mucilaginous cells beneath which is a yellowish-brown pigment layer containing numerous elongated prisms of calcium oxalate; thin-walled parenchyma of the endosperm and cotyledons containing aleurone grains and globules of fixed oil.

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

Test solution. To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methanol R* and heat in a water bath at 65 °C for 5 min, shaking frequently. Allow to cool to room temperature and filter. Dilute the filtrate to 10 ml with *methanol R*.

Reference solution. Dissolve 2 mg of *chlorogenic acid R*, 2 mg of *caffeic acid R*, 5 mg of *hyperoside R* and 5 mg of *rutin R* in 20 ml of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application: 30 µl of the test solution and 10 µl of the reference solution, as bands.

Development: over a path of 15 cm.

Drying: at 100-105 °C.

Detection: spray whilst hot with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, subsequently spray 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: the chromatogram obtained with the reference solution shows in the lower half, in order of increasing R_F values, a yellowish-brown fluorescent zone (rutin), a light blue fluorescent zone (chlorogenic acid) and a yellowish-brown fluorescent zone (hyperoside); in the upper third appears a light blue fluorescent zone (caffeic acid). The chromatogram obtained with the test solution shows 3 zones similar in position and fluorescence to the zones due to chlorogenic acid, hyperoside and caffeic acid in the chromatogram obtained with the reference solution, and 3 weak reddish fluorescent zones, one corresponding to the zone due to rutin in the chromatogram obtained with the reference solution and both of the others located above the zone due to hyperoside. Below and above the zone due to caffeic acid some light blue zones appear.

TESTS

Foreign matter (*2.8.2*): maximum 5 per cent of deteriorated false fruit and maximum 2 per cent of other foreign matter. It does not contain fruits of other *Crataegus* species (*C. nigra* Waldst. et Kit., *C. pentagyna* Waldst. et Kit. ex Willd. and *C. azarolus* L.) which are characterised by the presence of more than 3 hard stones.

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

Total ash (*2.4.16*): maximum 5.0 per cent.

ASSAY

To 2.50 g of the powdered drug (355) (*2.9.12*) add 30 ml of *ethanol (70 per cent V/V) R*. Heat under a reflux condenser for 30 min and filter. Wash the residue with 10.0 ml of *ethanol (70 per cent V/V) R*. Add to the filtrate 15.0 ml of *hydrochloric acid R1* and 10.0 ml of *water R*. Heat under a reflux condenser for 80 min. Allow to cool, filter and wash the residue with *ethanol (70 per cent V/V) R* until the filtrate is colourless. Dilute the filtrate to 250.0 ml with *ethanol (70 per cent V/V) R*. Evaporate 50.0 ml of this solution in a round-bottomed flask to about 3 ml and transfer to a separating funnel. Rinse the round-bottomed flask sequentially with 10 ml and 5 ml of *water R* and transfer to the separating funnel. Shake the combined solution with 3 quantities, each of 15 ml, of *butanol R*. Combine the organic layers and dilute to 100.0 ml with *butanol R*.

Measure the absorbance (*2.2.25*) of the solution at 545 nm.

Calculate the percentage content of procyanidins, expressed as cyanidin chloride, using the following expression:

$$\frac{A \times 500}{75 \times m}$$

i.e. taking the specific absorbance of cyanidin chloride to be 75.

A = absorbance at 545 nm,

m = mass of the substance to be examined, in grams.

01/2008:1432
corrected 6.0

HAWTHORN LEAF AND FLOWER

Crataegi folium cum flore

DEFINITION

Whole or cut, dried flower-bearing branches of *Crataegus monogyna* Jacq. (Lindm.), *C. laevigata* (Poiret) D.C. (*C. oxyacanthoides* Thuill.) or their hybrids or, more rarely, other European *Crataegus* species including *C. pentagyna* Waldst. et Kit. ex Willd., *C. nigra* Waldst. et Kit., *C. azarolus* L.

Content: minimum 1.5 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

IDENTIFICATION

A. The stems are dark brown, woody, 1-2.5 mm in diameter, bearing alternate, petiolate leaves with small, often deciduous stipules and corymbs of numerous small white flowers. The leaves are more or less deeply lobed with slightly serrate or almost entire margins; those of *C. laevigata* are pinnately lobed or pinnatifid with 3, 5 or 7 obtuse lobes, those of *C. monogyna* pinnatisect with 3 or 5 acute lobes; the adaxial surface is dark green or brownish-green, the abaxial surface is lighter greyish-green and shows a prominent, dense, reticulate venation. The leaves of *C. laevigata*, *C. monogyna* and *C. pentagyna* are glabrous or bear only isolated trichomes, those of *C. azarolus* and *C. nigra* are densely pubescent. The flowers have a brownish-green tubular calyx composed of 5 free, reflexed sepals, a corolla composed of 5 free, yellowish-white or brownish, rounded or broadly ovate and shortly unguiculate petals and numerous stamens. The ovary is fused to the calyx and consists of 1-5 carpels, each with a long style and containing a single ovule; in *C. monogyna* there is 1 carpel, in *C. laevigata* 2 or 3, in *C. azarolus* 2 or 3, or sometimes only 1, in *C. pentagyna* 5 or, rarely, 4.

B. Reduce to a powder (355) (*2.9.12*). The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: unicellular covering trichomes, usually with a thick wall and wide lumen, almost straight or slightly curved, pitted at the base; fragments of leaf epidermis with cells which have sinuous or polygonal anticlinal walls and with large anomocytic stomata (*2.8.3*) surrounded by 4-7 subsidiary cells; parenchymatous cells of the mesophyll containing calcium oxalate clusters, usually measuring 10-20 µm,

those associated with the veins containing groups of small prism crystals; fragments of petals showing rounded polygonal epidermal cells, strongly papillose, with thick walls, the cuticle of which clearly shows wavy striations; fragments of anthers showing endothecium with an arched and regularly thickened margin; fragments of stems containing collenchymatous cells, bordered pitted vessels and groups of lignified sclerenchymatous fibres with narrow lumina; numerous spherical to elliptical or triangular pollen grains up to 45 µm in diameter, with 3 germinal pores and a faintly granular exine.

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

Test solution. To 1.0 g of the powdered drug (355) (*2.9.12*) add 10 ml of *methanol R* and heat in a water-bath at 65 °C under a reflux condenser for 5 min. Cool and filter.

Reference solution. Dissolve 1.0 mg of *chlorogenic acid R* and 2.5 mg of *hyperoside R* in 10 ml of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, methyl ethyl ketone R, ethyl acetate R* (10:10:30:50 *V/V/V/V*).

Application: 20 µl, as bands.

Development: over a path of 15 cm.

Drying: at 100-105 °C.

Detection: spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, subsequently 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 the 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 yellowish-green fluorescent zone (vitexin)
Hyperoside: a yellowish-orange fluorescent zone	A yellowish-orange fluorescent zone (hyperoside)
Chlorogenic acid: a light blue fluorescent zone	A light blue fluorescent zone (chlorogenic acid)
	A yellowish-green fluorescent zone (vitexin-2″-rhamnoside)
_____	_____
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 8 per cent of lignified branches with a diameter greater than 2.5 mm and maximum 2 per cent of other foreign matter.

Loss on drying (*2.2.32*): maximum 10.0 per cent, determined on 1.000 g of powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 10.0 per cent.

ASSAY

Stock solution. Into a 200 ml flask introduce 0.400 g of the powdered drug (250) (*2.9.12*) and 40 ml of *ethanol (60 per cent V/V) R*. Heat in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter through a plug of absorbent cotton into a 100 ml volumetric flask. Transfer the absorbent cotton with the drug residue back into the 200 ml flask, add 40 ml of *ethanol (60 per cent V/V) R* and heat again in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter into the same 100 ml volumetric flask. Rinse the 200 ml flask with a further quantity of *ethanol (60 per cent V/V) R*, filter and transfer to the same 100 ml volumetric flask. Dilute to 100.0 ml with *ethanol (60 per cent V/V) R* and filter.

Test solution. Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the same 25 ml volumetric flask. Add 10.0 ml of a solution containing 25.0 g/l of *boric acid R* and 20.0 g/l of *oxalic acid R* in *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Compensation liquid. Introduce 5.0 ml of the stock solution into a round-bottomed flask and evaporate to dryness under reduced pressure. Take up the residue with 8 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into a 25 ml volumetric flask. Rinse the round-bottomed flask with 3 ml of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and transfer into the same 25 ml volumetric flask. Add 10.0 ml of *anhydrous formic acid R* and dilute to 25.0 ml with *anhydrous acetic acid R*.

Measure the absorbance (*2.2.25*) of the test solution after 30 min at 410 nm, by comparison with the compensation liquid.

Calculate the percentage content of total flavonoids, expressed as hyperoside, using the following expression:

$$\frac{A \times 1.235}{m}$$

i.e. taking the value of the specific absorbance of hyperoside to be 405.

A = absorbance at 410 nm,

m = mass of the drug to be examined, in grams.

01/2008:1865
corrected 6.0

HAWTHORN LEAF AND FLOWER DRY EXTRACT

Crataegi folii cum flore extractum siccum

DEFINITION

Dry extract produced from *Hawthorn leaf and flower (1432)*.

Content:

– for aqueous extracts: minimum 2.5 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried extract);

– for hydroalcoholic extracts: minimum 6.0 per cent of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried extract).

PRODUCTION

The extract is produced from the drug by a suitable procedure using either water or a hydroalcoholic solvent equivalent in strength to a minimum of 45 per cent *V/V* ethanol.

CHARACTERS

Appearance: light brown or greenish-brown powder.