Cervi Cornu Pantotrichum

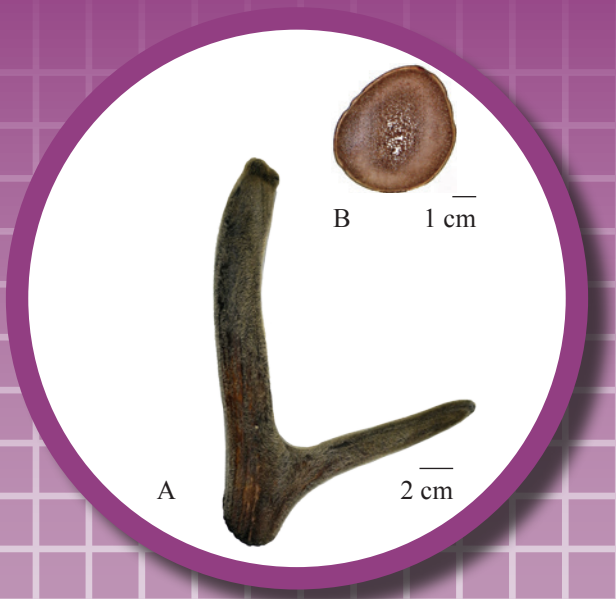

Figure 1 (i) A photograph of young unossified hairy antler of male *Cervus nippon* Temminck

A. A young unossified hairy male antler (Ergang) B. Slice

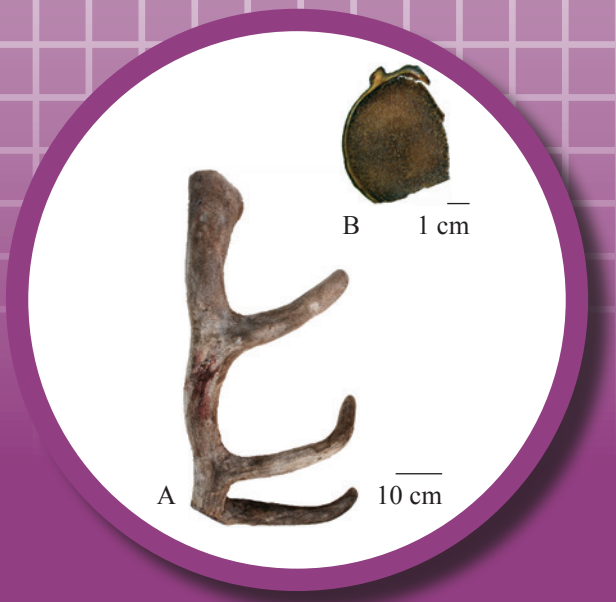

Figure 1 (ii) A photograph of young unossified hairy antler of male *Cervus elaphus* Linnaeus

A. A young unossified hairy male antler (Sancha) B. Slice

1. NAMES

Official Name: Cervi Cornu Pantotrichum

Chinese Name: 鹿茸

Chinese Phonetic Name: Lurong

2. SOURCE

Cervi Cornu Pantotrichum is the young unossified hairy antler of male *Cervus nippon* Temminck or *Cervus elaphus* Linnaeus (Cervidae). The former is known as "Hualurong" (sika deer pilose antler), the later known as "Malurong" (red deer pilose antler). The young unossified hairy antler is collected when the deer is aged 3, "Ergang" collected twice a year. The antler collected 45-50 days after Ching Ming festival is known as "Tou cha rong", the one collected after the beginning of Autumn is known as "Er cha rong". "San Cha" collect once a year, in late July. After the antler collected, then process: blood and dirt removed, washed clean, dried in the shaded area or baked to dryness below 60°C to obtain Cervi Cornu Pantotrichum.

3. DESCRIPTION

***Cervus nippon* Temminck:** Branched, cylindrical, commonly known as "Ergang" with one branch; the main branch as "Dating", 16-20 cm long, slightly triquetrous, apex obtuse and rounded. The side branch arising at about 2 cm from the cut face or at the cut face, known as "Menzhuang", 8-12 cm long, slightly smaller than the main branch in diameter. The out skin reddish-brown or brown, usually lustrous, densely covered with reddish-yellow or brownish-yellow soft hairs, relatively dense at the upper end, and sparse at the lower end, a greyish-black vein at the base between the main and side branches, the skin and hairs nestled each other. The cut surface yellowish-white, without bony element in the outer part, small pores densely distributed in the central part. Texture light in weight. Odour slightly stinky; taste slightly salty [Fig. 1 (i)].

***Cervus elaphus* Linnaeus:** Larger and thicker than Hualurong, with relatively more branches, commonly known as "Sancha" with three branches. The main branch (Dating) thick and strong, 25-100 cm long, 4-5 cm in diameter. The two branches arising from the base of antler, closed each other, the second branch farther away from the third one. Apex obtuse and rounded. The out skin greyish-brown, soft hairs thick and strong, dense, greyish-yellow to greyish-brown. The cut face greyish-black, small pores densely distributed in the central part. Texture tender or bony. Odour stinky; taste salty [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

***Cervus nippon* Temminck:** The outer surface of skin sinuous, soft hairs numerous and dense, or relatively sparse, easily fallen off during transverse section processing. Transverse section consists of layers of skin, mesenchyme and bone. Layers of skin consist of epidermis and dermis. The outermost layer of epidermis composed of the stratum corneum, arranged in scale-shape; the inner consists of several layers of stratum germinativum, with papilla protruding from the dermis. Dermis relatively thick, mainly consists of dense connective tissues, scattered with hair follicles, sebaceous glands and blood vessels. Sebaceous glands scattered singly or several in groups; blood vessels varied in size, with thickened wall and indistinct lumen, concentric and ring-shape striations distinct, surrounded by relatively dense or sparse tissues. Mesenchyme consists of sparse connective tissues. Bone mainly consists of bone trabecula. Bone trabecula spongy, covered with numerous bone lacunae and bone tubes on surface [Fig. 2 (i)].

***Cervus elaphus* Linnaeus:** Soft hairs relatively numerous, easily fallen off during transverse section processing. Papillary layer flattened, without obviously denticulate protruding. The inner part of dermis scattered with large long-oblate and elliptical blood vessels, elongated tangentially, lumen closed, surrounded by small blood vessels. Bone trabecula near mesenchyme relatively thin and sparse, and those in the central part relatively thick, sparsely reticulated, bone lacunae relatively less [Fig. 2 (ii)].

Powder

***Cervus nippon* Temminck:** Colour pale yellow. Stratum corneum pale yellow, externally granular, rough and uneven, alveoli rounded and hole-shaped after soft hairs fall off, edge even. Fragments of bone nearly colourless or pale yellowish-brown, irregular pieces, with fine and dense longitudinal striations and dotted pores externally; bone lacunae relatively numerous, rounded, subtriangular or subfusiform, varing in size, 8-22 μm in diameter, arranged irregularly, edge tubes visible faintly, showing radial furrows. Hairs mostly broken, the middle part of hair shaft 11-56 μm in diameter, externally covered with many scale-shaped and transparent hair cuticle cells, imbricate, the free edge faced the hair point, spiny, with longitudinal and straight striations, medulla interrupted or absent, greyish-black or greyish-brown; bright white under the polarized microscope. Hair base often connected with hair follicle, base expanded, elliptical or subrounded [Fig. 3 (i)].

***Cervus elaphus* Linnaeus:** Colour greyish-yellow. Stratum corneum pale yellow or brown, with relatively more alveoli. Bone lacunae of fragments of bone relatively large, arranged sparsely, 11-26 μm in diameter, with indistinct fine and dense longitudinal striations externally. The middle part of hair shaft 15-70 μm in diameter, scale-shaped cells arranged parallel externally, medulla beaded or reticulate; bright white under the polarized microscope. Hair base often connected with hair follicle, base expanded, elliptical or subrounded [Fig. 3 (ii)].

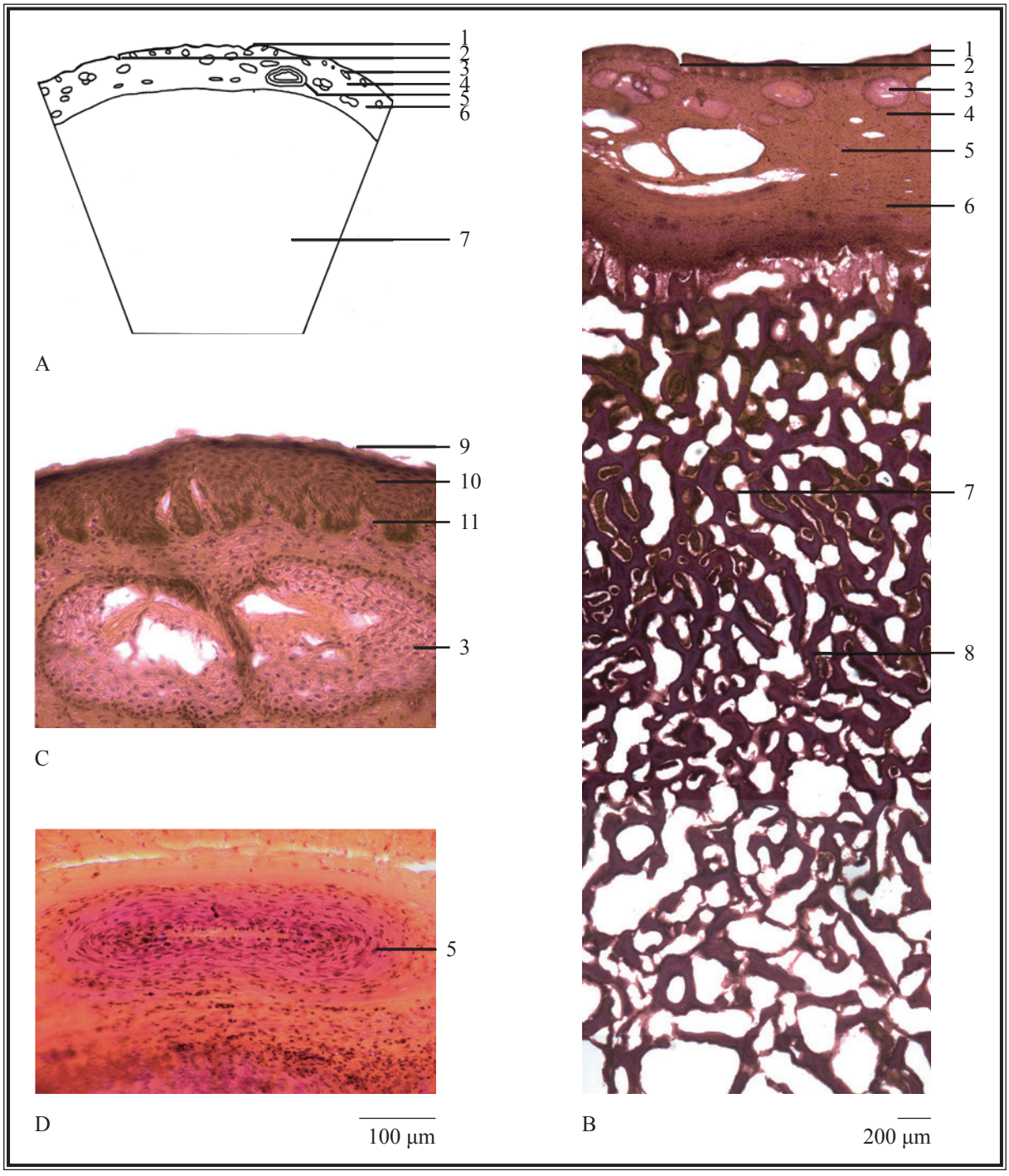

Figure 2 (i) Microscopic features of transverse section of young unossified hairy antler of male *Cervus nippon* Temminck

A. Sketch B. Section illustration C. Sebaceous glands D. Blood vessel

1. Epidermis 2. Hair follicle 3. Sebaceous gland 4. Dermis 5. Blood vessel 6. Mesenchyme 7. Bone 8. Bone trabecula 9. Stratum corneum 10. Stratum germinativum 11. Papillary layer

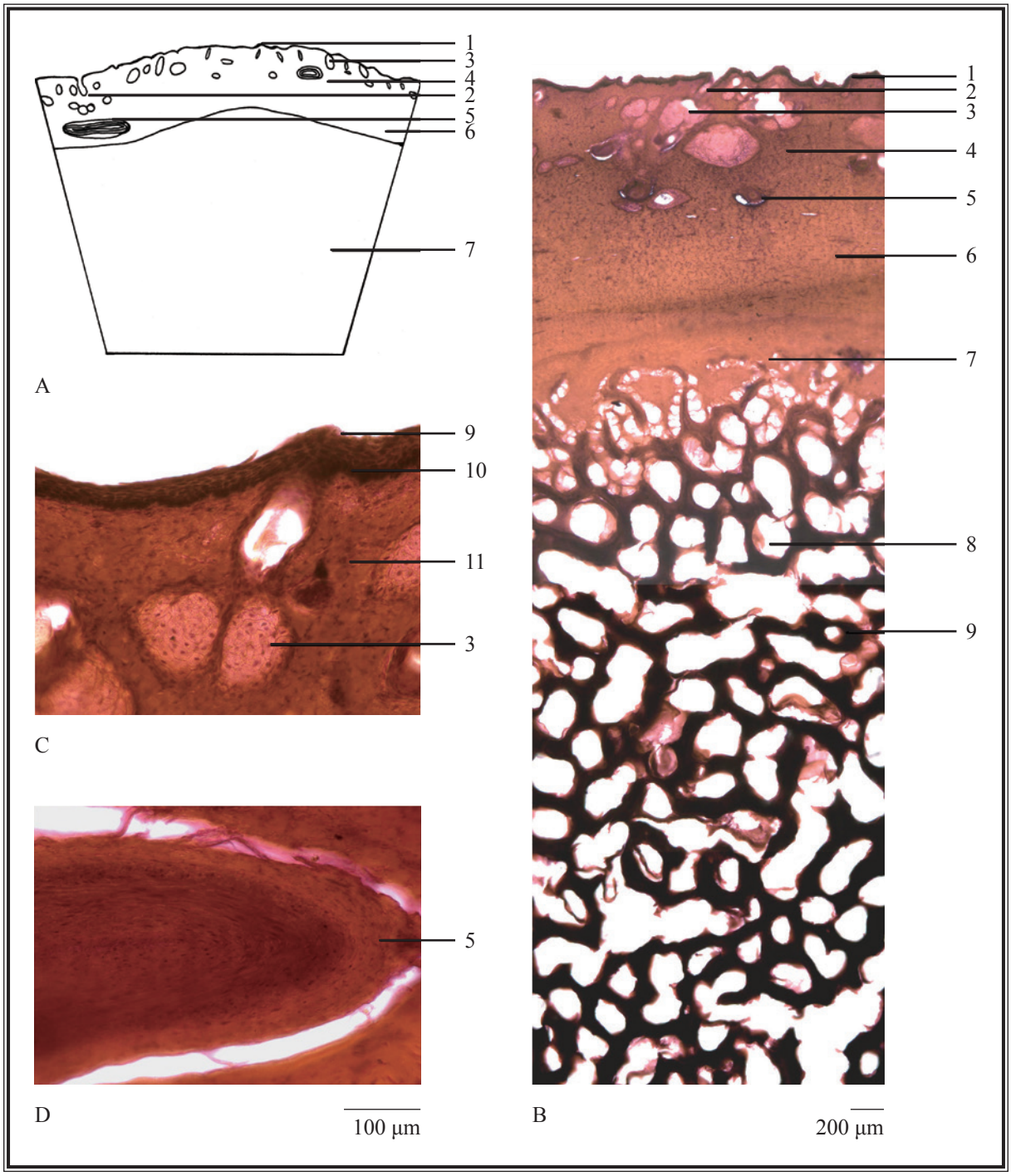

Figure 2 (ii) Microscopic features of transverse section of young unossified hairy antler of male *Cervus elaphus* Linnaeus

A. Sketch B. Section illustration C. Sebaceous glands D. Blood vessel

1. Epidermis 2. Hair follicle 3. Sebaceous gland 4. Dermis 5. Blood vessel 6. Mesenchyme
7. Bone 8. Bone trabecula 9. Stratum corneum 10. Stratum germinativum 11. Papillary layer

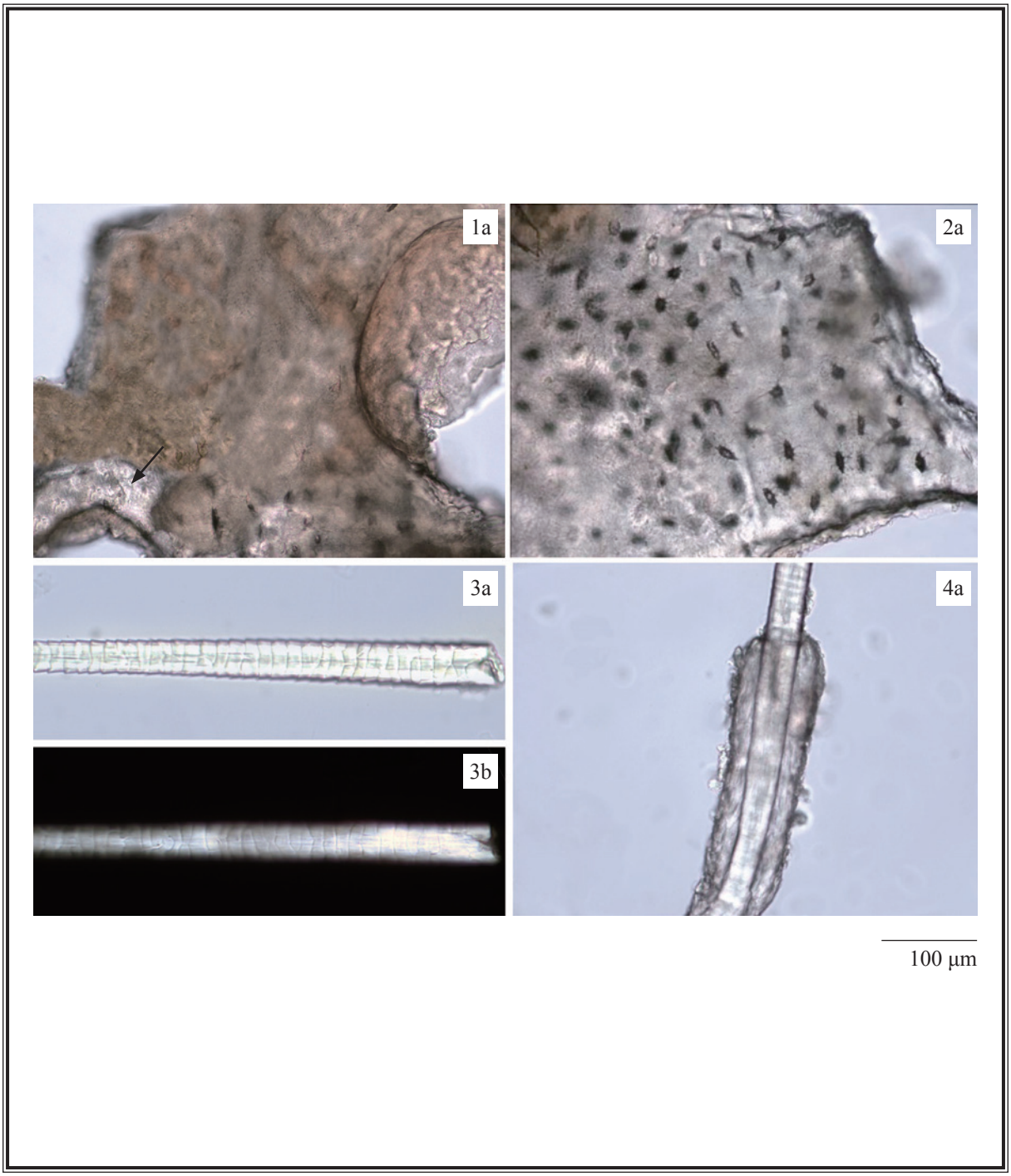

Figure 3 (i) Microscopic features of powder of young unossified hairy antler of male *Cervus nippon* Temminck

1. Stratum corneum (alveolus ⟶) 2. Fragments of bone contain bone lacunae 3. Hair shaft
4. Hair base with hair follicle

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

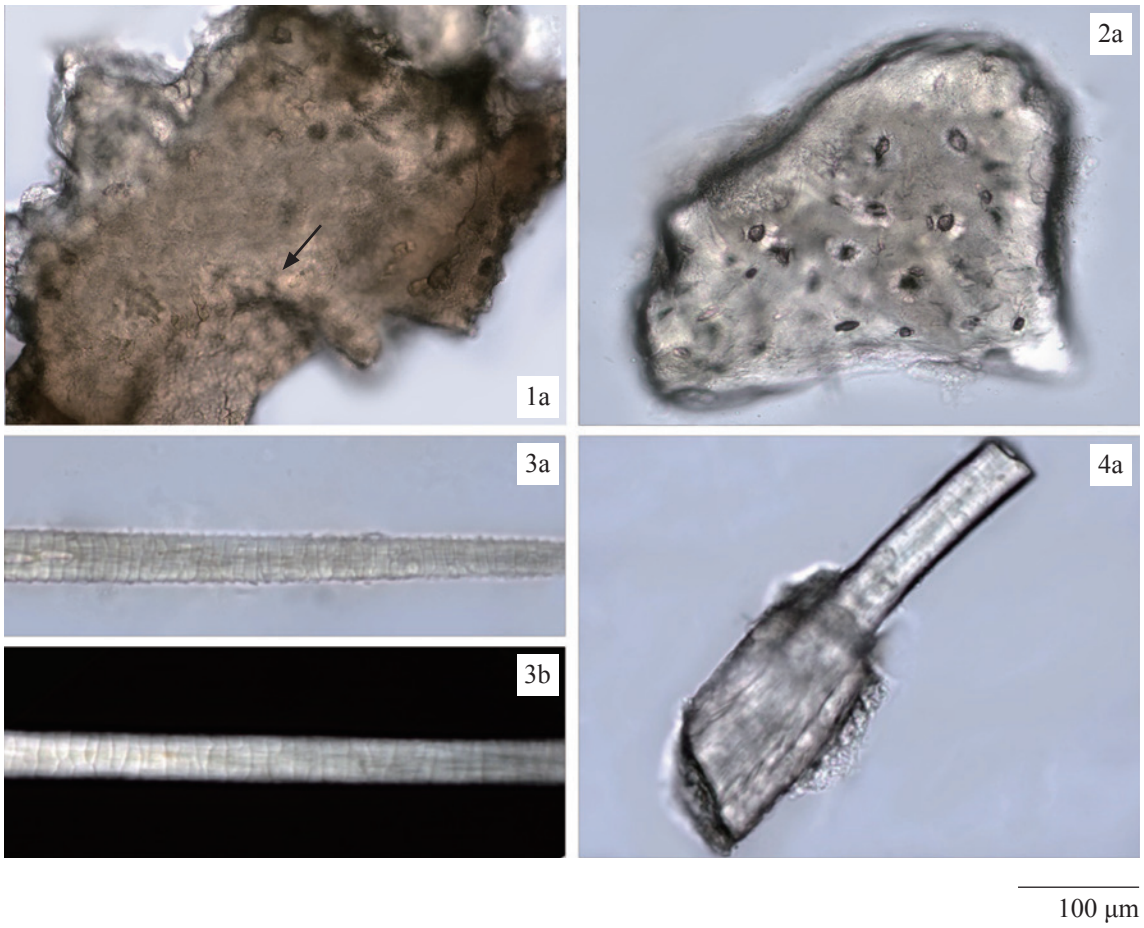

Figure 3 (ii) Microscopic features of powder of young unossified hairy antler of male *Cervus elaphus* Linnaeus

1. Stratum corneum (alveolus ⟶) 2. Fragments of bone containing bone lacunae 3. Hair shaft
4. Hair base with hair follicle

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

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

Standard solution

Cholesterol standard solution

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

Developing solvent system

Prepare a mixture of petroleum ether (40-60°C), diethyl ether and glacial acetic acid (5:5:0.1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of methanol. Sonicate (100 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 methanol.

Procedure

Carry out the method by using a TLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately cholesterol standard solution (3 μL) and the test solution (4 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the TLC 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 TLC plate for development. Develop over a path of about 7 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 10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

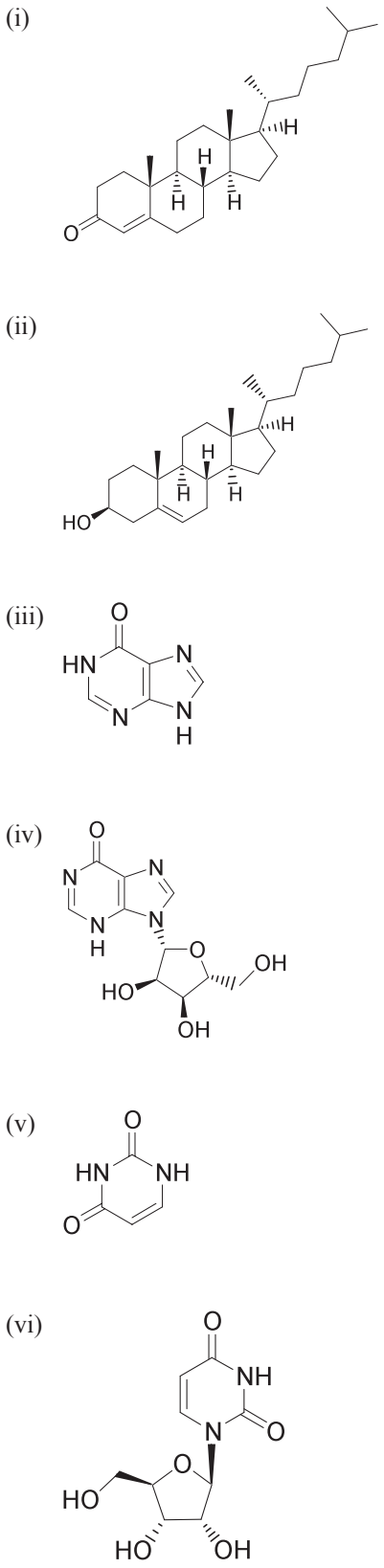

Figure 4 Chemical structures of (i) cholest-4-en-3-one (ii) cholesterol (iii) hypoxanthine (iv) inosine (v) uracil and (vi) uridine

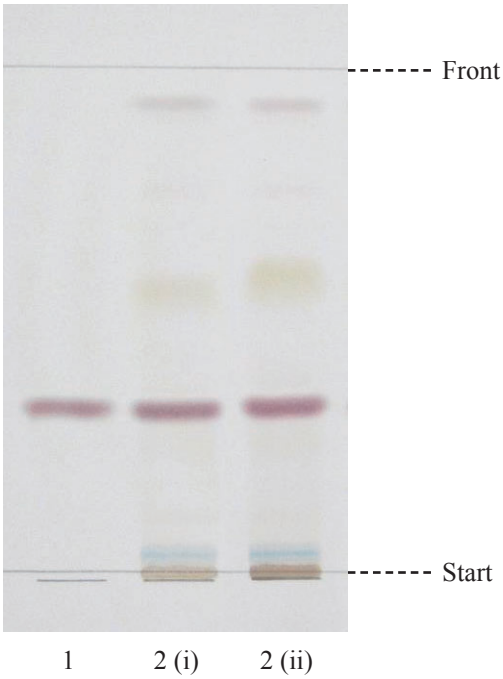

Figure 5 A reference TLC chromatogram of Cervi Cornu Pantotrichum extract observed under visible light after staining

1. Cholesterol standard solution
2. Test solution of
(i) young unossified hairy antler of male *Cervus nippon* Temminck
(ii) young unossified hairy antler of male *Cervus elaphus* Linnaeus

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 cholesterol (Fig. 5).

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

Reagent

5 mM Ammonium acetate solution

Weigh 0.385 g of ammonium acetate and dissolve in 1000 mL of water.

Standard solutions

Hypoxanthine standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of hypoxanthine CRS (Fig. 4) and dissolve in 50 mL of methanol (10%).

Inosine standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of inosine CRS (Fig. 4) and dissolve in 50 mL of methanol (10%).

Uracil standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of uracil CRS (Fig. 4) and dissolve in 50 mL of methanol (10%).

Uridine standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 1.0 mg of uridine CRS (Fig. 4) and dissolve in 50 mL of methanol (10%).

Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of methanol (10%). Sonicate (100 W) the mixture for 45 min. Centrifuge at about 3000 x *g* for 10 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (260 nm) and a column (4.6 × 250 mm) packed with alkyl reversed-phase bonded silica gel with diisopropyl side chain (5 μm particle size). The flow rate is about 0.4-1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	5 mM Ammonium acetate solution (%, v/v)	Flow rate (mL/min)	Elution
0 – 18	1	99	0.4 → 1.0	linear gradient
18 – 20	1 → 4	99 → 96	1.0	linear gradient
20 – 25	4 → 5	96 → 95	1.0	linear gradient
25 – 40	5 → 20	95 → 80	1.0	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of hypoxanthine Std-FP, inosine Std-FP, uracil Std-FP and uridine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of hypoxanthine, inosine, uracil and uridine should not be more than 5.0%; the RSD of the retention times of hypoxanthine, inosine, uracil and uridine peaks should not be more than 2.0%; the column efficiencies determined from hypoxanthine, inosine, uracil and uridine peaks should not be less than 16000 theoretical plates.

The *R* value between peak 1 and the closest peak; the *R* value between peak 2 and the closest peak; the *R* value between peak 3 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 1.0 [Fig. 6 (i) or (ii)].

Procedure

Separately inject hypoxanthine Std-FP, inosine Std-FP, uracil Std-FP, uridine Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of hypoxanthine, inosine, uracil and uridine peaks in the chromatograms of hypoxanthine Std-FP, inosine Std-FP, uracil Std-FP, uridine Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify hypoxanthine, inosine, uracil and uridine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of hypoxanthine Std-FP, inosine Std-FP, uracil Std-FP and uridine Std-FP. The retention times of hypoxanthine, inosine, uracil and uridine 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 Cervi Cornu Pantotrichum extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Cervi Cornu Pantotrichum extract

Peak No.	RRT	Acceptable Range
1 (uracil)	0.54	± 0.04
2 (uridine)	0.64	± 0.04
3 (hypoxanthine)	0.93	± 0.03
4 (marker, inosine)	1.00	-

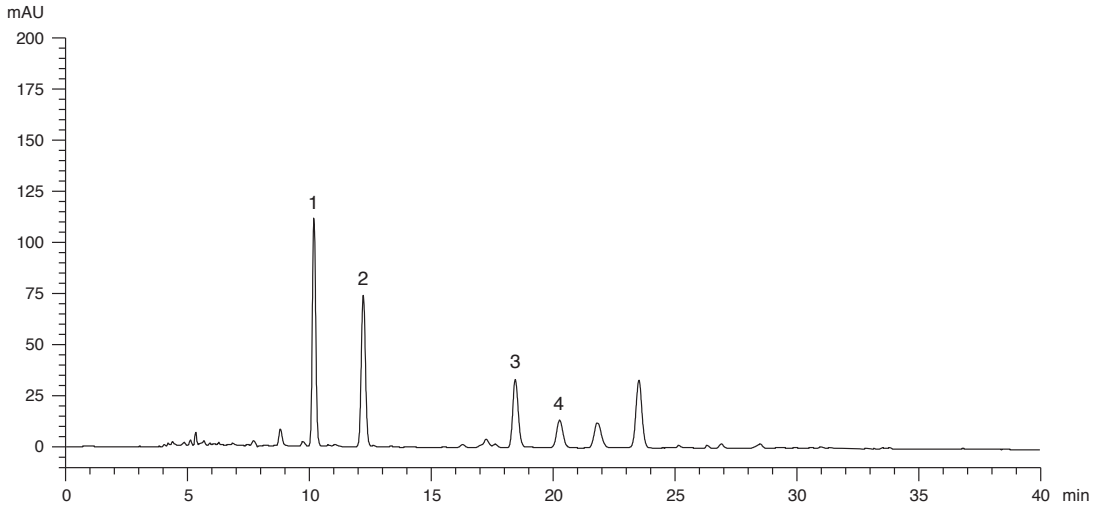

Figure 6 (i) A reference fingerprint chromatogram of young unossified hairy antler of male *Cervus nippon* Temminck extract

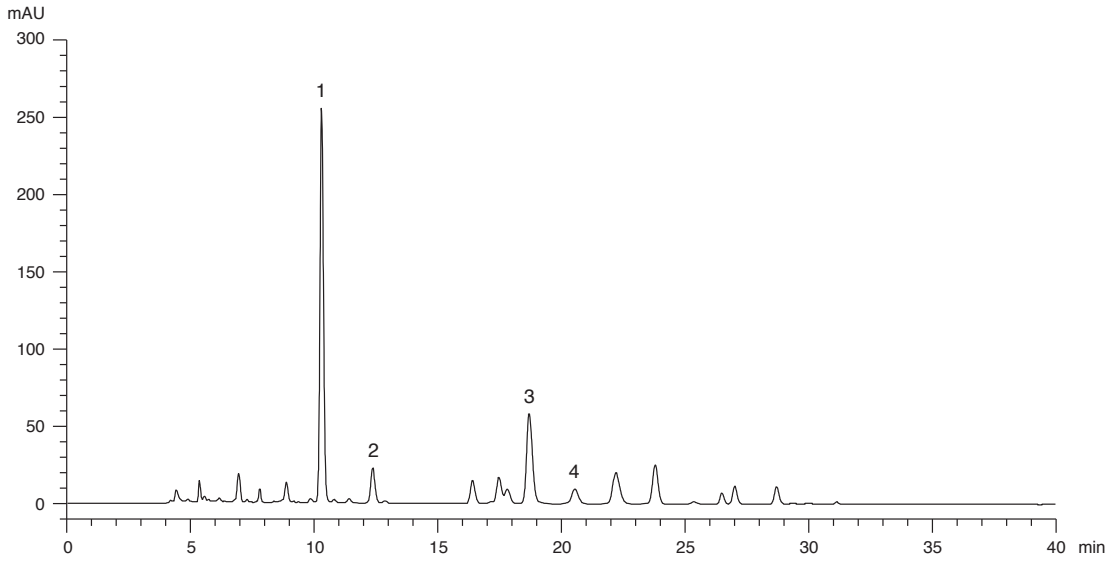

Figure 6 (ii) A reference fingerprint chromatogram of young unossified hairy antler of male *Cervus elaphus* Linnaeus extract

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 respective reference fingerprint chromatogram [Fig. 6 (i) or (ii)].

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 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 37.0%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 15.0%.
Ethanol-soluble extractives (hot extraction method): not less than 5.0%.

7. ASSAY

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

A. Assay of Cholest-4-en-3-one

Standard solution

Cholest-4-en-3-one standard stock solution, Std-Stock (50 mg/L)

Weigh accurately 5.0 mg of cholest-4-en-3-one CRS (Fig. 4) and dissolve in 100 mL of methanol.

Cholest-4-en-3-one standard solution for assay, Std-AS

Measure accurately the volume of the cholest-4-en-3-one Std-Stock, dilute with methanol to produce a series of solutions of 1, 5, 10, 30, 50 mg/L for cholest-4-en-3-one.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 x *g* for 5 min. Transfer the supernatant to a 50-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (243 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of methanol and water (98:2, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of cholest-4-en-3-one Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of cholest-4-en-3-one should not be more than 5.0%; the RSD of the retention time of cholest-4-en-3-one peak should not be more than 2.0%; the column efficiency determined from cholest-4-en-3-one peak should not be less than 7500 theoretical plates.

The *R* value between cholest-4-en-3-one 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 cholest-4-en-3-one Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of cholest-4-en-3-one against the corresponding concentrations of cholest-4-en-3-one 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 cholest-4-en-3-one peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of cholest-4-en-3-one Std-AS. The retention times of cholest-4-en-3-one 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 cholest-4-en-3-one in the test solution, and calculate the percentage content of cholest-4-en-3-one in the sample by using the equations as indicated in Appendix IV (B).

B. Assay of Cholesterol

Standard solution

Cholesterol standard stock solution, Std-Stock (1000 mg/L)

Weigh accurately 10.0 mg of cholesterol CRS and dissolve in 10 mL of methanol.

Cholesterol standard solution for assay, Std-AS

Measure accurately the volume of the cholesterol Std-Stock, dilute with methanol to produce a series of solutions of 50, 100, 250, 500, 750 mg/L for cholesterol.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3000 x *g* for 5 min. Transfer the supernatant to a 50-mL round-bottomed flask. Repeat the extraction for one more time. Combine the supernatants. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 35°C; nebulizer gas (N_2) pressure: 3.5 bar] and a column (4.6 × 250 mm) packed with ODS bonded silica gel

(5 µm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of methanol and water (98:2, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 10 µL of cholesterol Std-AS (250 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of cholesterol should not be more than 5.0%; the RSD of the retention time of cholesterol peak should not be more than 2.0%; the column efficiency determined from cholesterol peak should not be less than 10000 theoretical plates.

The *R* value between cholesterol 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 cholesterol Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of cholesterol against the natural logarithm of the corresponding concentrations of cholesterol 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 cholesterol peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of cholesterol Std-AS. The retention times of cholesterol 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 cholesterol in the test solution by using the following equation –

$$\text{Concentration of cholesterol in the test solution} = e^{[\text{Ln (A)}-I]/m}$$

Where A = the peak area of cholesterol in the test solution,

I = the y-intercept of the 5-point calibration curve of cholesterol,

m = the slope of the 5-point calibration curve of cholesterol.

Calculate the percentage content of cholesterol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.25% of the total content of cholest-4-en-3-one ($C_{27}H_{44}O$) and cholesterol ($C_{27}H_{46}O$), calculated with reference to the dried substance.