ASSAY

Carry out the determination of essential oils in herbal drugs (*2.8.12*). Use 20.0 g of the freshly, coarsely powdered drug, a 1000 mL round-bottomed flask, 10 drops of *liquid paraffin R* or other antifoam, 500 mL of *water R* as distillation liquid and 0.5 mL of *xylene R* in the graduated tube. Distil at a rate of 2-3 mL/min for 4 h.

04/2008:1827

GINKGO DRY EXTRACT, REFINED AND QUANTIFIED

Ginkgonis extractum siccum raffinatum et quantificatum

DEFINITION

Refined and quantified dry extract produced from *Ginkgo leaf (1828)*.

Content:

– *flavonoids, expressed as flavone glycosides* (M_r 756.7): 22.0 per cent to 27.0 per cent (dried extract);

– *bilobalide*: 2.6 per cent to 3.2 per cent (dried extract);

– *ginkgolides A, B and C*: 2.8 per cent to 3.4 per cent (dried extract);

– *ginkgolic acids*: maximum 5 ppm (dried extract).

PRODUCTION

The extract is produced from the herbal drug by an appropriate procedure using organic solvents and their mixtures with water, physical separation steps as well as other suitable processes.

CHARACTERS

Appearance: bright yellow-brown, powder or friable mass.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 20.0 mg of the extract to be examined in 10 mL of a mixture of 2 volumes of *water R* and 8 volumes of *methanol R*.

Reference solution. Dissolve 1.0 mg of *chlorogenic acid R* and 3.0 mg of *rutin R* in 20 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* (7.5:7.5:17.5:67.5 *V/V/V/V*).

Application: 20 µL [or 5 µL], as bands.

Development: over a path of 17 cm [or 6 cm].

Drying: at 100-105 °C.

Detection: spray the plate whilst still hot with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, then spray with a 50 g/L solution of *macrogol 400 R* in *methanol R*; allow to dry in air for about 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, weaker fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	A blue fluorescent zone
	Several faint coloured zones
———	———
	A brown fluorescent zone
	A green fluorescent zone
	An intense light blue fluorescent zone sometimes overlapped by a greenish-brown fluorescent zone
Chlorogenic acid: a light blue fluorescent zone	
	One or two green fluorescent zones
Rutin: a yellowish-brown fluorescent zone	One or two yellowish-brown fluorescent zones
———	———
	Several green and yellowish-brown fluorescent zones
Reference solution	**Test solution**

ASSAY

Flavonoids. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.200 g of the extract to be examined in 20 mL of *methanol R*. Add 15.0 mL of *dilute hydrochloric acid R* and 5 mL of *water R* and dilute to 50.0 mL with *methanol R*. Transfer 10.0 mL of this solution into a 10 mL brown-glass vial. Close the vial with a tight rubber membrane stopper and secure with an aluminium crimped cap. Heat on a water-bath for 25 min. Allow to cool to 20 °C.

Reference solution. Dissolve 10.0 mg of *quercetin dihydrate CRS* in 20 mL of *methanol R*. Add 15.0 mL of *dilute hydrochloric acid R* and 5 mL of *water R* and dilute to 50.0 mL with *methanol R*.

Column:

– *size*: *l* = 0.125 m, Ø = 4 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 25 °C.

Mobile phase:

– *mobile phase A*: 0.3 g/L solution of *phosphoric acid R* adjusted to pH 2.0;

– *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	60	40
1 - 20	60 → 45	40 → 55
20 - 21	45 → 0	55 → 100
21 - 25	0	100

Flow rate: 1.0 mL/min.

Detector: spectrophotometer at 370 nm.

Injection: 10 µL.

Relative retention with reference to quercetin (retention time = about 12.5 min): kaempferol = about 1.4; isorhamnetin = about 1.5.

System suitability: test solution:

– *resolution*: minimum 1.5 between the peaks due to kaempferol and isorhamnetin.

Determine the sum of the areas including all the peaks from the peak due to quercetin to the peak due to isorhamnetin in the chromatogram obtained with the test solution (see Figure 1827.-1).

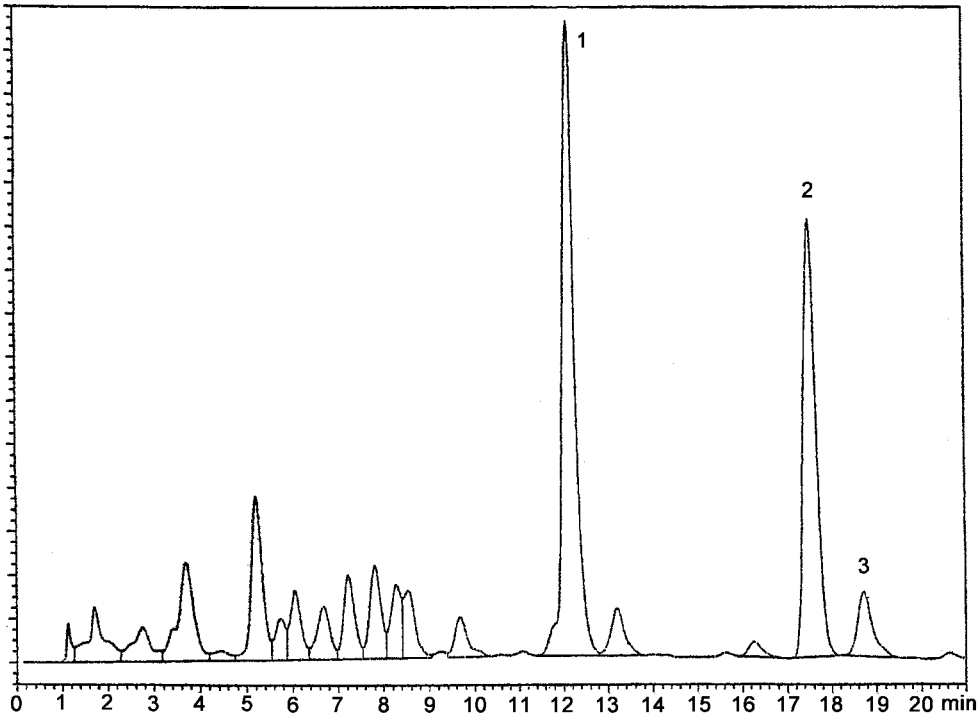

Figure 1827.-1. – *Chromatogram for the assay of flavonoids in refined and quantified ginkgo dry extract*

Calculate the percentage content of flavonoids, expressed as flavone glycosides, using the following expression:

$$\frac{F_1 \times m_1 \times 2.514 \times p}{F_2 \times m_2}$$

F_1 = sum of the areas of all the peaks from the peak due to quercetin to the peak due to isorhamnetin in the chromatogram obtained with the test solution;

F_2 = area of the peak due to quercetin in the chromatogram obtained with the reference solution;

m_1 = mass of *quercetin dihydrate CRS* in the reference solution, in grams;

m_2 = mass of the extract to be examined used to prepare the test solution, in grams;

p = percentage content of anhydrous quercetin in *quercetin dihydrate CRS*.

Terpene lactones. Liquid chromatography (*2.2.29*).

Test solution. Place 0.120 g of the extract to be examined in a 25 mL beaker and dissolve it in 10 mL of *phosphate buffer solution pH 5.8 R* by stirring. Transfer the solution into a chromatography column, about 0.15 m long and about 30 mm in internal diameter, containing 15 g of *kieselguhr for chromatography R*. Wash the beaker with 2 quantities, each of 5 mL, of *phosphate buffer solution pH 5.8 R* and transfer the washings to the chromatography column. Allow to stand for 15 min. Elute with 100 mL of *ethyl acetate R*. Evaporate the eluate to dryness at a pressure not exceeding 4 kPa in a water-bath at 50 °C. The residue of solvent is eliminated by an air-current. Take up the residue in 2.5 mL of the mobile phase.

Reference solution (a). Dissolve 30.0 mg of *benzyl alcohol CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (b). Place 0.120 g of the *ginkgo dry extract for peak identification CRS* in a 25 mL beaker and dissolve it in 10 mL of *phosphate buffer solution pH 5.8 R* by stirring, then proceed as described for the test solution.

Column:

– *size*: *l* = 0.25 m, Ø = 4 mm;

– *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 25 °C.

Mobile phase: *tetrahydrofuran R, methanol R, water R* (10:20:75 *V/V/V*).

Flow rate: 1.0 mL/min.

Detection: refractometer maintained at 35 °C.

Injection: 100 µL.

Identification of peaks: use the chromatogram supplied with *ginkgo dry extract for peak identification CRS* and the chromatogram obtained with the reference solution (b) to identify the peaks due to bilobalide and ginkgolides A, B and C.

System suitability:

– the chromatogram obtained with reference solution (b) is similar to the chromatogram supplied with *ginkgo dry extract for peak identification CRS*.

Calculate the percentage content of bilobalide, using the following expression:

$$\frac{F_1 \times m_1 \times p \times 0.025 \times 1.20}{F_5 \times m_2}$$

Calculate the percentage content of ginkgolide A, using the following expression:

$$\frac{F_2 \times m_1 \times p \times 0.025 \times 1.22}{F_5 \times m_2}$$

Calculate the percentage content of ginkgolide B, using the following expression:

$$\frac{F_3 \times m_1 \times p \times 0.025 \times 1.19}{F_5 \times m_2}$$

Calculate the percentage content of ginkgolide C, using the following expression:

$$\frac{F_4 \times m_1 \times p \times 0.025 \times 1.27}{F_5 \times m_2}$$

F_1 = area of the peak due to bilobalide in the chromatogram obtained with the test solution;

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

F_3 = area of the peak due to ginkgolide B in the chromatogram obtained with the test solution;

F_4 = area of the peak due to ginkgolide C in the chromatogram obtained with the test solution;

F_5 = area of the peak due to benzyl alcohol in the chromatogram obtained with reference solution (a);

m_1 = mass of *benzyl alcohol CRS* in reference solution (a), in grams;

m_2 = mass of the extract to be examined used to prepare the test solution, in grams;

p = percentage content of benzyl alcohol in *benzyl alcohol CRS*.

Calculate the percentage content of the sum of ginkgolides A, B and C, using the following expression:

$$G_A + G_B + G_C$$

G_A = percentage content of ginkgolide A;

G_B = percentage content of ginkgolide B;

G_C = percentage content of ginkgolide C.

Ginkgolic acids. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.500 g of the powdered extract to be examined in 8 mL of *methanol R*, sonicating if necessary, and dilute to 10.0 mL with the same solvent. Centrifuge if necessary.

Reference solution. Dissolve 10.0 mg of *ginkgolic acids CRS* in 8 mL of *methanol R*, sonicating if necessary, and dilute to 10.0 mL with the same solvent. Dilute 2.0 mL of this solution to 10.0 mL with *methanol R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 35 °C.

Mobile phase:

- *mobile phase A*: dilute 0.1 mL of *trifluoroacetic acid R* to 1000 mL with *water R*;
- *mobile phase B*: dilute 0.1 mL of *trifluoroacetic acid R* to 1000 mL with *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	25 → 10	75 → 90
30 - 35	10	90
35 - 36	10 → 25	90 → 75
36 - 45	25	75

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 50 µL.

Identification of components: use the chromatogram supplied with *ginkgolic acids CRS* and the chromatogram obtained with the test solution to identify the peaks due to ginkgolic acids C13, C15 and C17.

System suitability: reference solution:

- *resolution*: minimum 2.0 between the peaks due to ginkgolic acids C13 and C15;
- *symmetry factor*: 0.8 to 2.0 for the peaks due to ginkgolic acids C13, C15 and C17.

Calculate the content in parts per million of ginkgolic acids expressed as ginkgolic acid C17, using the following expression:

$$\frac{A_1 \times m_2 \times p \times 2000}{A_2 \times m_1}$$

A_1 = sum of the areas of the peaks due to the ginkgolic acids C13, C15 and C17 in the chromatogram obtained with the test solution;

A_2 = area of the peak due to ginkgolic acid C17 in the chromatogram obtained with the reference solution;

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

m_2 = mass of *ginkgolic acids CRS* used to prepare the reference solution, in grams;

p = percentage content of ginkgolic acid C17 in *ginkgolic acids CRS*.

01/2011:1828

GINKGO LEAF

Ginkgonis folium

DEFINITION

Whole or fragmented, dried leaf of *Ginkgo biloba* L.

Content: not less than 0.5 per cent of flavonoids, expressed as flavone glycosides (M_r 757) (dried drug).

IDENTIFICATION

A. The leaf is greyish or yellowish-green or yellowish-brown. The upper surface is slightly darker than the lower surface. The petioles are about 4-9 cm long. The lamina is about 4-10 cm wide, fan-shaped, usually bilobate or sometimes undivided. Both surfaces are smooth, and the venation dichotomous, the veins appearing to radiate from the base; they are equally prominent on both surfaces. The distal margin is incised, irregularly and to different degrees, and irregularly lobate or emarginate. The lateral margins are entire and taper towards the base.

B. Reduce to a powder (355) (*2.9.12*). The powder is greyish or yellowish-green or yellowish-brown. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 1828.-1): irregularly-shaped fragments of the lamina [A, B, D, E], with the upper epidermis, in surface view (D) and transverse section (E), consisting of elongated cells with irregularly sinuous walls [Da], often accompanied by palisade parenchyma [Db], and the lower epidermis, in surface view (A) and transverse section (B), consisting of small cells, with a finely striated cuticle and each cell shortly papillose [Aa], and stomata [Ab] about 60 µm, wide, deeply sunken with 6-8 subsidiary cells; fragments of vascular tissue from the petiole and veins [C] with xylem [Ca] and parenchyma, some cells containing abundant cluster crystals of calcium oxalate of various sizes [Cb].