Corydalis Rhizoma

Figure 1 A photograph of Corydalis Rhizoma

1. NAMES

Official Name: Corydalis Rhizoma

Chinese Name: 延胡索

Chinese Phonetic Name: Yanhusuo

2. SOURCE

Corydalis Rhizoma is the dried tuber of *Corydalis yanhusuo* W. T. Wang (Papaveraceae). The tuber is collected in early summer when the plant withered, removed the fibrous root, washed clean, boiled in water until no dry core visible, then dried under the sun to obtain Corydalis Rhizoma.

3. DESCRIPTION

Irregularly flattened spherical, 5-27 mm in diameter. Externally yellow to yellowish-brown, with irregular reticulate wrinkles. Top end with slightly sunken stem scars, base usually with warty protuberances, or a slight dent like a navel. Texture hard and fragile, fractured surface yellow to yellowish-brown, cuticular, with waxy lustre. Odour slight; taste bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Epidermis occasionally remains as remnants. Cortex broad, consisting 2-3 layers of sclerenchymatous cells at outer side, and with several layers of flattened cells. Laticifer intermittently arranged in several ring. Phloem narrow. Cambium indistinct. Vessels elements small, 2-7 in a bundle, and arranged in a interrupted ring. Pith visible in the centre. Parenchymatous cells filled with starch granules and starch gelatinous masses (Fig. 2).

Powder

Colour greenish-yellow. Starch gelatinous masses nearly colourless to pale yellow. Simple starch granules subglobular, hilum distinct; compound granules composed of 2-5 units; black and cruciate in shape under the polarized microscope. Sclerenchymatous cells at outer side of cortex greenish-yellow, polygonal, subsquare or subrounded, 7-129 μm wide, or elongated, 42-283 μm

long; cell walls slightly curved, lignified, some beaded, finely pitted, 2-17 µm thick. Stone cells yellowish-green, subsquare, subrounded to subpolygonal, 17-87 µm wide, 28-157 µm long; wall 2-18 µm thick. Vessels spiral, 5-52 µm in diameter (Fig. 3).

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

Standard solutions

Corydaline standard solution

Weigh 1.0 mg of corydaline CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Tetrahydropalmatine standard solution

Weigh 1.0 mg of tetrahydropalmatine CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of petroleum ether (60-80°C), ethyl acetate and isopropanol (8:2:1, v/v).

Staining reagent

Iodine

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 (70%). Sonicate (90 W) the mixture for 10 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately corydaline standard solution (0.5 µL), tetrahydropalmatine standard solution (0.5 µL) and the test solution (1 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 3 min until the spots or bands become visible. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

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 corydaline and tetrahydropalmatine.

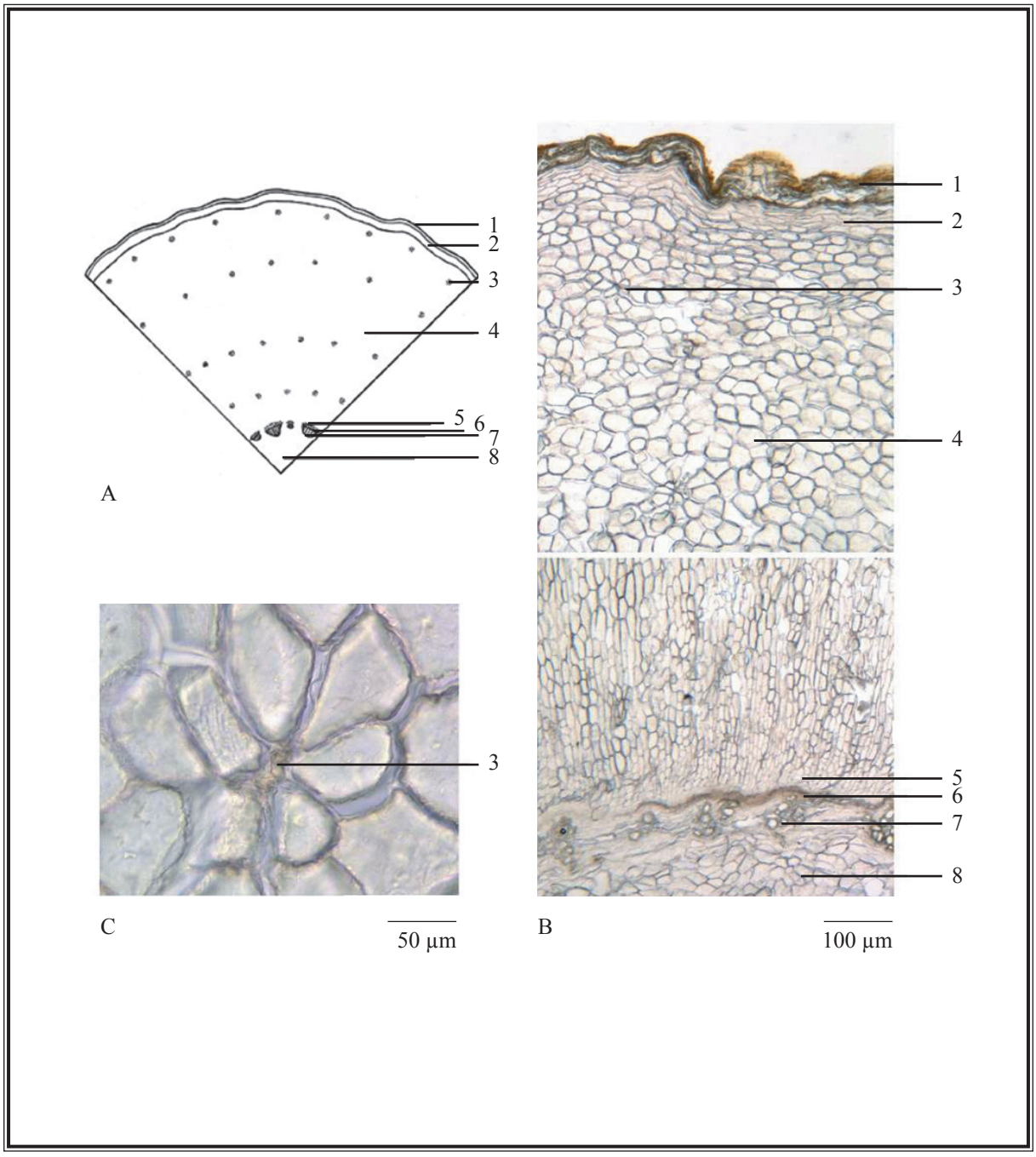


Figure 2 Microscopic features of transverse section of Corydalis Rhizoma

A. Sketch B. Section illustration C. Laticifer

1. Epidermis 2. Flattened cells 3. Laticifer 4. Cortex 5. Phloem 6. Cambium 7. Xylem 8. Pith

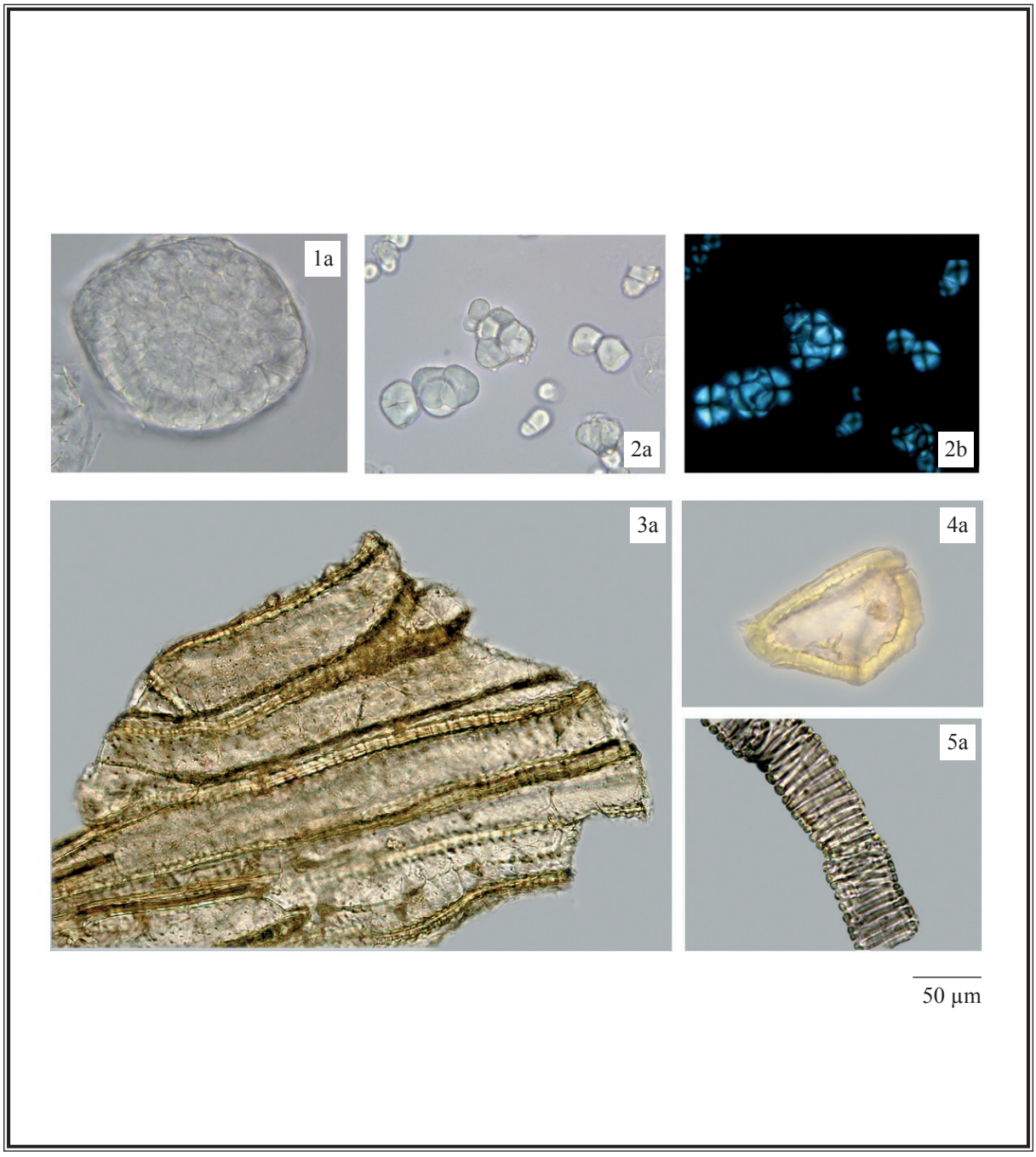
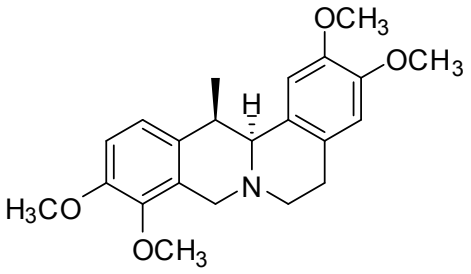


Figure 3 Microscopic features of powder of Corydalis Rhizoma

1. Starch gelatinous mass 2. Starch granules 3. Sclerenchymatous cells 4. Stone cell 5. Spiral vessel

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

(i)



(ii)

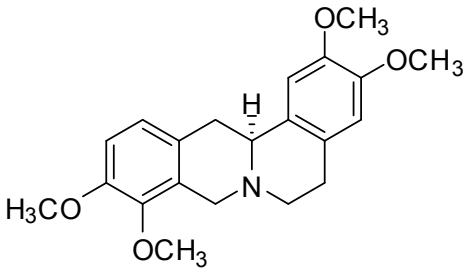


Figure 4 Chemical structures of (i) corydaline and (ii) tetrahydropalmatine

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

Standard solutions

Corydaline standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 2.0 mg of corydaline CRS and dissolve in 50 mL of ethanol (70%).

Tetrahydropalmatine standard solution for fingerprinting, Std-FP (30 mg/L)

Weigh 1.5 mg of tetrahydropalmatine CRS and dissolve in 50 mL of ethanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (70%). Sonicate (150 W) the mixture for 1 h. Centrifuge at about 3000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 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. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Acetonitrile (%, v/v)	0.1% Phosphoric acid and 0.22% Triethylamine (%, v/v)	Elution
0 – 27	20 → 32	80 → 68	linear gradient
27 – 29	32 → 42	68 → 58	linear gradient
29 – 36	42 → 47	58 → 53	linear gradient
36 – 40	47 → 65	53 → 35	linear gradient
40 – 49	65 → 75	35 → 25	linear gradient
49 – 60	75 → 100	25 → 0	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of corydaline Std-FP and tetrahydropalmatine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of corydaline and tetrahydropalmatine should not be more than 5.0%; the RSD of the retention times of corydaline and tetrahydropalmatine peaks should not be more than 2.0%; the column efficiencies determined from corydaline and tetrahydropalmatine peaks should not be less than 200000 theoretical plates.

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. 5).

Procedure

Separately inject corydaline Std-FP, tetrahydropalmatine Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of corydaline and tetrahydropalmatine peaks in the chromatograms of corydaline Std-FP, tetrahydropalmatine Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify corydaline and tetrahydropalmatine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of corydaline Std-FP and tetrahydropalmatine Std-FP. The retention times of corydaline and tetrahydropalmatine 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 five characteristic peaks of Corydalis Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Corydalis Rhizoma extract

Peak No.	RRT	Acceptable Range
1	0.63	± 0.03
2 (dehydrocorydaline)	0.70	± 0.03
3 (marker, tetrahydropalmatine)	1.00	-
4 (corydaline)	1.14	± 0.03
5	1.18	± 0.04

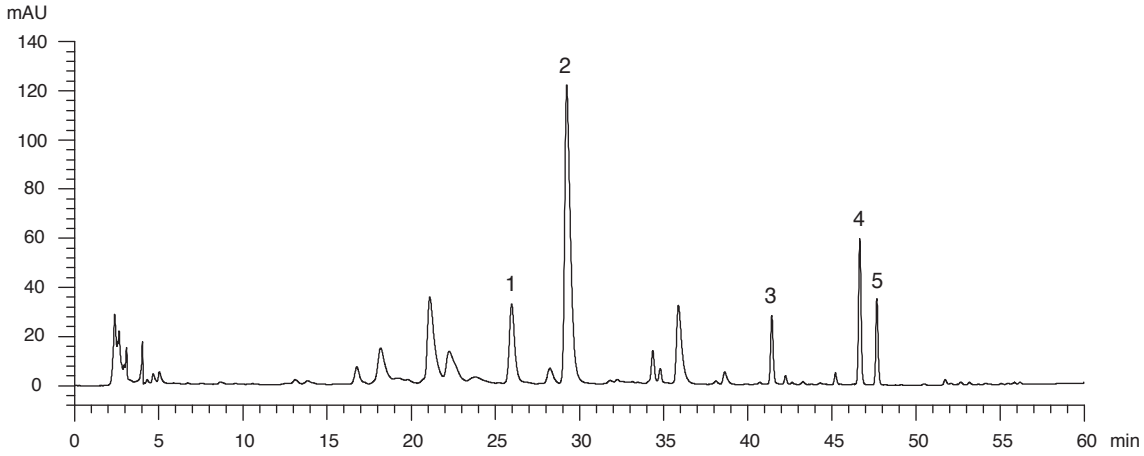


Figure 5 A reference fingerprint chromatogram of Corydalis Rhizoma extract

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. 5).

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 XVIII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 3.5%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 15.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 24.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

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

Standard solution

Mixed corydaline and tetrahydropalmatine standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 2.0 mg of corydaline CRS and 2.0 mg of tetrahydropalmatine CRS, and dissolve in 10 mL of ethanol (50%).

Mixed corydaline and tetrahydropalmatine standard solution for assay, Std-AS

Measure accurately the volume of the mixed corydaline and tetrahydropalmatine Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 8, 20, 30, 60, 80 mg/L for both corydaline and tetrahydropalmatine. `

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 40 mL of ethanol (50%). Reflux the mixture for 1 h. Cool down to room temperature. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Wash the residue with ethanol (50%). Centrifuge at about 3000 × *g* for 5 min. Combine the supernatants and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 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. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Methanol (%, v/v)	0.1% Phosphoric acid* (%, v/v)	Elution
0 – 20	60 → 65	40 → 35	linear gradient
20 – 30	65 → 80	35 → 20	linear gradient
30 – 35	80	20	isocratic

*Adjust the pH to 6 with triethylamine

System suitability requirements

Perform at least five replicate injections, each using 5 µL of the mixed corydaline and tetrahydropalmatine Std-AS (30 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of corydaline and tetrahydropalmatine should not be more than 5.0%; the RSD of the retention times of corydaline and tetrahydropalmatine peaks should not be more than 2.0%; the column efficiencies determined from corydaline and tetrahydropalmatine peaks should not be less than 30000 and 9000 theoretical plates respectively.

The R value between corydaline peak and the closest peak; and the R value between tetrahydropalmatine 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 corydaline and tetrahydropalmatine Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of corydaline and tetrahydropalmatine against the corresponding concentrations of the mixed corydaline and tetrahydropalmatine Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify corydaline and tetrahydropalmatine peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed corydaline and tetrahydropalmatine Std-AS. The retention times of corydaline and tetrahydropalmatine 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 corydaline and tetrahydropalmatine in the test solution, and calculate the percentage contents of corydaline and tetrahydropalmatine in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.10% of the total content of corydaline ($C_{22}H_{27}NO_4$) and tetrahydropalmatine ($C_{21}H_{25}NO_4$), calculated with reference to the dried substance.