Calculate the percentage content of the sum of costunolide and dehydrocostus lactone using the following expression:

$$\frac{(A_1 + (1.52 \times A_3)) \times m_2 \times p}{A_2 \times m_1}$$

A_1 = area of the peak due to costunolide in the chromatogram obtained with the test solution;

A_2 = area of the peak due to costunolide in the chromatogram obtained with reference solution (a);

A_3 = area of the peak due to dehydrocostus lactone in the chromatogram obtained with the test solution;

m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *costunolide CRS* used to prepare reference solution (a), in grams;

p = percentage content of costunolide in *costunolide CRS*;

1.52 = correlation factor between dehydrocostus lactone and costunolide.

04/2011:2438

BAICAL SKULLCAP ROOT

Scutellariae baicalensis radix

DEFINITION

Dried, peeled, usually fragmented root of *Scutellaria baicalensis* Georgi without rootlets. It is collected in spring or autumn.

Content: not less than 9.0 per cent of baicalin ($C_{21}H_{18}O_{11}$; M_r 446.4) (dried drug).

IDENTIFICATION

A. The root is conical, twisted and, if not reduced in size, 8-25 cm long and 1-3 cm in diameter. The outer surface is brownish-yellow or dark yellow, bearing sparse, warty traces of rootlets, the upper part rough, with twisted longitudinal wrinkles or irregular reticula, the lower part with longitudinal striations and fine wrinkles. Texture hard and fragile, easily broken, fracture yellow, reddish-brown in the centre; the central part of an old root dark brown or brownish-black, withered or hollowed.

B. Microscopic examination (*2.8.23*). The powder is yellow or light brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: phloem fibres, single or in bundles, fusiform, 60-250 µm long, 9-33 µm in diameter, with thick, channelled walls; stone cells sub-spherical, square or rectangular with rounded edges, with thickened walls, sometimes heavily; cork cells polygonal and brownish-yellow; numerous reticulated vessels, 24-72 µm in diameter; lignified fibres frequently broken, about 12 µm in diameter, with sparse, oblique pits. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows abundant starch granules, simple, spheroidal, 2-10 µm in diameter, with a distinct hilum, or compound with 2-3 components.

C. Thin-layer chromatography (*2.2.27*).

Test solution. To 1 g of the powdered herbal drug (355) (*2.9.12*) add 10 mL of *methanol R* and sonicate for 10 min. Centrifuge and use the supernatant.

Reference solution. Dissolve 1 mg of *baicalin R* and 1 mg of *acteoside R* in 10 mL of *methanol R*.

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *acetic acid R*, *anhydrous formic acid R*, *water R*, *ethyl acetate R* (1:1:2:15 *V/V/V/V*).

Application: 10 µL as bands.

Development: over a path of 6 cm.

Drying: in air.

Detection: heat at 100-105 °C for 3 min, treat with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, then treat with a 50 g/L solution of *macrogol 400 R* in *methanol R*, allow to dry in air for 30 min and examine in ultraviolet light at 365 nm.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint blue fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	3-4 fluorescent zones
_____	_____
	2 fluorescent zones
_____	_____
Verbascoside: a blue fluorescent zone	A strong blue fluorescent zone
	A blue fluorescent zone
Baicalin: a black zone	A black zone
	A weak yellow fluorescent zone
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 6.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 2.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. To 0.300 g of the powdered herbal drug (355) (*2.9.12*) add 40 mL of *ethanol (70 per cent V/V) R*, heat under a reflux condenser on a water bath for 3 h, cool and filter. Transfer the filtrate to a 100 mL volumetric flask. Wash both the container and the residue several times with a small volume of *ethanol (70 per cent V/V) R* and filter the washings into the same flask. Dilute to 100.0 mL with *ethanol (70 per cent V/V) R*. Mix well. Dilute 1.0 mL of the solution to 10.0 mL with *methanol R*. Mix well.

Reference solution (a). Dissolve 5.0 mg of *baicalin CRS* in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (b). Dissolve 2 mg of *methyl parahydroxybenzoate R* in *methanol R*, add 20 mL of reference solution (a) and dilute to 100 mL with *methanol R*.

Column:

- *size*: *l* = 0.125 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: 0.1 per cent *V/V* solution of *phosphoric acid R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	90 → 60	10 → 40

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 280 nm.

Injection: 10 µL.

Retention time: methyl parahydroxybenzoate = about 15 min; baicalin = about 16 min.

System suitability: reference solution (b):

– *resolution*: minimum 3 between the peaks due to methyl parahydroxybenzoate and baicalin.

Calculate the percentage content of baicalin using the following expression:

$$\frac{m_2 \times S_1 \times 10 \times p}{S_2 \times m_1}$$

m_1 = mass of the herbal drug, in grams;

m_2 = mass of baicalin used to prepare reference solution (a), in grams;

S_1 = area of the peak due to baicalin in the chromatogram obtained with the test solution;

S_2 = area of the peak due to baicalin in the chromatogram obtained with reference solution (a);

p = percentage content of baicalin in *baicalin CRS*.

STORAGE

Protected from moisture.

01/2018:2612

BARBARY WOLFBERRY FRUIT

Lycii fructus

DEFINITION

Dried, whole, ripe fruit of *Lycium barbarum* L.

IDENTIFICATION

A. The berry is elliptical, fusiform or ovoid and frequently flattened, about 6-20 mm long and 3-10 mm in diameter. The apex of the fruit shows a ring-shaped scar of the nectar-bearing base of the style and the base of the fruit bears the whitish to light brown remnants of the cut stalk. The external surface is orange-red or dark red. The pericarp is fleshy, wrinkled, soft and viscous. It contains 20-50 hard, flat, subreniform seeds, bent upwards. Each pale yellow or yellowish-brown seed is about 1.7 mm long and 1.5 mm wide.

B. Microscopic examination (*2.8.23*). The powder is orange-red or reddish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 2612.-1): fragments of the epicarp (surface view [A]) with polygonal or elongated cells, about 60 µm in diameter, with straight or slightly wavy walls, covered with a thick cuticle, with distinct, more or less parallel striations; fragments of the epicarp (transverse section [F]) covered by a crenate cuticle [Fa], consisting of 1 or 2 layers of cells [Fb] associated with the mesocarp [Fc]; fragments of the mesocarp [C] with thin-walled subpolygonal cells containing reddish-orange or brownish-red spherical granules [Ca, Fd] or microsphenoidal crystals of calcium oxalate [Cb]; fragments of the seeds (surface view [B]) with a testa consisting of greenish-yellow sclereids with heavily thickened, striated and lobed walls and (transverse section [E]) with a testa having thin external walls [Ea] and deeply lobed, irregularly thickened, striated, radial internal walls [Eb]; fragments of endosperm containing oil droplets [D]; fragments of vascular tissue with narrow, spiral or annular vessels [G].

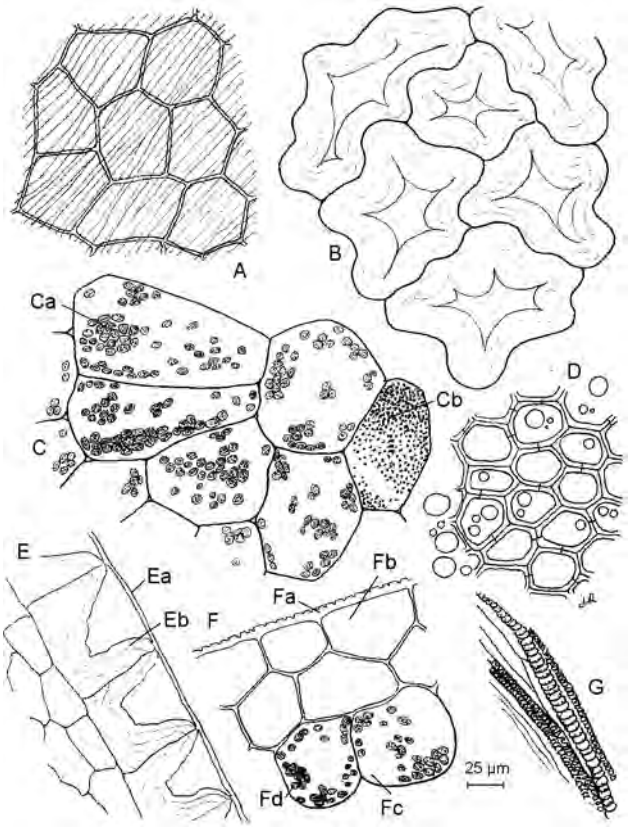

Figure 2612.-1. – *Illustration for identification test B of powdered herbal drug of barbary wolfberry fruit*

C. Thin-layer chromatography (*2.2.27*).

Test solution. To 0.1 g of the powdered herbal drug (355) (*2.9.12*) add 7 mL of *water R*. Sonicate for 10 min and centrifuge. Prepare a ready-to-use sample preparation cartridge containing 0.50 g of octadecylsilyl silica gel (50 µm) using 3 mL of *methanol R*, drying with a stream of air, then using 3 mL of *water R*. The flow rate does not exceed 6 mL/min. Apply 4 mL of the supernatant to the top of the cartridge. Wash the cartridge twice with 1 mL of a mixture of 1 volume of *methanol R* and 9 volumes of *water R*. Elute the cartridge with 1 mL of *methanol R*; collect the eluate and use it as the test solution.

Reference solution. Dissolve 1 mg of *scopoletin R* in 10 mL of *methanol R*. Dilute 1 mL of the solution to 10 mL with *methanol R* to obtain solution (a). Dissolve 1 mg of *rutoside trihydrate R* in 5 mL of solution (a).

Plate: *TLC silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *anhydrous formic acid R*, *glacial acetic acid R*, *water R*, *ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application: 2 µL as bands of 8 mm.

Development: over a path of 6 cm.

Drying: in air.

Detection: heat at 100 °C for 3 min; treat the still-warm plate with a 5 g/L solution of *diphenylboric acid aminoethyl ester R* in *ethyl acetate R*, then treat with a 50 g/L solution of *macrogol 400 R* in *methylene chloride R*; examine in ultraviolet light at 365 nm after 5 min.