IDENTIFICATION

Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution. Dissolve 1.0 g of the substance to be examined in *ethylene chloride R* and dilute to 10 ml with the same solvent.

Apply to the plate 2 µl of the test solution. Develop over a path of 12 cm with a mixture of 10 volumes of *ether R* and 90 volumes of *ethylene chloride R*. Allow the plate to dry in air and expose to iodine vapour until the spots appear. Examine in daylight. The chromatogram shows a spot with an R_f value of about 0.6, corresponding to triglycerides (R_{st} 1) and spots corresponding to 1,3-diglycerides (R_{st} 0.5) and to 1,2-di-glycerides (R_{st} 0.3). A spot corresponding to 1-monoglycerides may also be visible (R_{st} 0.05).

TESTS

Alkaline impurities. Dissolve 2.00 g in a mixture of 1.5 ml of *alcohol R* and 3.0 ml of *ether R*. Add 0.05 ml of *bromophenol blue solution R*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

Melting point (*2.2.15*): 30 °C to 45 °C; the melting point does not differ by more than 2 °C from the nominal value. Introduce the melted substance into the capillary tube and allow to stand at a temperature below 10 °C for 24 h.

Acid value (*2.5.1*). Not more than 0.5. Dissolve 5.0 g in 50 ml of the prescribed mixture of solvents.

Hydroxyl value (*2.5.3, Method A*). The hydroxyl value is not more than 50 and does not differ by more than 5 units from the nominal value. If the nominal value is less than 5, the hydroxyl value is not more than 5.

Iodine value (*2.5.4*). Not more than 3.

Peroxide value (*2.5.5*). Not more than 3.

Saponification value (*2.5.6*): 210 to 260, determined on 2.0 g. The saponification value does not differ by more than 5 per cent from the nominal value.

Unsaponifiable matter (*2.5.7*). Not more than 0.6 per cent, determined on 5.0 g.

Heavy metals (*2.4.8*). 2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Total ash (*2.4.16*). Not more than 0.05 per cent, determined on 2.00 g.

STORAGE

Store protected from light and heat.

LABELLING

The label states:

– the nominal melting point,
– the nominal hydroxyl value,
– the nominal saponification value.

01/2005:1220

HAWTHORN BERRIES

Crataegi fructus

DEFINITION

Hawthorn berries consist of the dried false fruits of *Crataegus monogyna* Jacq. (Lindm.), or *Crataegus laevigata* (Poir.) D.C. (synonym: *Crataegus oxyacantha* L.) or their hybrids or a mixture of these false fruits. They contain not less than 1.0 per cent of procyanidins, calculated as cyanidin chloride ($C_{15}H_{11}ClO_6$; M_r 322.7) with reference to the dried drug.

CHARACTERS

The false fruit has a sweet mucilaginous taste.

It has the macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION

A. The false fruit of *Crataegus monogyna* is obovate to globular. It is generally 6 mm to 10 mm long and 4 mm to 8 mm wide, reddish-brown to dark red. The surface is pitted or, more rarely, reticulated. The upper end of the fruit is crowned by the remains of five reflexed sepals surrounding a small sunken disc with a shallow, raised rim. The remains of the style occur in the centre of the disc with tufts of stiff, colourless hairs at the base. At the lower end of the fruit a short length of pedicel or, more frequently, a small pale circular scar where the pedicel was attached. The receptacle is fleshy and encloses a yellowish-brown, ovoid fruit with a hard, thick wall containing a single, elongated, pale brown, smooth and shiny seed.

   The false fruit of *Crataegus laevigata* is up to 13 mm long. It contains two to three stony fruits, ventrally flattened, with short hairs at the top. Frequently, in the centre of the disc of the false fruit occur the remains of the two styles.

B. Reduce to a powder (355). The powder is greyish-red. Examine under a microscope using *chloral hydrate solution R*. The powder shows covering trichomes from inside the disc which are long, unicellular, frequently bent, tapering to a point, with smooth, much thickened and lignified walls, parenchymatous receptacle fragments the outer layer with red colouring matter, some cells of the inner layers containing small cluster crystals of calcium oxalate; occasional fragments including groups of sclereids and vascular strands with associated files of cells containing prisms of calcium oxalate; pericarp fragments consisting of large thick-walled sclereids with numerous pits, some of which are conspicuously branched; a few fragments of the testa having an epidermal layer composed of hexagonal, 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. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

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

   Apply separately to the plate, as bands, 30 µl of the test solution and 10 µl of the reference solution. Develop over a path of 15 cm using as the mobile phase a mixture of 10 volumes of *anhydrous formic acid R*, 10 volumes of *water R*, 30 volumes of *methyl ethyl ketone R* and 50 volumes of *ethyl acetate R*. Dry the plate at 100-105 °C and spray whilst hot with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Subsequently spray the plate with a 50 g/l solution

of *macrogol 400 R* in *methanol R*. Allow the plate to dry in air for 30 min and examine in ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows in the lower half, in order of increasing R_f values, the yellowish-brown fluorescent zone of rutin, the light blue fluorescent zone of chlorogenic acid and the yellowish-brown fluorescent zone of hyperoside; in the upper third appears the light blue fluorescent zone of caffeic acid. The chromatogram obtained with the test solution shows three 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 three weak reddish fluorescent zones, one corresponding to the rutin zone in the chromatogram obtained with the reference solution and both of the others located above the hyperoside zone. Below and above the caffeic acid zone some light blue zones appear.

TESTS

Foreign matter (*2.8.2*). Not more than 2 per cent. Not more than 5 per cent of deteriorated false fruit. 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 three hard stones.

Loss on drying (*2.2.32*). Not more than 12.0 per cent, determined on 1.000 g of the powdered drug (355) by drying in an oven at 100-105 °C for 2 h.

Total ash (*2.4.16*). Not more than 5.0 per cent.

ASSAY

To 2.50 g of the powdered drug (355) add 30 ml of *alcohol (70 per cent V/V) R*. Heat under a reflux condenser for 30 min and filter. Wash the residue with 10.0 ml of *alcohol (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 *alcohol (70 per cent V/V) R* until the filtrate is colourless. Dilute the filtrate to 250.0 ml with *alcohol (70 per cent V/V) R*. Evaporate 50.0 ml of this solution in a round-bottomed flask to about 3 ml and transfer it 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 three 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, as cyanidin chloride, from the 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.

STORAGE

Store protected from light.

01/2005:1432

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).

CHARACTERS

Macroscopic and microscopic characters described under identification tests A and B.

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 to 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 to brownish, rounded or broadly ovate and shortly unguiculate petals and numerous stamens. The ovary is fused to the calyx and consists of 1 to 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). The powder is yellowish-green. Examine under a microscope using *chloral hydrate solution R*. The powder shows unicellular covering trichomes, usually with a thick wall and wide lumen, almost straight to slightly curved, pitted at the base; fragments of leaf epidermis with cells which have sinuous to polygonal anticlinal walls and with large anomocytic stomata (*2.8.3*) surrounded by 4 to 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) 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.