Calculate the percentage of lactone sesquiterpenes, expressed as dihydrohelenalin tiglate, using the following expression:

$$\frac{F_1 \times C \times V \times 1.187}{F_2 \times m \times 10}$$

F_1 = area of all peaks appearing between the peaks due to santonin and butyl 4-hydroxybenzoate in the chromatogram obtained with the test solution;

F_2 = area of the peak due to santonin in the chromatogram obtained with the test solution;

m = mass of the tincture to be examined, in grams;

C = concentration of santonin in the internal standard solution used to prepare the test solution, in milligrams per millilitre;

V = volume of the internal standard solution used to prepare the test solution, in millilitres;

1.187 = peak correlation factor between dihydrohelenalin tiglate and santonin.

01/2013:1866

ARTICHOKE LEAF

Cynarae folium

DEFINITION

Whole or cut, dried leaf of *Cynara cardunculus* L. (syn. *C. scolymus* L.).

Content: minimum 0.8 per cent of chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3) (dried drug).

IDENTIFICATION

A. The entire leaf may be up to 70 cm long and 30 cm wide. The lamina is deeply lobed in the upper part to within 1-2 cm of the petiole on either side, in the lower part the leaf becomes pinnate; all the segments have markedly dentate margins and taper at the apex. Spines are absent. The upper surface of the lamina is green with a fine covering of whitish hairs, the lower surface is pale green or white and densely tomentose with long, tangled hairs. The petiole and main veins are flat on the upper surface, prominently raised and longitudinally ridged on the lower surface, with conspicuous hairs on both surfaces.

B. Reduce to a powder (1000) (*2.9.12*). The powder is greenish-grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 1866.-1): fragments of the epidermises of the lamina, in surface view; the upper epidermis [F] is composed of cells with straight or slightly sinuous walls [Fa], accompanied by palisade parenchyma [Fb]; the lower epidermis [C] is composed of more sinuous-walled cells; abundant anomocytic stomata (*2.8.3*) on both surfaces [D] and multicellular, uniseriate covering trichomes in felted masses, the majority fragmented [Ca] with a short stalk composed of several cells and a very long, narrow and frequently curled terminal cell, others consisting of 4-6 cylindrical cells; very occasional glandular trichomes with a short stalk and a uniseriate or biseriate head, in surface view [E] or in transverse section [Ba]; abundant fragments of covering trichomes [G]; fragments of the lamina, in transverse section [B]; abundant fragments of vascular tissue from the petiole and veins [A].

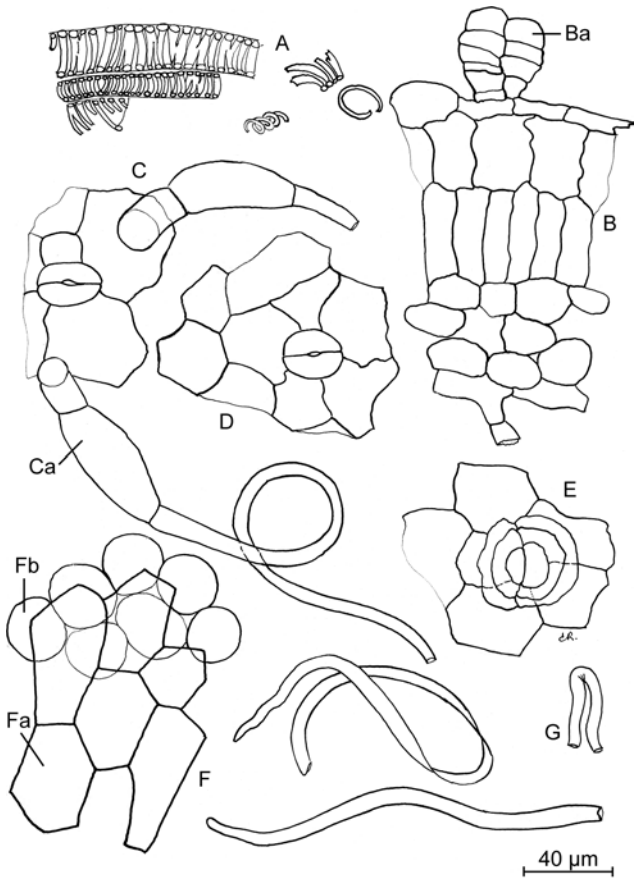


Figure 1866.-1. – *Illustration for identification test B of powdered herbal drug of artichoke leaf*

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

Test solution. To 2.0 g of the powdered herbal drug (1000) (*2.9.12*) add 20 mL of *ethanol (60 per cent V/V) R*. Allow to stand for 2 h with occasional stirring. Filter.

Reference solution. Dissolve 5 mg of *luteolin-7-glucoside R* and 5 mg of *chlorogenic acid CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel 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: 10 µL [or 2 µL] as bands of 10 mm [or 8 mm].

Development: over a path of 13 cm [or 6 cm].

Drying: in air.

Detection: heat at 100 °C for 5 min; spray the warm plate with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R* followed by a 50 g/L solution of *macrogol 400 R* in *methanol R*; examine in ultraviolet light at 365 nm.

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

Top of the plate	
	A light blue fluorescent zone
———	———
Luteolin-7-glucoside: a yellow or orange fluorescent zone Chlorogenic acid: a light blue fluorescent zone	A yellow or orange fluorescent zone (luteolin-7-glucoside) A light blue fluorescent zone (chlorogenic acid)
———	———
Reference solution	**Test solution**

TESTS

Total ash (*2.4.16*): maximum 20.0 per cent.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the powdered herbal drug (710) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. To 0.500 g of the powdered herbal drug (1000) (*2.9.12*) add 50.0 mL of *methanol R* and heat under a reflux condenser on a water-bath at 70 °C for 1 h. Centrifuge and transfer the supernatant to a 200 mL volumetric flask. Repeat the procedure and dilute to 200.0 mL with *water R*.

Reference solution. Dissolve 5.0 mg of *chlorogenic acid CRS* in 50.0 mL of *methanol R*. Transfer 5.0 mL of this solution to a volumetric flask, add 5 mL of *methanol R* and dilute to 20.0 mL with *water R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: *phosphoric acid R*, *water R* (0.5:99.5 *V/V*);
- *mobile phase B*: *phosphoric acid R*, *acetonitrile R* (0.5:99.5 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	92	8
1 - 20	92 → 75	8 → 25
20 - 33	75	25
33 - 35	75 → 0	25 → 100

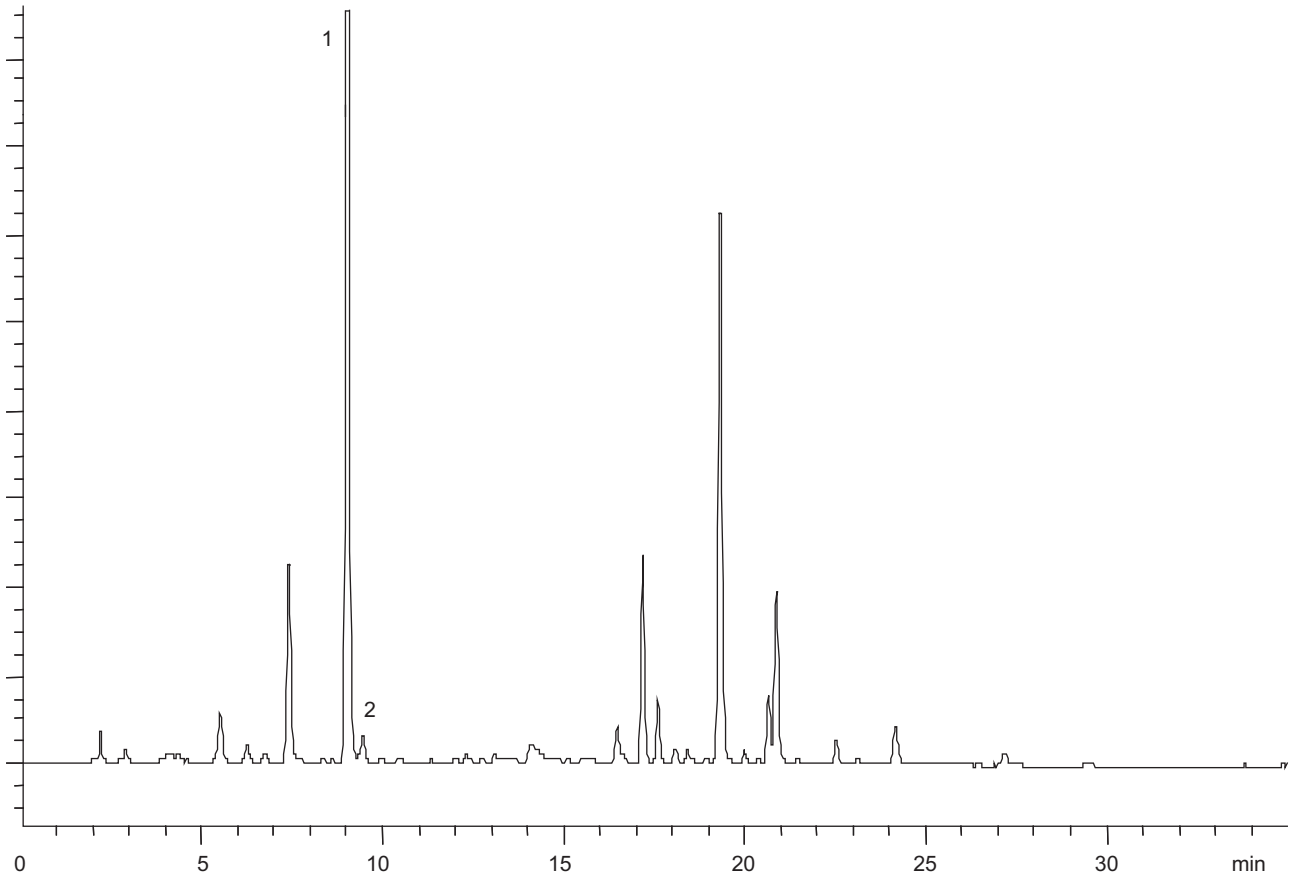
Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 330 nm.

Injection: 25 µL.

System suitability: test solution:

- the chromatogram obtained is similar to the chromatogram shown in Figure 1866.-2;
- *resolution*: minimum 2.0 between the peak due to chlorogenic acid and the subsequent peak (peak 2).



1. chlorogenic acid 2. unknown substance

Figure 1866.-2. – *Chromatogram for the assay of artichoke leaf: test solution*

Calculate the percentage content of chlorogenic acid using the following expression:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1}$$

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

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;

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

m_2 = mass of *chlorogenic acid CRS* in the reference solution, in grams;

p = percentage content of chlorogenic acid in *chlorogenic acid CRS*.

01/2010:2389

ARTICHOKE LEAF DRY EXTRACT

Cynarae folii extractum siccum

DEFINITION

Dry extract produced from *Artichoke leaf (1866)*.

Content: minimum 0.6 per cent of chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3) (dried extract).

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using water of minimum 80 °C.

CHARACTERS

Appearance: light brown or brown, amorphous powder.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 1.0 g of the extract to be examined in 10 mL of *ethanol (60 per cent V/V) R*. Sonicate for 5 min and filter.

Reference solution. Dissolve 5 mg of *luteolin-7-glucoside R* and 5 mg of *chlorogenic acid R* in 10 mL of *methanol R*.

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel 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: 10 µL [or 2 µL] as bands of 10 mm [or 8 mm].

Development: over a path of 13 cm [or 6 cm].

Drying: in air.

Detection: heat at 100 °C for 5 min; spray the warm plate with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R* followed by a 50 g/L solution of *macrogol 400 R* in *methanol R*; examine in ultraviolet light at 365 nm.

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

Top of the plate	
	A light blue fluorescent zone
Luteolin-7-glucoside: a yellow or orange fluorescent zone	A yellow or orange fluorescent zone (luteolin-7-glucoside)
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
Reference solution	**Test solution**

TESTS

Loss on drying (*2.8.17*): maximum 6.0 per cent.

Total ash (*2.4.16*): maximum 30.0 per cent.

ASSAY

Liquid chromatography (*2.2.29*).

Solvent mixture: *methanol R*, *water R* (30:70 *V/V*).

Test solution. Dissolve 30.0 mg of the extract to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a). Dissolve 5.0 mg of *chlorogenic acid CRS* in 50.0 mL of *methanol R*. Transfer 5.0 mL of this solution to a volumetric flask, add 5 mL of *methanol R* and dilute to 20.0 mL with *water R*.

Reference solution (b). Dissolve 30 mg of the *artichoke leaf dry extract HRS* in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: *phosphoric acid R*, *water R* (0.5:99.5 *V/V*);
- *mobile phase B*: *phosphoric acid R*, *acetonitrile R* (0.5:99.5 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	92	8
1 - 20	92 → 75	8 → 25
20 - 33	75	25
33 - 35	75 → 0	25 → 100

Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 330 nm.

Injection: 25 µL.

System suitability: reference solution (b):

- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak immediately after the peak due to chlorogenic acid and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to chlorogenic acid;
- the chromatogram obtained is similar to the chromatogram supplied with the *artichoke leaf dry extract HRS*.

Calculate the percentage content of chlorogenic acid using the following expression:

$$\frac{A_1 \times m_2 \times p \times 0.125}{A_2 \times m_1}$$

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

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

m_1 = mass of the extract to be examined used to prepare the test solution, in milligrams;

m_2 = mass of *chlorogenic acid CRS* used to prepare reference solution (a), in milligrams;

p = percentage content of chlorogenic acid in *chlorogenic acid CRS*.

07/2012:1600

ASH LEAF

Fraxini folium

DEFINITION

Dried leaf of *Fraxinus excelsior* L. or *Fraxinus angustifolia* Vahl (syn. *Fraxinus oxyphylla* M. Bieb) or of hybrids of these 2 species or of a mixture.

Content: minimum 2.5 per cent of total hydroxycinnamic acid derivatives, expressed as chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3) (dried drug).

IDENTIFICATION

A. The leaf consists of leaflets that are sometimes detached and separated from the rachis. The leaflet is about 6 cm long and 3 cm wide. Each leaflet is subsessile or shortly petiolate, oblong, lanceolate, somewhat unequal at the base, acuminate at the apex, with fine, acute teeth on the margins; the upper surface is dark green and the lower surface is greyish-green. The midrib and secondary veins are whitish and prominent on the lower surface.

B. Microscopic examination (*2.8.23*). The powder is greyish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 1600.-1): fragments of the upper epidermis of the lamina in surface view [B], with some of the cells showing cuticular striations, accompanied by underlying palisade parenchyma [Ba]; fragments of the lower epidermis in surface view [A] consisting of cells covered by fine cuticular striations [Aa], numerous anomocytic stomata (*2.8.3*) [Ab] and rare peltate glandular trichomes with a unicellular stalk and a glandular head composed of radiating cells [Ac]; fragments of lamina in transverse section [F] with 2 layers of palisade parenchyma [Fa], spongy parenchyma [Fb] and, occasionally, glandular trichomes embedded in the epidermis [Fc]; occasional multicellular, uniseriate, conical covering trichomes composed of cells with thick striated walls, either on an epidermis [C] or fragmented [D]; fragments of vascular tissue from the leaflets [E] composed of spiral vessels [Ea], short fibres [Eb] and sometimes palisade parenchyma [Ec]; fragments of vascular tissue from the veins [G] composed of fibres [Ga], sometimes accompanied by cells with thick, pitted walls from the medullary rays [Gb].

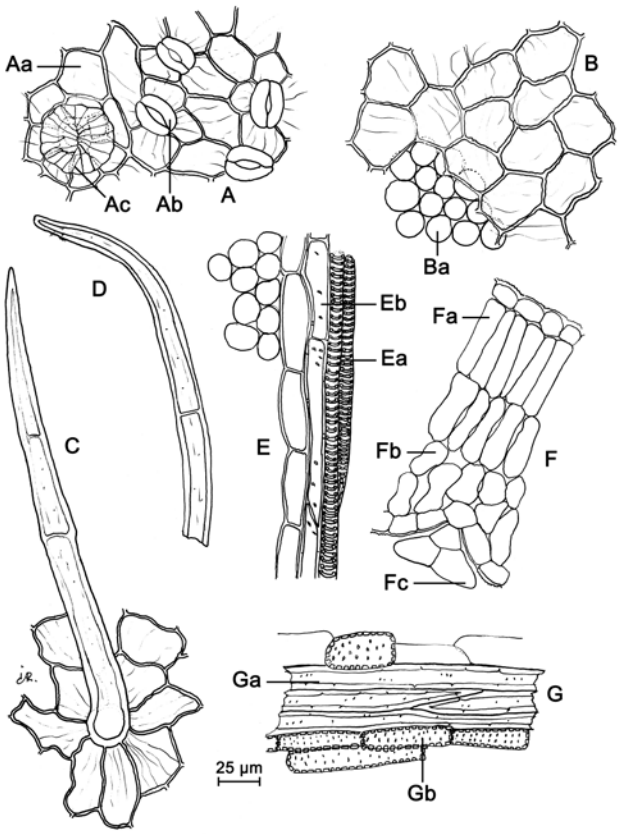


Figure 1600.-1. – *Illustration for identification test B of powdered herbal drug of ash leaf*

C. Examine the chromatograms obtained in the test for *Fraxinus ornus*.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. The intensity of the zones present in the chromatogram obtained with the test solution may vary depending on the presence of *F. excelsior*, *F. angustifolia*, their hybrids or their concentration in a mixture. Furthermore, other fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
———	———
	A light blue fluorescent zone (acteoside)
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone may be present (chlorogenic acid)
———	———
	A light blue fluorescent zone
Rutin: an orange fluorescent zone	An orange fluorescent zone (rutin)
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 3.0 per cent of stems and maximum 2.0 per cent of other foreign matter.

Fraxinus ornus. Thin-layer chromatography (*2.2.27*).

Test solution. To 1 g of the powdered herbal drug (355) (*2.9.12*) add 20 mL of *methanol R*. Stir with a magnetic stirrer for 10 min. Filter.

Reference solution. Dissolve 5 mg of *rutin R* and 5 mg of *chlorogenic acid R* in 10 mL of *methanol R*.

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].