Bistortae Rhizoma

Figure 1 A photograph of Bistortae Rhizoma

A. Bistortae Rhizoma B. Transverse section of rhizome

1. NAMES

Official Name: Bistortae Rhizoma

Chinese Name: 拳參

Chinese Phonetic Name: Quanshen

2. SOURCE

Bistortae Rhizoma is the dried rhizome of *Polygonum bistorta* L. (Polygonaceae). The rhizome is collected in early spring when bud or in autumn just before withering, soil removed, dried under the sun, then rootlets removed to obtain Bistortae Rhizoma.

3. DESCRIPTION

Compressed-cylindrical, usually curved into a shrimp-like shape, both ends obtuse or slightly narrowed, 2-11 cm long, 8-25 mm in diameter. Externally purple brown to dark brown, rough, one side protuberant and the other side flat or slightly furrowed, with thick annulated striations and remnants of rootlets or root scars. Texture hard. Fracture roundish or nearly reniform, pale brown to brown, dotted vascular bundles yellowish-white, arranged interruptedly in a ring. Odour slightly aromatic; taste bitter and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of several layers of brown cells, containing brown masses. Cortex occupies about 1/4 of rhizome. Vascular bundles collateral, arranged in an interrupted ring. Phloem narrow. Xylem consists of vessels and xylem fibres. Pith broad, parenchymatous cells contain clusters of calcium oxalate (Fig. 2).

Powder

Colour pale brownish-red. Starch granules fairly abundant, broadly ovoid, long ellipsoid or subspheroid, 2-13 μm in diameter, hilum pointed, V-shaped or slit-shaped, striations distinct; black and cruciate-shaped under the polarized microscope; compound starch granules rare, composed of 2-3 units. Clusters of calcium oxalate numerous, scattered singly, 19-65 μm in diameter; polychromatic under the polarized microscope. Vessels mainly bordered-pitted, spiral vessels occasionally visible, 10-55 μm in diameter. Fibres scattered or in bundles, 6-30 μm in diameter, walls relatively thick, pit canals distinct. Cork cells subsquare, containing brownish-red masses (Fig. 3).

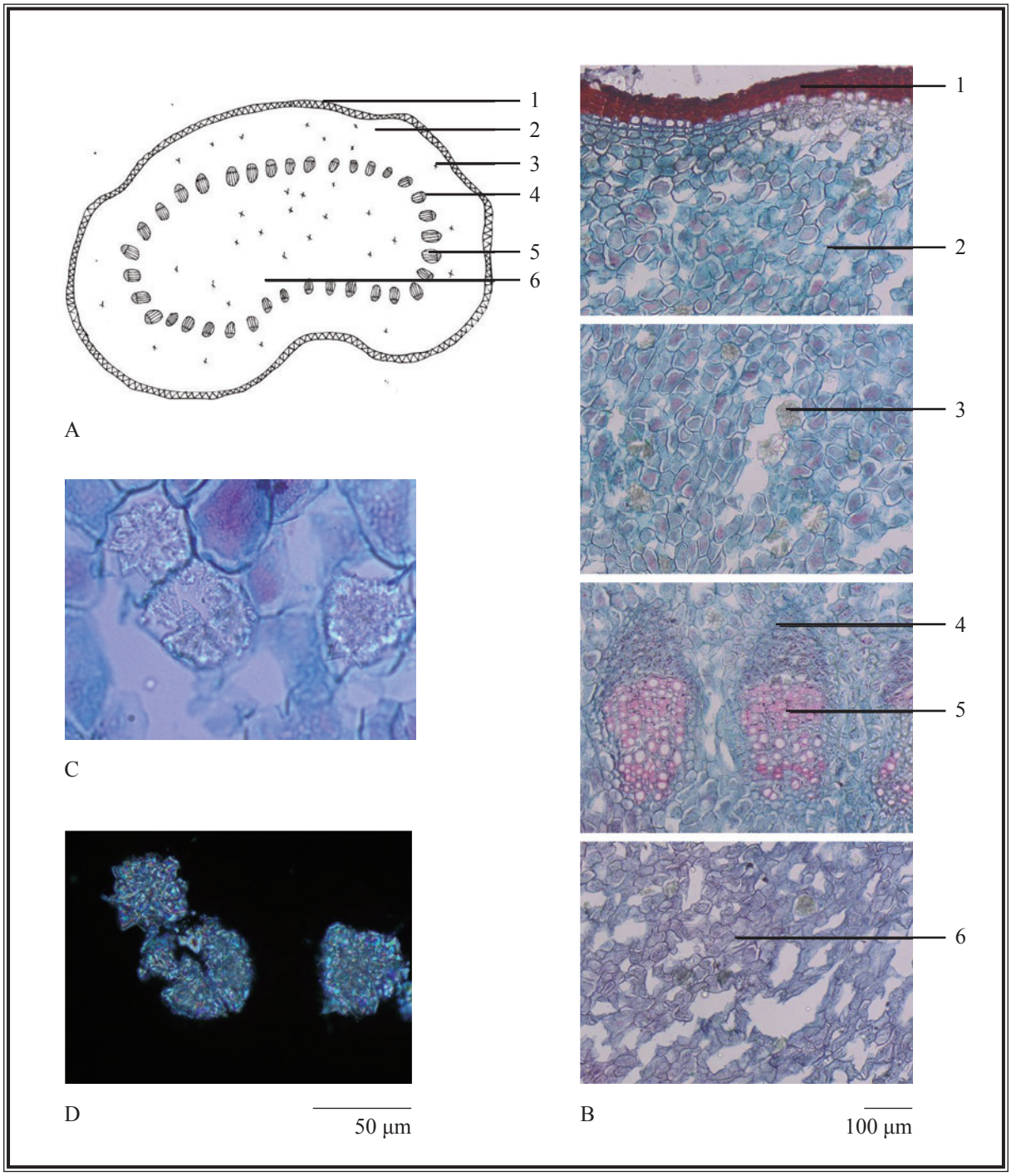

Figure 2 Microscopic features of transverse section of Bistortae Rhizoma

A. Sketch B. Section illustration C. Clusters of calcium oxalate
D. Clusters of calcium oxalate (under the polarized microscope)

1. Cork 2. Cortex 3. Clusters of calcium oxalate 4. Phloem 5. Xylem 6. Pith

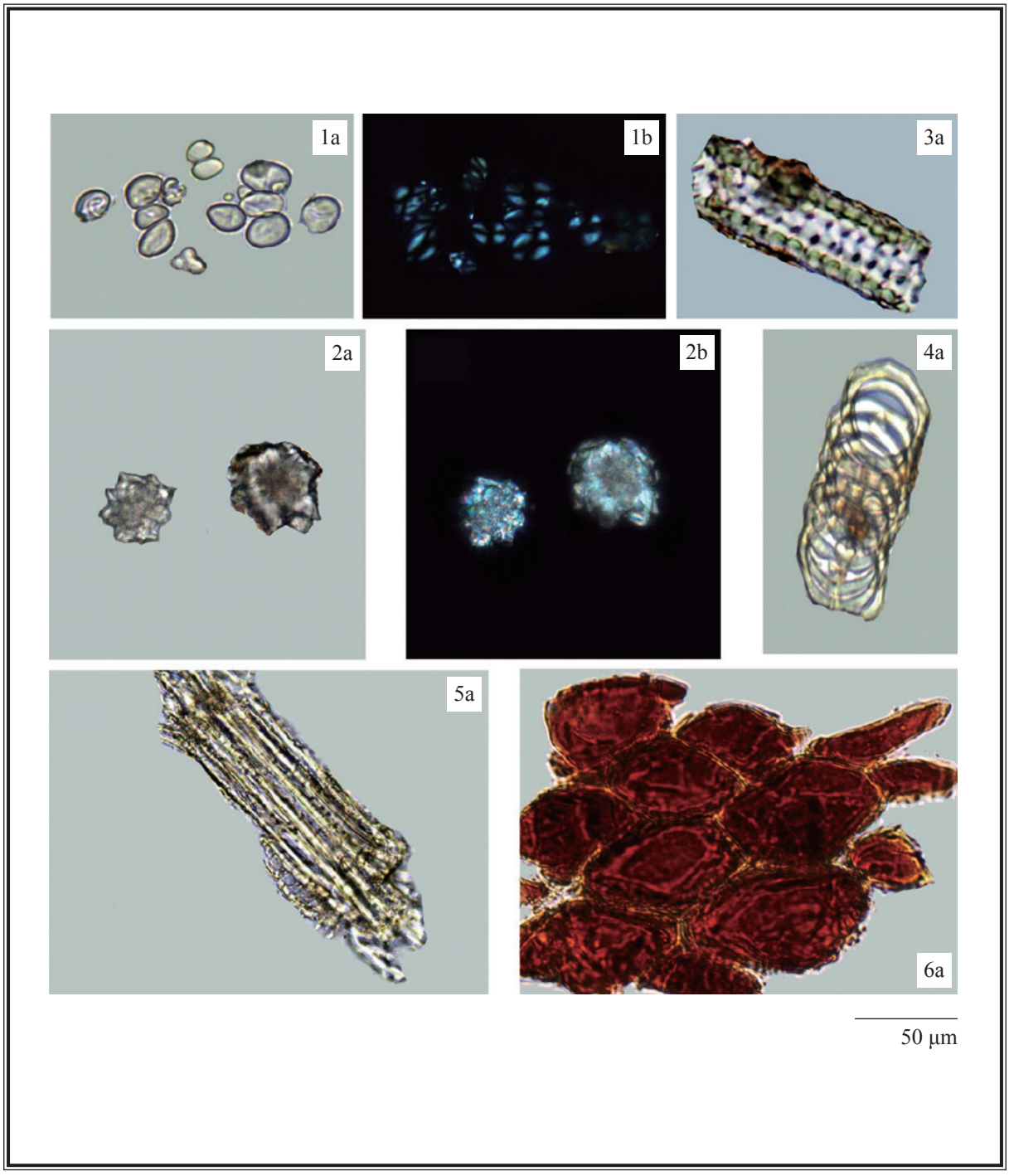

Figure 3 Microscopic features of powder of Bistortae Rhizoma

1. Starch granules 2. Clusters of calcium oxalate 3. Bordered-pitted vessel
4. Spiral vessel 5. Fibres 6. Cork cells

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

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

Standard solution

Chlorogenic acid standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, acetone, water and formic acid (20:3:1.5:1.5, v/v).

Staining reagent

Ammonia.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol (30%). Sonicate (350 W) the mixture for 30 min. 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 chlorogenic acid standard solution (4 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 5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Fumigate the plate with ammonia vapor chamber for about 1 min until the spots or bands become visible. Examine the plate under UV light (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

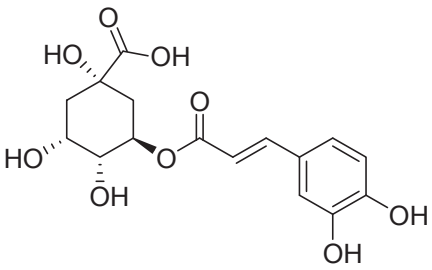

Figure 4 Chemical structure of chlorogenic acid

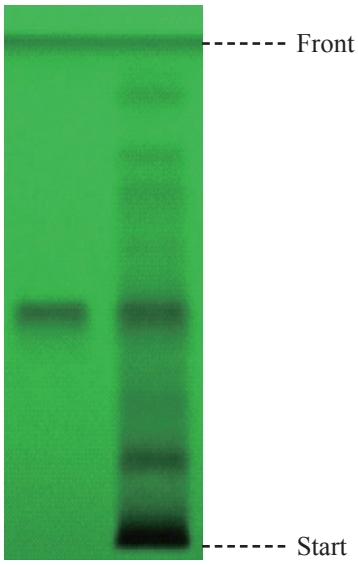

Figure 5 A reference HPTLC chromatogram of Bistortae Rhizoma extract observed under UV light (254 nm) after staining

1. Chlorogenic 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 chlorogenic acid (Fig. 5).

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

Standard solution

Chlorogenic acid standard solution for fingerprinting, Std-FP (70 mg/L)

Weigh 0.7 mg of chlorogenic acid CRS and dissolve in 10 mL of methanol (30%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (30%). Sonicate (350 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (270 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)	0.2% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	96.5	3.5	isocratic
10 – 20	96.5 → 93	3.5 → 7	linear gradient
20 – 30	93 → 92	7 → 8	linear gradient
30 – 60	92	8	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of chlorogenic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of chlorogenic acid should not be more than 5.0%; the RSD of the retention time of chlorogenic acid peak should not be more than 2.0%; the column efficiency determined from chlorogenic acid peak should not be less than 30000 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 chlorogenic acid Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of chlorogenic acid peak in the chromatogram of chlorogenic acid Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify chlorogenic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of chlorogenic acid Std-FP. The retention times of chlorogenic 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 Bistortae Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Bistortae Rhizoma extract

Peak No.	RRT	Acceptable Range
1 (gallic acid)	0.23	± 0.03
2	0.28	± 0.03
3 (3-*O*-methylgallic acid)	0.72	± 0.05
4 (marker, chlorogenic acid)	1.00	-

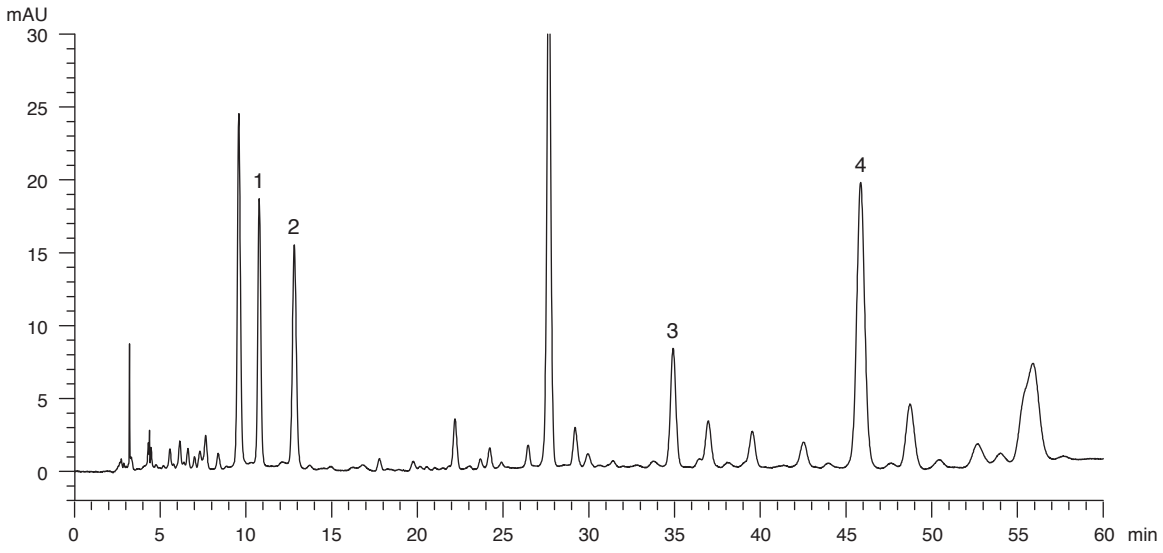

Figure 6 A reference fingerprint chromatogram of Bistortae Rhizoma extract

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 – 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 9.0%.
Acid-insoluble ash: not more than 1.0%.

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 29.0%.
Ethanol-soluble extractives (cold extraction method): not less than 21.0%.

7. ASSAY

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

Standard solution

Chlorogenic acid standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 1.0 mg of chlorogenic acid CRS and dissolve in 10 mL of ethanol (30%).

Chlorogenic acid standard solution for assay, Std-AS

Measure accurately the volume of the chlorogenic acid Std-Stock, dilute with ethanol (30%) to produce a series of solutions of 1, 5, 10, 50, 100 mg/L for chlorogenic acid.

Test solution

Weigh accurately 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (30%). Sonicate (350 W) the mixture for 15 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with ethanol (30%). Combine the solutions and make up to the mark with ethanol (30%). Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (326 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)	0.2% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	93	7	isocratic
10 – 20	93 → 91	7 → 9	linear gradient
20 – 40	91	9	isocratic

System suitability requirements

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

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

Limits

The sample contains not less than 0.10% of chlorogenic acid ($C_{16}H_{18}O_9$), calculated with reference to the dried substance.