperature is maintained at 35ºC during the separation. The flow rate is about 1.2 mL/min. The mobile phase is a mixture of 0.2% phosphoric acid* and acetonitrile (35:65, v/v). The elution time is about 35 min.

*Adjust the pH to 3.8 with ammonium hydroxide solution (28%, v/v)

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed arecaidine hydrochloride and arecoline hydrobromide Std-AS (3.2 mg/L for arecaidine hydrochloride and 30.4 mg/L for arecoline hydrobromide). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of arecaidine and arecoline should not be more than 5.0%; the RSD of the retention times of arecaidine and arecoline peaks should not be more than 2.0%; the column efficiencies determined from arecaidine and arecoline peaks should not be less than 6000 theoretical plates.

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

Calibration curves

Inject a series of the mixed arecaidine hydrochloride and arecoline hydrobromide Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of arecaidine and arecoline against the corresponding concentrations of the mixed arecaidine hydrochloride and arecoline hydrobromide Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify arecaidine and arecoline peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed arecaidine hydrochloride and arecoline hydrobromide Std-AS. The retention times of arecaidine and arecoline peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of arecaidine and arecoline in the test solution, and calculate the percentage contents of arecaidine (the percentage content of arecaidine hydrochloride × 0.795, where 0.795 is the molar mass ratio of arecaidine and arecaidine hydrochloride) and arecoline (the percentage content of arecoline hydrobromide × 0.657, where 0.657 is the molar mass ratio of arecoline and arecoline hydrobromide) in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.23% of the total content of arecaidine ($C_7H_{11}NO_2$) and arecoline ($C_8H_{13}NO_2$), calculated with reference to the dried substance.