FUNCTIONALITY-RELATED CHARACTERISTICS

This section provides information on characteristics that are recognised as being relevant control parameters for one or more functions of the substance when used as an excipient (see chapter 5.15). This section is a non-mandatory part of the monograph and it is not necessary to verify the characteristics to demonstrate compliance. Control of these characteristics can however contribute to the quality of a medicinal product by improving the consistency of the manufacturing process and the performance of the medicinal product during use. Where control methods are cited, they are recognised as being suitable for the purpose, but other methods can also be used. Wherever results for a particular characteristic are reported, the control method must be indicated.

The following characteristic may be relevant for acacia used as a viscosity-increasing agent and/or suspending agent in aqueous preparations.

Apparent viscosity. Determine the dynamic viscosity using a capillary viscometer (*2.2.9*) or a rotating viscometer (*2.2.10*) on a 100 g/L solution of acacia (dried substance).

01/2009:0310

AGAR

Agar

DEFINITION

Polysaccharides from various species of Rhodophyceae mainly belonging to the genus *Gelidium*. It is prepared by treating the algae with boiling water; the extract is filtered whilst hot, concentrated and dried.

CHARACTERS

Appearance: powder or crumpled strips 2-5 mm wide or sometimes flakes, colourless or pale yellow, translucent, somewhat tough and difficult to break, becoming more brittle on drying.

Mucilaginous taste.

IDENTIFICATION

A. Examine under a microscope. When mounted in *0.005 M iodine*, the strips or flakes are partly stained brownish-violet. Magnified 100 times, they show the following diagnostic characters: numerous minute, colourless, ovoid or rounded grains on an amorphous background; occasional brown, round or ovoid spores with a reticulated surface, measuring up to 60 µm, may be present. Reduce to a powder, if necessary. The powder is yellowish-white. Examine under a microscope using *0.005 M iodine*. The powder presents angular fragments with numerous grains similar to those seen in the strips and flakes; some of the fragments are stained brownish-violet.

B. Dissolve 0.1 g with heating in 50 mL of *water R*. Cool. To 1 mL of the mucilage carefully add 3 mL of *water R* so as to form 2 separate layers. Add 0.1 mL of *0.05 M iodine*. A dark brownish-violet colour appears at the interface. Mix. The liquid becomes pale yellow.

C. Heat 5 mL of the mucilage prepared for identification test B on a water-bath with 0.5 mL of *hydrochloric acid R* for 30 min. Add 1 mL of *barium chloride solution R1*. A white turbidity develops within 30 min.

D. Heat 0.5 g with 50 mL of *water R* on a water-bath until dissolved. Only a few fragments remain insoluble. During cooling, the solution gels between 35 °C and 30 °C. Heat the gel thus obtained on a water-bath; it does not liquefy below 80 °C.

TESTS

Swelling index (*2.8.4*): minimum 10 and within 10 per cent of the value stated on the label, determined on the powdered drug (355) (*2.9.12*).

Insoluble matter: maximum 1.0 per cent.

To 5.00 g of the powdered drug (355) (*2.9.12*) add 100 mL of *water R* and 14 mL of *dilute hydrochloric acid R*. Boil gently for 15 min with frequent stirring. Filter the hot liquid through a tared, sintered-glass filter (160) (*2.1.2*), rinse the filter with hot *water R* and dry at 100-105 °C. The residue weighs a maximum of 50 mg.

Gelatin. To 1.00 g add 100 mL of *water R* and heat on a water-bath until dissolved. Allow to cool to 50 °C. To 5 mL of this solution add 5 mL of *picric acid solution R*. No turbidity appears within 10 min.

Loss on drying (*2.2.32*): maximum 20.0 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C.

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

Microbial contamination

TAMC: acceptance criterion 10^3 CFU/g (*2.6.12*).

TYMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

LABELLING

The label states the swelling index.

01/2008:2147
corrected 6.2

AGNUS CASTUS FRUIT

Agni casti fructus

DEFINITION

Whole, ripe, dried fruit of *Vitex agnus-castus* L.

Content: minimum 0.08 per cent of casticin ($C_{19}H_{18}O_8$; M_r 374.3) (dried drug).

IDENTIFICATION

A. Agnus castus fruit is oval or almost globular, with a diameter of up to 5 mm. The persistent calyx is greenish-grey, finely pubescent, ends in 4-5 short teeth and envelops 2/3 to 3/4 of the surface of the fruit. The blackish-brown fruit consists of a pericarp that becomes progressively sclerous up to the endocarp. The style scar is often visible. Some of the fruits may retain a stalk, about 1 mm long. A transverse section of the fruit shows 4 locules, each containing an elongated seed.

B. Reduce to a powder (355) (*2.9.12*). Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of the outer epidermis of the calyx composed of polygonal cells densely covered with short, bent or undulate, uni-, bi- or tri-cellular uniseriate covering trichomes; cells of the epicarp with thick walls and well-marked, large pits; isolated glandular trichomes with a unicellular stalk and a uni- or multi-cellular head; layers of parenchyma from the outer part of the mesocarp, some containing brown pigment, others extending into septa; fragments from the inner part of the mesocarp composed of thin-walled, pitted, sclerenchymatous cells and of typical isodiametric sclerous cells with very thick, deeply grooved walls and a narrow, stellate lumen; small brown cells of the endocarp; fragments of the testa containing areas of fairly large, thin-walled lignified cells with reticulate bands of thickening; numerous fragments of the endosperm composed of thin-walled parenchymatous cells containing aleurone grains and oil droplets.

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*. Heat in a water-bath at 60 °C for 10 min. Allow to cool and filter.

Reference solution. Dissolve 0.5 mg of *aucubin R* and 1 mg of *agnuside R* in *methanol R* and dilute to 1.0 mL with the same solvent.

Plate: *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F_{254} plate R* (2-10 µm)].

Mobile phase: *water R, methanol R, ethyl acetate R* (8:15:77 *V/V/V*).

Application: 10 µL [or 8 µL] as bands.

Development: over a path of 8 cm [or 5 cm].

Drying: in air.

Detection: spray with *formic acid R* and heat at 120 °C for 10 min; examine in daylight.

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

Top of the plate	
———	———
Agnuside: a blue zone	A blue zone (agnuside)
———	———
Aucubin: a blue zone	A blue zone (aucubin)
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): maximum 3.0 per cent.

Other species of *Vitex*, in particular *Vitex negundo*. No fruit of other species with a much greater diameter is present.

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

Loss on drying (*2.2.32*): maximum 10.0 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.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. Extract 1.000 g of the powdered drug (355) (*2.9.12*) with 40 mL of *methanol R* for 2 min using a suitable-speed homogeniser. Collect the supernatant liquid and filter into a 250 mL flask. Repeat the extraction with a further 40 mL of *methanol R*, collecting the supernatant liquid and filtering as before. Rinse the residue carefully with a small quantity of *methanol R*. Combine the methanol extracts and rinsings and evaporate to dryness *in vacuo* in a water-bath at not more than 30 °C. With the aid of ultrasound, dissolve the residue obtained in *methanol R* and dilute to 20.0 mL with the same solvent. Filter the solution through a membrane filter (nominal pore size 0.45 µm). Dilute 1.0 mL to 10.0 mL with *methanol R*.

Reference solution. Dissolve 100.0 mg of *agnus castus fruit standardised dry extract CRS* in 20.0 mL of *methanol R* with the aid of ultrasound for 20 min, then dilute to 25.0 mL with the same solvent. Filter the solution through a membrane filter (nominal pore size 0.45 µm).

Column:

- *size*: *l* = 0.125 m, Ø = 3.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 25 °C.

Mobile phase:

- *mobile phase A*: 5.88 g/L solution of *phosphoric acid R*;
- *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 13	50 → 35	50 → 65
13 - 18	35 → 0	65 → 100
18 - 23	0 → 50	100 → 50

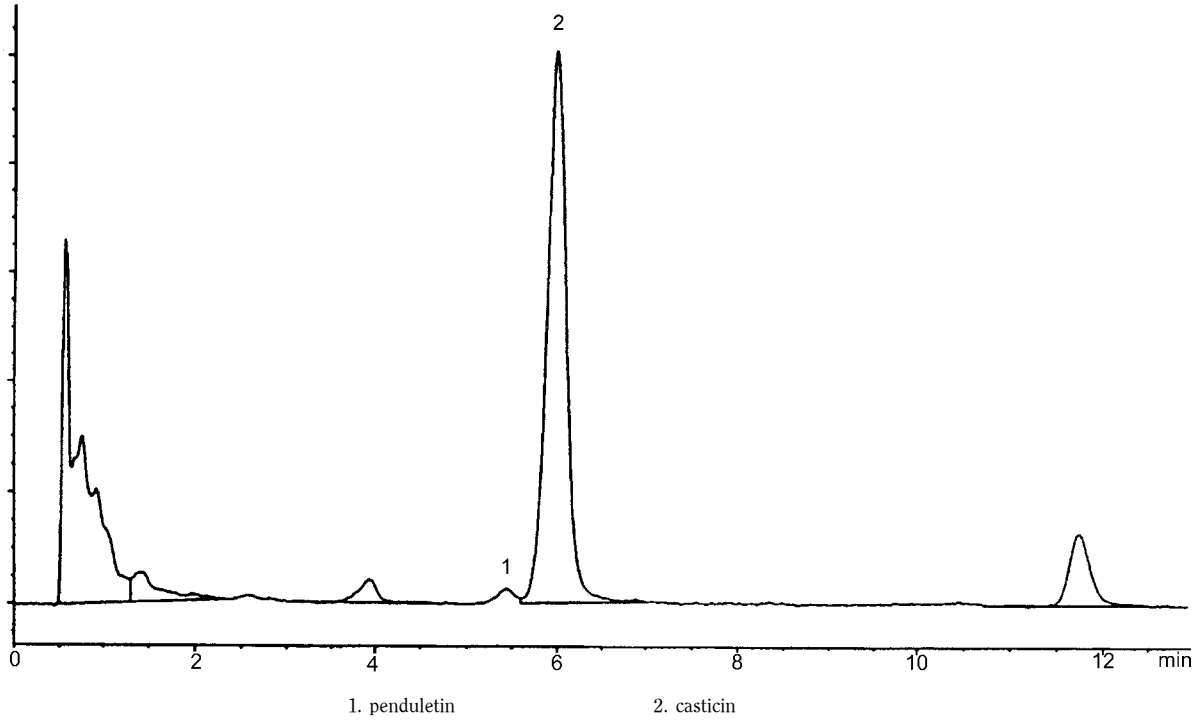
Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 348 nm.

Injection: 10 µL.

System suitability: test solution:

- *resolution*: minimum 1.5 between the peaks due to penduletin and casticin (see Figure 2147.-1).



1. penduletin 2. casticin

Figure 2147.-1. – *Chromatogram for the assay of casticin in Agnus castus fruit: test solution*

Calculate the percentage content of casticin using the following expression:

$$\frac{F_1 \times m_2 \times p_1 \times 8}{F_2 \times m_1}$$

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

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

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

m_2 = mass of *agnus castus fruit standardised dry extract CRS* used to prepare the reference solution, in grams;

p_1 = percentage content of casticin in *agnus castus fruit standardised dry extract CRS*.

01/2011:1587

AGRIMONY

Agrimoniae herba

DEFINITION

Dried flowering tops of *Agrimonia eupatoria* L.

Content: minimum 2.0 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$; M_r 126.1) (dried drug).

IDENTIFICATION

A. The stem is green or, more usually, reddish, cylindrical and infrequently branched. It is covered with long, erect or tangled hairs. The leaves are compound imparipennate with 3 or 6 opposite pairs of leaflets, with 2 or 3 smaller leaflets between. The leaflets are deeply dentate to serrate, dark green on the upper surface, greyish and densely tomentose on the lower face. The flowers are small and form a terminal spike. They are pentamerous and borne in the axils of hairy bracts, the calyces closely surrounded by numerous terminal hooked spires, which occur on the rim of the hairy receptacle. The petals are free, yellow and deciduous. Fruit-bearing obconical receptacles, with deep furrows and hooked bristles, are usually present at the base of the inflorescence.

B. Reduce to a powder (355) (*2.9.12*). The powder is yellowish-green or grey. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 1587.-1): numerous straight or bent, unicellular, long, thick-walled (about 500 µm) covering trichomes [Ab, Ca, F], finely warty, and sometimes spirally marked, often fragmented (F); fragments of the epidermis of the stems [A] with stomata [Aa], covering trichomes (Ab) and glandular trichomes [Ac]; fragments of upper leaf epidermis in surface view [C] with straight walls bearing covering trichomes (Ca), accompanied by palisade parenchyma [Cb], with some of the cells containing calcium oxalate prisms [Cc]; fragments of lower leaf epidermis in surface view [J] with sinuous walls and abundant stomata [Ja], mostly anomocytic (*2.8.3*) but occasionally anisocytic, and glandular trichomes [Jb]; ovoid to subspherical pollen grains, with 3 pores and a smooth exine [D]; glandular trichomes with a multicellular, uniseriate stalk and a unicellular to quadricellular head [B, Jb]; fragments of the stems [H] with groups of fibres [Ha] and parenchymatous cells, some of which contain cluster crystals of calcium oxalate [Hb]; small spiral vessels from the leaflets [G]; fragments of large, spiral or bordered-pitted vessels from the stem [E].

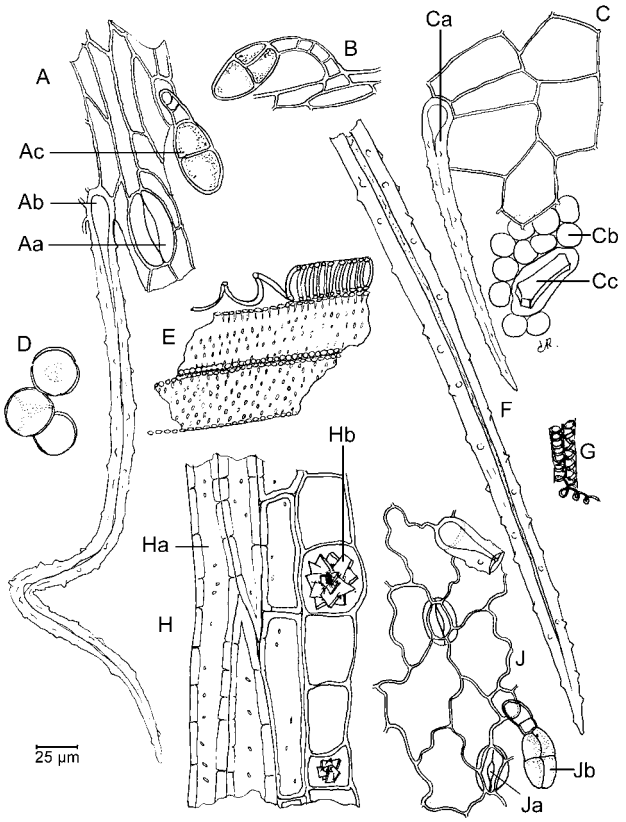


Figure 1587.-1.– *Illustration for identification test B of powdered herbal drug of agrimony*

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

Test solution. To 2.0 g of the powdered drug (355) (*2.9.12*) add 20 mL of *methanol R*. Heat with shaking at 40 °C for 10 min. Filter.

Reference solution. Dissolve 1.0 mg of *isoquercitroside R* and 1.0 mg of *rutin R* in 2 mL of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, ethyl acetate R* (10:10:80 *V/V/V*).

Application: 10 µL as bands.

Development: over a path of 12 cm.

Drying: at 100-105 °C.

Detection: spray the still-warm plate with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R* and then 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.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution.

Top of the plate	
	An orange fluorescent zone may be present (quercitroside)
Isoquercitroside: an orange fluorescent zone	An orange fluorescent zone (isoquercitroside)
	An orange fluorescent zone (hyperoside)
Rutin: an orange fluorescent zone	An orange fluorescent zone (rutin)
Reference solution	**Test solution**

TESTS

Loss on drying (*2.2.32*): maximum 10.0 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 10.0 per cent.

Herbal drugs