Herba Ephedrae

Figure 1 A photograph of Herba Ephedrae

1. NAMES

Official Name: Herba Ephedrae

Chinese Name: 麻黃

Chinese Phonetic Name: Mahuang

2. SOURCE

Herba Ephedrae is the dried herbaceous stem of *Ephedra sinica* Stapf (Ephedraceae). The green herbaceous stem is collected in early autumn dried in shade, or dried to a half-dry condition in shade and then fully dried under the sun to obtain Herba Ephedrae.

3. DESCRIPTION

Stem very slender, cylindrical, slightly flattened, with few branches, 1-2 mm in diameter. Some plants have brown, woody stems. Externally pale green to yellowish-green, with fine longitudinal ridges, slightly rough. Nodes distinct, internodes 2-6 cm long. Scaly leaves on the nodes membranous, 3-4 mm long, lower part 1/4-1/2 connate, tubular, reddish-brown in colour, the upper part with 2 lobes (rarely 3), lobes acutely triangular, apex greyish-white, reflexed. Texture of stem light, fragile, easily broken, fracture slightly fibrous with yellowish-green edge and dark reddish-brown suborbicular pith. Odour slightly fragrant; taste slightly bitter and astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Subround and slightly oblate. Epidermal cells covered with thick cuticle. Ridges 20-32, in an undulating formation, with sunken stomata located between two ridges. Hypodermal fibre bundles are located just below the epidermis of the ridges, with thickened and unlignified wall. Cortex relatively broad, with few fibre bundles. Pericycle fibre bundles crescent-shaped. Collateral vascular bundles about 10, interruptedly arranged in a ring, phloem narrow, cambium ring subround, the

xylem triangular. The xylem of young stems consists of several separate triangular bundles, while that of old ones forming a ring. Pith broad, parenchyma cells containing brown masses, occasionally showing perimedullary fibres. The outer wall of epidermal cells, cortex fibres and cortex parenchyma cells are covered by numerous fine sandy crystals or prisms of calcium oxalate (Fig. 2).

Powder

Pale brown in colour. Epidermis fragments commonly observed, transverse section view of cells subrectangular, covered with cuticle layer which up to 31 μm; outer wall covered with numerous sandy crystals of calcium oxalate. Stomata peculiar, sunken, long-rounded, guard cells phone-shaped and with two rather thick ends in lateral view. Fibres slender, 3-26 μm in diameter, with thickened wall; sometimes the wall covered with sandy crystals of calcium oxalate to form crystal-inlaid fibres. Polychrome when observed under the polarized microscope. Sandy crystals of calcium oxalate numerous, occurring on the outer wall of epidermal cells, cortex fibres and cortex parenchyma cells. Intensely white to polychrome under the polarized microscope. Spiral and bordered pitted vessels, fine, 3-42 μm in diameter, perforation plates with numerous round perforations (Fig. 3).

4.2 Physicochemical Identification

Reagent

Ninhydrin solution

Weigh 0.2 g of ninhydrin and dissolve in 100 mL of methanol.

Procedure

Weigh 0.2 g of the powdered sample and put into a test tube, then add 4 mL of hydrochloric acid (6.2%, v/v). Sonicate (490 W) the mixture for 15 min. Allow the solid residue to settle. Transfer the supernatant to another test tube, adjust the pH to about 12 with sodium hydroxide solution (20%, w/v), then add 4 mL of dichloromethane, vortex. Collect the lower layer and evaporate to dryness on a warm water bath (50-60˚C). Dissolve the residue in 2 mL of methanol. Spot the solution onto a filter paper with a capillary. Spray the filter paper evenly with ninhydrin solution and heat at about 110˚C (about 5 min). A purplish-blue spot is observed.

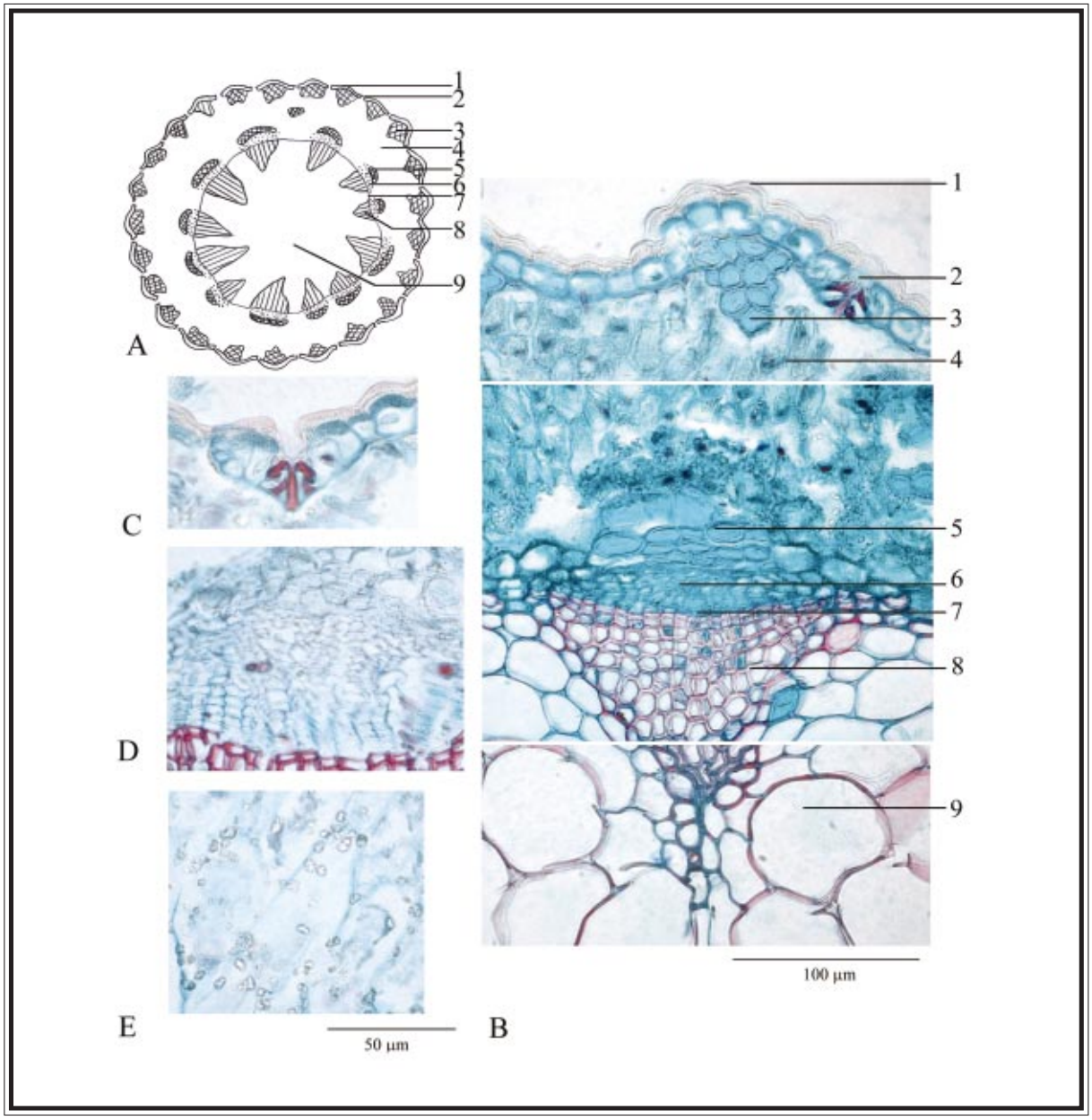


Figure 2 Microscopic features of transverse section of Herba Ephedrae

A. Sketch (of the young stem) B. Section illustration C. Epidermis and sunken stoma
D. Pericycle fibre bundles E. Sandy crystals of calcium oxalate

1. Cuticle 2. Stomata 3. Hypodermal fibre bundles 4. Cortex 5. Pericycle fibre bundles 6. Phloem
7. Cambium 8. Xylem 9. Pith

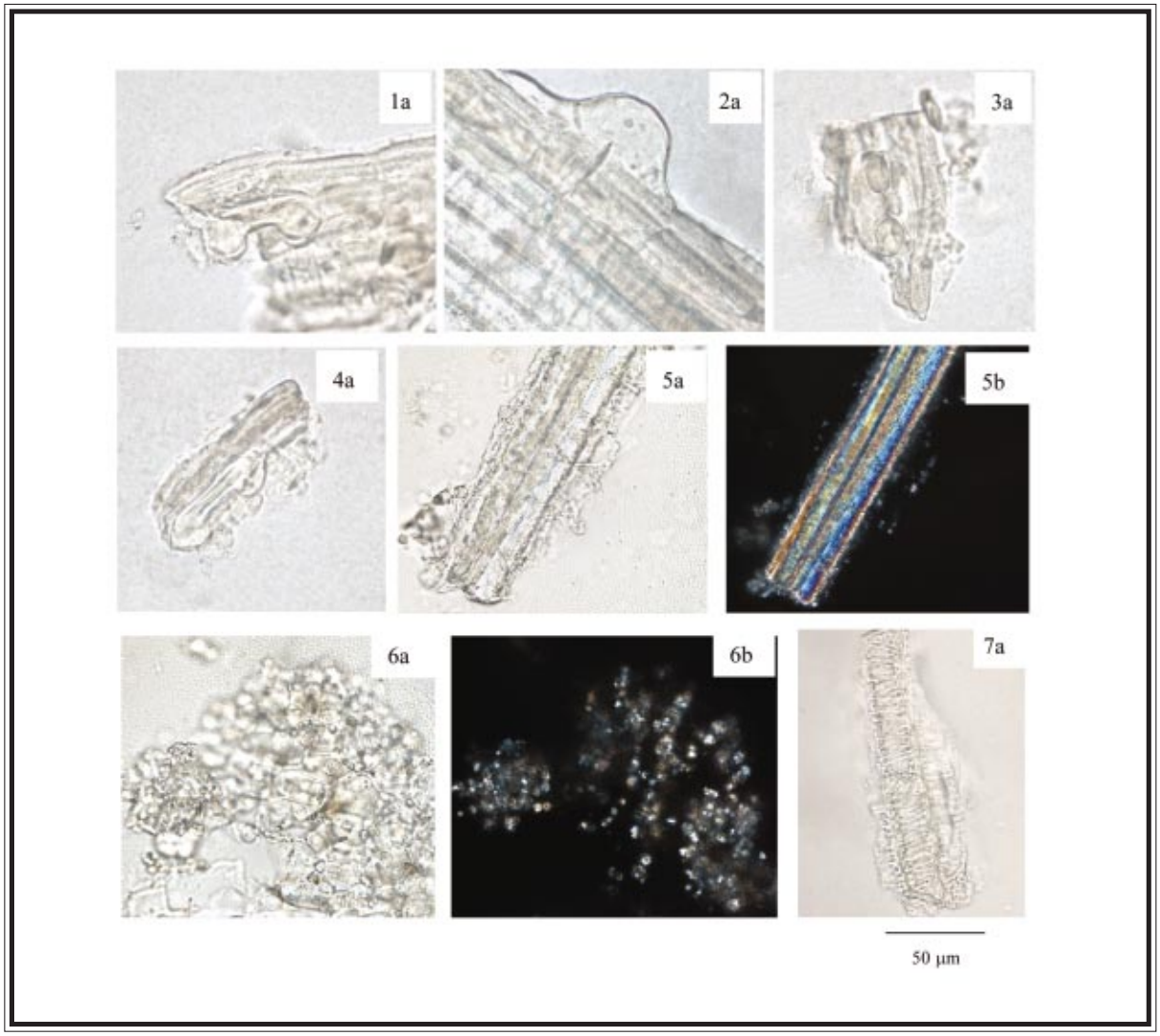


Figure 3 Microscopic features of powder of Herba Ephedrae

1. Epidermis fragments with stoma 2. Epidermis fragments with cuticle 3. Surface view of stomata
4. Lateral view of stomata 5. Phloem fibres 6. Sandy crystals of calcium oxalate 7. Vessels

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

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

Standard solution

Ephedrine hydrochloride standard solution

Weigh 2.0 mg of ephedrine hydrochloride CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane, methanol and ammonium solution (20:5:0.5, v/v).

Spray reagent

Weigh 0.2 g of ninhydrin and dissolve in 100 mL of ethanol.

Test solution

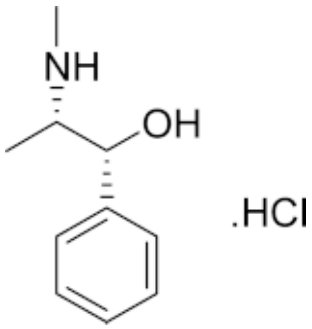
Weigh 1.0 g of the powdered sample and put into a 50-mL round-bottomed flask, then add 0.5 mL of ammonium solution and 10 mL of dichloromethane. Reflux the mixture for 60 min. Cool down to room temperature. Transfer the solution to a 50-mL centrifugal tube. Centrifuge at about 1800 × *g* for 10 min. Filter and evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol and filter.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately ephedrine hydrochloride standard solution and the test solution (1.5 µL each) to the plate. 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. Spray the plate evenly with the spray reagent and heat at about 110°C until the spots or bands become visible (about 5 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV(A).

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 ephedrine hydrochloride.

(i)



(ii)

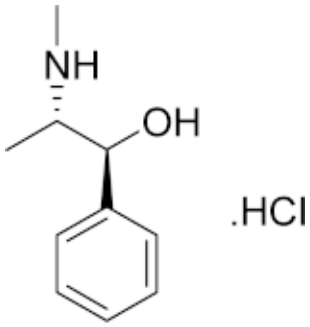


Figure 4 Chemical structures of (i) ephedrine hydrochloride and (ii) pseudoephedrine hydrochloride

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

Standard solution

Ephedrine hydrochloride standard solution for fingerprinting, Std-FP (24 mg/L)

Weigh 2.4 mg of ephedrine hydrochloride CRS and dissolve in 100 mL of methanol.

Test solution

Weigh 0.1 g of the powdered sample and put into a 50-mL centrifugal tube, then add 50 mL of a mixture of methanol and 0.01 M potassium phosphate, monobasic solution (3:97, v/v). Sonicate (490 W) the mixture for 60 min. Centrifuge at about 1800 × *g* for 5 min. Filter the supernatant through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (210 nm) and a Ether-linked Phenyl bonded silica gel with Polar Endcapping column (4.6 × 150 mm) (4 μm particle size, pH:1.5-7.0). The flow rate is about 1.5 mL/min. The mobile phase is a mixture of methanol and 0.1 M potassium phosphate, monobasic solution (3:97, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections each with 20 μL of ephedrine hydrochloride Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of ephedrine hydrochloride should not be more than 3.0%; the RSD of the retention time of ephedrine hydrochloride peak should not be more than 2.0%; the column efficiency determined from ephedrine hydrochloride peak should not be less than 3000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject ephedrine hydrochloride Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of ephedrine hydrochloride peak in the chromatogram of the ephedrine hydrochloride Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify ephedrine hydrochloride peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of ephedrine hydrochloride Std-FP. The retention times of ephedrine hydrochloride 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 Herba Ephedrae extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the four characteristic peaks of Herba Ephedrae extract

Peak No.	RRT	Acceptable Range
1	0.58	±0.03
2	0.68	±0.03
3 (marker, ephedrine hydrochloride)	1.00	-
4 (pseudoephedrine hydrochloride)	1.18	±0.03

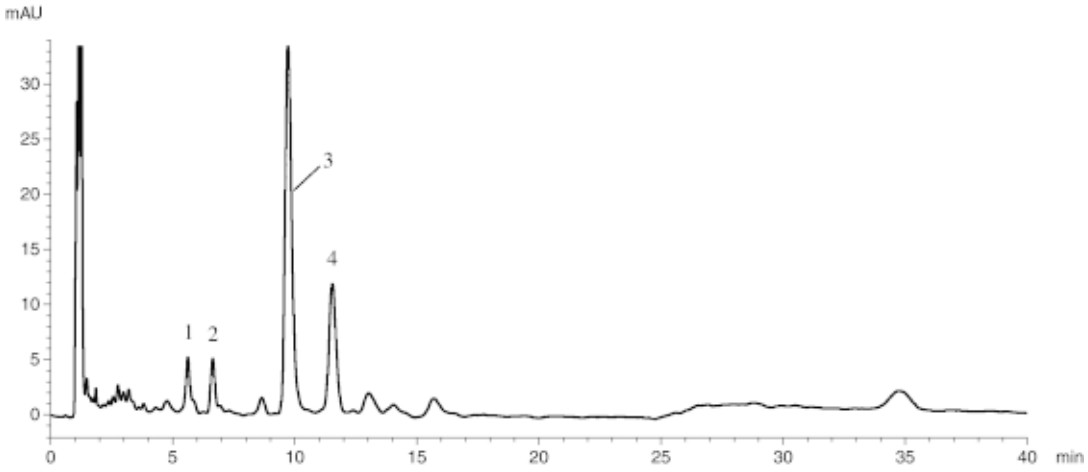


Figure 5 A reference fingerprint chromatogram of Herba Ephedrae extract

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. 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 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 10.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 **Water Content** *(Appendix X)*: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 22.0%.

7. ASSAY

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

Standard solution

Mixed ephedrine hydrochloride and pseudoephedrine hydrochloride standard stock solution, Std-Stock (1220 mg/L each)

Weigh accurately 6.1 mg of ephedrine hydrochloride CRS and 6.1 mg of pseudoephedrine hydrochloride CRS (Fig. 4) and dissolve in 5 mL of a mixture of methanol and 0.01 M potassium phosphate, monobasic solution (3:97, v/v).

Mixed ephedrine hydrochloride and pseudoephedrine hydrochloride standard solution for assay, Std-AS

Measure accurately the volume of the mixed ephedrine hydrochloride and pseudoephedrine hydrochloride Std-Stock, dilute with the above mixture to produce a series of solutions of 1.2, 12.2, 24.4, 48.8, 73.2 mg/L for both ephedrine hydrochloride and pseudoephedrine hydrochloride.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of a mixture of methanol and 0.01 M potassium phosphate, monobasic solution (3:97, v/v). Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 5 min. Transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction for thrice. Combine the extracts and make up to the mark with the above mixture. Mix and filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (210 nm) and a Ether-linked Phenyl bonded silica gel with Polar Endcapping column (4.6 × 150 mm) (4 μm particle size, pH:1.5-7.0). The flow rate is about 1.5 mL/min. The mobile phase is a mixture of methanol and 0.1 M potassium phosphate, monobasic solution (3:97, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections each with 20 μL of the mixed ephedrine hydrochloride and pseudoephedrine hydrochloride Std-AS (24.4 mg/L each). The requirements of the system suitability

parameters are as follows: the RSD of the peak area of ephedrine hydrochloride should not be more than 3.0%; the RSD of the retention time of ephedrine hydrochloride peak should not be more than 2.0%; the column efficiency determined from ephedrine hydrochloride peak should not be less than 3000 theoretical plates.

The *R* value between ephedrine hydrochloride 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 ephedrine hydrochloride and pseudoephedrine hydrochloride Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of ephedrine hydrochloride and pseudoephedrine hydrochloride against the corresponding concentrations of the mixed ephedrine hydrochloride and pseudoephedrine hydrochloride Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 μL of the test solution into the HPLC system and record the chromatogram. Identify ephedrine hydrochloride peak and pseudoephedrine hydrochloride peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed ephedrine hydrochloride and pseudoephedrine hydrochloride Std-AS. The retention times of ephedrine hydrochloride peaks and pseudoephedrine hydrochloride peaks from the two chromatograms should not differ from their counterparts by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of ephedrine hydrochloride and pseudoephedrine hydrochloride in the test solution, and calculate the percentage contents of ephedrine and pseudoephedrine (the percentage contents of ephedrine hydrochloride and pseudoephedrine hydrochloride × 0.819) in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.78% of the total content of ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$), calculated with reference to the dried substance.