EUROPEAN PHARMACOPOEIA

Cannabis flower

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CANNABIS FLOWER

Cannabis flos

DEFINITION

Dried, whole or fragmented, fully developed female inflorescence of *Cannabis sativa* L.

Content: if the herbal drug is to be prescribed to patients as a medicinal product, the measured contents of total tetrahydrocannabinol and total cannabidiol, respectively, do not deviate from the values stated on the label by more than ± 10 per cent.

THC-dominant type:

- *total tetrahydrocannabinol, expressed as Δ^9-tetrahydrocannabinol* ($C_{21}H_{30}O_2$; M_r 314.5): minimum 5.0 per cent (dried drug);
- *total cannabidiol, expressed as cannabidiol* ($C_{21}H_{30}O_2$; M_r 314.5): maximum 1.0 per cent (dried drug).

THC/CBD-intermediate type:

- *total tetrahydrocannabinol, expressed as Δ^9-tetrahydrocannabinol* ($C_{21}H_{30}O_2$; M_r 314.5): minimum 1.0 per cent (dried drug);
- *total cannabidiol, expressed as cannabidiol* ($C_{21}H_{30}O_2$; M_r 314.5): minimum 1.0 per cent (dried drug);
- *total tetrahydrocannabinol / total cannabidiol ratio*: 0.2 to 5.0 (dried drug).

CBD-dominant type:

- *total tetrahydrocannabinol, expressed as Δ^9-tetrahydrocannabinol* ($C_{21}H_{30}O_2$; M_r 314.5): maximum 1.0 per cent (dried drug);
- *total cannabidiol, expressed as cannabidiol* ($C_{21}H_{30}O_2$; M_r 314.5): minimum 5.0 per cent (dried drug).

PRODUCTION

If the herbal drug is to be prescribed to patients as a medicinal product, the inflorescence is cut at the base with minimal rachis remaining.

IDENTIFICATION

A. Depending on the variety, the colour of the herbal drug varies from dark green to pale yellow or from light brown to reddish-brown. The whole female inflorescence is a dense or more or less lax panicle, comprising sessile or almost sessile, elongated bracts (about 10 mm long) with dentate margins, intermingled with the flowers. The fragmented inflorescence, comprises parts of the axis of the inflorescence, the bracts and panicle, together with individual flowers or floral organs. The female flowers are very small (about 2 mm) with a short pedicel. The perianth is monosepalous and apetalous. The sepal, often referred to as the bracteole, is wrapped around the unilocular ovary which bears two styles, each terminating in a fine, orange-brown stigma that is longer than the calyx. The inflorescence is more or less densely pilose, with covering trichomes and glandular trichomes that produce a sticky resin with an aromatic odour.

B. Microscopic examination (*2.8.23*), on the milled or ground herbal drug (not sieved). The colour varies from dark green to yellowish-green or from light brown to reddish-brown. Examine under a microscope using *chloral hydrate solution R*. The milled or ground herbal drug shows the following diagnostic characters (Figure 3028.-1): very numerous glandular or covering trichomes, free or attached to epidermis, of different types: a) whole glandular trichomes, with a multiseriate, multicellular stalk and a multicellular head covered by a domed cuticle (transverse section [E]), or fragments of these trichomes comprising the stalk or head [A] only; some have a very short stalk [Ha], others are sessile; some still have the domed cuticle over the glandular cells (surface view [Da], transverse section [Ea]) while others no longer have it [A]; b) small glandular trichomes with a uni- or biseriate stalk and a uni-, bi- or quadricellular head containing orange-yellow droplets (surface view [Bc, Ca, J], side view [Cb, La, Lb]); c) cystolithic [Fa, Ka] and non-cystolithic unicellular covering trichomes; the conical, cystolithic covering trichomes have either thickened walls, a broad base and a curved, pointed end, with a clearly visible, lumpy, globular calcium carbonate deposit (surface view [Ba], transverse section [Ka]), or a narrower base and markedly pitted walls [Fa]; the non-cystolithic covering trichomes are more elongated and have thickened, smooth walls [Hb]; fragments of the upper epidermis of the bracts (surface view [B, F, L]) sometimes covered by a fine, striated cuticle composed of polygonal cells with rigid walls [Bb], cystolithic covering trichomes [Ba, Fa] and small glandular trichomes (surface view [Bc], side view [La, Lb]); the upper epidermis is usually associated with palisade parenchyma with some cells containing small cluster crystals of calcium oxalate [Bd]; fragments of the lower epidermis of the bracts [D] comprising cells with slightly sinuous walls [Db], anomocytic stomata (*2.8.3*) [Dc], small glandular trichomes [Dd] and glandular trichomes with a multicellular stalk and a multicellular head [Da]; fragments of the lamina of the bracts (transverse section [K]) comprising the upper epidermis covered by a cuticle [Kb], with rectangular cells and cystolithic covering trichomes [Ka], and the palisade parenchyma layer with some cells containing a small cluster crystal of calcium oxalate [Kc]; fragments of the lower epidermis of the bracteoles [H] with slightly wavy cells [Hc], glandular trichomes with a short stalk [Ha], anomocytic stomata [Hd], non-cystolithic covering trichomes [Hb] and small glandular trichomes [He]; small cluster crystals of the underlying mesophyll are clearly visible in the fragments of the bracteole epidermises [Hf]; fragments of the orange-brown stigmas showing epidermal cells with very fine, faintly visible walls, terminating in large papillae with a rounded end [G]; fragments of the axis of the inflorescence [N] comprising cellulose fibres, spiral [Na] or annular vessels, and cells of the pith with reticulate walls [Nb], some of which contain cluster crystals of calcium oxalate of about 30 µm in diameter; free cluster crystals of calcium oxalate [M].

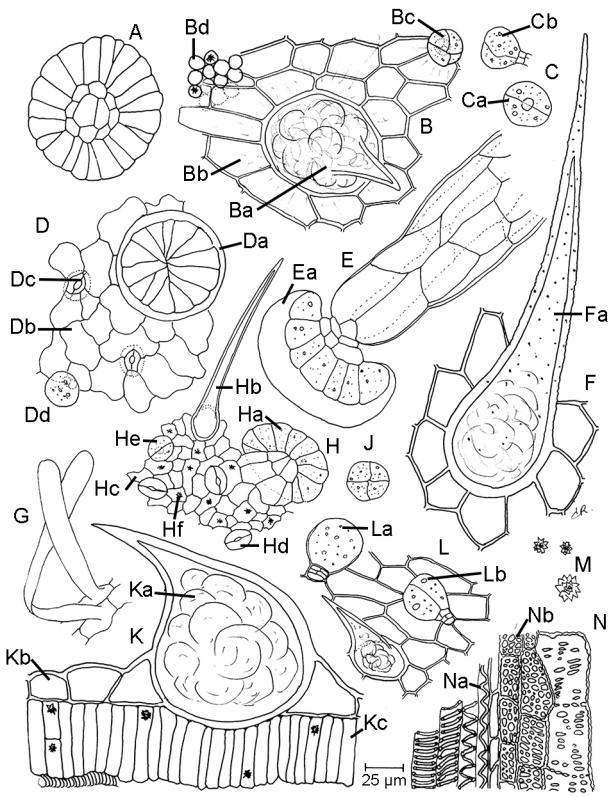

Figure 3028.-1. – *Illustration for identification test B of milled or ground herbal drug of cannabis flower*

C. High-performance thin-layer chromatography (*2.8.25*).

Test solution. Introduce 0.5 g of the cut or milled herbal drug (not sieved) into a test tube and add 5.0 mL of *methanol R*. Stopper the tube and mix using a vortex mixer for 10 s. Sonicate for 5 min, then mix using a vortex mixer for 10 s. Repeat this operation twice. Centrifuge and use the supernatant.

Reference solution (a). Dissolve 5.0 mg of *cannabidiol R* in 1.0 mL of *Δ^9-tetrahydrocannabinol solution R*.

Reference solution (b). Dilute 0.25 mL of reference solution (a) to 1.0 mL with *methanol R*.

Reference solution (c). Dissolve 1 mg of *cannabidiol R* and 1 mg of *cannabidiolic acid R* in *methanol R* and dilute to 1 mL with the same solvent.

Intensity marker: reference solutions (a) and (b):

– Δ^9-tetrahydrocannabinol.

Plate: *TLC octadecylsilyl silica gel F_{254} plate R* (2-10 µm).

Mobile phase: *water R*, *glacial acetic acid R*, *methanol R* (10:10:80 *V/V/V*).

Application: 2.0 µL, as bands of 8 mm.

Development: 70 mm from the lower edge of the plate.

Drying: in a current of air at room temperature for 5 min.

Detection: treat with *vanillin reagent R*, heat at 100 °C for 3 min and then allow to cool for 3 min; examine in daylight.

System suitability: reference solution (c):

– the chromatogram shows in the middle third 2 distinct zones, which may be touching; the lower zone (cannabidiolic acid) and the upper zone (cannabidiol) are grey to reddish-violet.

Results: see below the sequence of zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other very faint zones may be present. If present, the zone due to Δ^9-tetrahydrocannabinolic acid is more intense than the zone due to Δ^9-tetrahydrocannabinol. If present, the zone due to cannabidiolic acid is more intense than the zone due to cannabidiol.

Top of the plate			
Cannabidiol: a reddish-violet zone		A reddish-violet zone, faint to very faint (cannabidiol) A reddish-violet zone, intense (cannabidiolic acid)	A reddish-violet zone, faint to very faint (cannabidiol) A reddish-violet zone, intense (cannabidiolic acid)
Δ^9-Tetrahydrocannabinol: a reddishviolet zone	A reddish-violet zone, faint to equivalent (Δ^9-tetrahydrocannabinol)	A reddish-violet zone, faint (Δ^9-tetrahydrocannabinol)	A grey to reddish-violet zone, very faint, may be absent (Δ^9-tetrahydrocannabinol)
	A reddish-violet zone, intense (Δ^9-tetrahydrocannabinolic acid)	A reddish-violet zone (Δ^9-tetrahydrocannabinolic acid)	A reddish-violet zone, very faint (Δ^9-tetrahydrocannabinolic acid)
Reference solution (a)	**Test solution (THC-dominant type)**	**Test solution (THC/CBD-intermediate type)**	**Test solution (CBD-dominant type)**

TESTS

Total CBN. Liquid chromatography (*2.2.29*).

Test solution (a). To 0.50 g of the cut or milled herbal drug (not sieved) in a suitable centrifuge tube fitted with a screw cap, add 40 mL of *ethanol (96 per cent) R* and shake for 15 min. Centrifuge at about 1700 *g* and transfer the clear supernatant into a flask. Repeat the extraction twice with 25 mL of *ethanol (96 per cent) R*. Combine the supernatants and dilute to 100.0 mL with *ethanol (96 per cent) R*. Filter through a membrane filter (nominal pore size 0.22 µm).

Test solution (b). Dilute 1.0 mL of test solution (a) to 10.0 mL with *methanol R*.

Reference solution (a). Dissolve 20.0 mg of *cannabidiol for cannabis CRS* in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (b). Dilute 5.0 mL of reference solution (a) to 20.0 mL with *methanol R*.

Reference solution (c). Dilute 10.0 mL of reference solution (a) to 25.0 mL with *methanol R*.

Reference solution (d). To 50 mg of *cannabis flower for system suitability HRS* in a suitable centrifuge tube fitted with a screw cap, add 4 mL of *ethanol (96 per cent) R* and shake for 15 min. Centrifuge the solution at about 1700 *g* and transfer the clear supernatant into a flask. Repeat the extraction twice with 2.5 mL of *ethanol (96 per cent) R*. Combine the supernatants and dilute to 10 mL with *ethanol (96 per cent) R*. Filter through a membrane filter (nominal pore size 0.22 µm).

Reference solution (e). Dilute 1 mL of reference solution (d) to 10 mL with *methanol R*.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped solid core polar-embedded octadecylsilyl silica gel for chromatography R* (2.7 µm);
– *temperature*: 35 °C.

Mobile phase: 0.1 per cent *V/V* solution of *trifluoroacetic acid R*, *acetonitrile for chromatography R* (41:59 *V/V*).

Flow rate: 2.0 mL/min.

Detection: spectrophotometer at 228 nm.

Injection: 5 µL of test solution (a) and reference solutions (b) and (d).

Run time: 5.0 times the retention time of cannabidiol.

Identification of peaks: use the chromatogram obtained with reference solution (b) to identify the peak due to cannabidiol; use the chromatogram supplied with *cannabis flower for system suitability HRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to Δ^9-tetrahydrocannabinol, Δ^9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabinol, cannabinolic acid, cannabichromene, cannabigerol and cannabigerolic acid.

Relative retention with reference to cannabidiol (retention time = about 6.9 min): cannabidiolic acid = about 1.10; cannabigerol = about 1.17; cannabinol = about 1.48; cannabigerolic acid = about 1.63; Δ^9-tetrahydrocannabinol = about 1.76; cannabinolic acid = about 2.38; cannabichromene = about 2.48; Δ^9-tetrahydrocannabinolic acid = about 2.78.

System suitability: reference solution (d):

- *resolution*: minimum 2.0 between the peaks due to cannabigerolic acid and Δ^9-tetrahydrocannabinol;
- *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to cannabigerol and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to cannabidiolic acid; minimum 5.0, where H_p = height above the baseline of the peak due to cannabinolic acid and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to cannabichromene.

Calculate the percentage content of total CBN, using the following expression:

$$\frac{((A_1 \times 0.405) + (A_3 \times 0.901 \times 0.876)) \times m_2 \times p}{A_2 \times m_1 \times 4}$$

A_1 = area of the peak due to cannabinol in the chromatogram obtained with test solution (a);

A_2 = area of the peak due to cannabidiol in the chromatogram obtained with reference solution (b);

A_3 = area of the peak due to cannabinolic acid in the chromatogram obtained with test solution (a);

m_1 = mass of the herbal drug to be examined used to prepare test solution (a), in grams;

m_2 = mass of *cannabidiol for cannabis CRS* used to prepare reference solution (a), in grams;

p = percentage content of cannabidiol in *cannabidiol for cannabis CRS*;

0.405 = correction factor of cannabinol with reference to cannabidiol;

0.901 = correction factor of cannabinolic acid with reference to cannabidiol;

0.876 = ratio of the molecular mass of cannabinol to that of cannabinolic acid.

Limit:

- *total CBN*: maximum 1.0 per cent.

Foreign matter (*2.8.2*): maximum 2 per cent; if the herbal drug is to be prescribed to patients as a medicinal product, it does not contain any seeds and the whole herbal drug does not contain any leaves more than 1.0 cm in length.

Carry out the determination using 25-50 g.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of the cut or milled herbal drug (not sieved) by drying over about 100 g of *molecular sieve R* at a pressure between 1.5 kPa and 2.5 kPa at 40 °C for 24 h.

Arsenic (*2.4.27*): maximum 0.2 ppm if the herbal drug is to be prescribed to patients as a medicinal product.

Cadmium (*2.4.27*): maximum 1.0 ppm, or maximum 0.3 ppm if the herbal drug is to be prescribed to patients as a medicinal product.

Lead (*2.4.27*): maximum 5.0 ppm, or maximum 0.5 ppm if the herbal drug is to be prescribed to patients as a medicinal product.

Mercury (*2.4.27*): maximum 0.1 ppm.

ASSAY

This procedure has been validated for an analytical range of 0.2 per cent to 32.0 per cent of Δ^9-tetrahydrocannabinol, Δ^9-tetrahydrocannabinolic acid, cannabidiol and cannabidiolic acid respectively.

Liquid chromatography (*2.2.29*) as described in the test for total CBN, with the following modifications.

Injection: test solution (b) and reference solutions (c) and (e).

System suitability: reference solution (e):

- *resolution*: minimum 2.0 between the peaks due to cannabidiol and cannabidiolic acid.

Calculate the percentage content of total tetrahydrocannabinol, expressed as Δ^9-tetrahydrocannabinol, using the following expression:

$$\frac{((A_1 \times 1.097) + (A_3 \times 0.691 \times 0.877)) \times m_2 \times p \times 4}{A_2 \times m_1}$$

A_1 = area of the peak due to Δ^9-tetrahydrocannabinol in the chromatogram obtained with test solution (b);

A_2 = area of the peak due to cannabidiol in the chromatogram obtained with reference solution (c);

A_3 = area of the peak due to Δ^9-tetrahydrocannabinolic acid in the chromatogram obtained with test solution (b);

m_1 = mass of the herbal drug to be examined used to prepare test solution (a), in grams;

m_2 = mass of *cannabidiol for cannabis CRS* used to prepare reference solution (a), in grams;

p = percentage content of cannabidiol in *cannabidiol for cannabis CRS*;

1.097 = correction factor of Δ^9-tetrahydrocannabinol with reference to cannabidiol;

0.691 = correction factor of Δ^9-tetrahydrocannabinolic acid with reference to cannabidiol;

0.877 = ratio of the molecular mass of Δ^9-tetrahydrocannabinol to that of Δ^9-tetrahydrocannabinolic acid.

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

$$\frac{(A_1 + (A_3 \times 0.596 \times 0.877)) \times m_2 \times p \times 4}{A_2 \times m_1}$$

A_1 = area of the peak due to cannabidiol in the chromatogram obtained with test solution (b);

A_2 = area of the peak due to cannabidiol in the chromatogram obtained with reference solution (c);

A_3 = area of the peak due to cannabidiolic acid in the chromatogram obtained with test solution (b);

m_1 = mass of the herbal drug to be examined used to prepare test solution (a), in grams;

m_2 = mass of *cannabidiol for cannabis CRS* used to prepare reference solution (a), in grams;

p = percentage content of cannabidiol in *cannabidiol for cannabis CRS*;

0.596 = correction factor of cannabidiolic acid with reference to cannabidiol;

0.877 = ratio of the molecular mass of cannabidiol to that of cannabidiolic acid.

STORAGE

In an airtight container.

LABELLING

The label states the percentage contents of total tetrahydrocannabinol and total cannabidiol.

In addition, the label states if the herbal drug is to be prescribed to patients as a medicinal product.

Cannabidiol. $C_{21}H_{30}O_2$. (M_r 314.5). *1221500.* [13956-29-1]. (1′*R*,2′*R*)-5′-Methyl-4-pentyl-2′-(prop-1-en-2-yl)-1′,2′,3′,4′-tetrahydro[1,1′-biphenyl]-2,6-diol.

Content: minimum 95.0 per cent.

Δ^9-Tetrahydrocannabinol solution. *1222301.*

A 5.0 mg/mL solution of *Δ^9-tetrahydrocannabinol R* in *methanol R*.

Δ^9-Tetrahydrocannabinol. $C_{21}H_{30}O_2$. (M_r 314.5). *1222300.* [1972-08-3]. (6a*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran-1-ol.

Content: minimum 95.0 per cent.

Cannabidiolic acid. $C_{22}H_{30}O_4$. (M_r 358.5). *1221600.* [1244-58-2]. (1′*R*,2′*R*)-2,6-Dihydroxy-5′-methyl-4-pentyl-2′-(prop-1-en-2-yl)-1′,2′,3′,4′-tetrahydro[1,1′-biphenyl]-3-carboxylic acid.

Content: minimum 95.0 per cent.

Silica gel for chromatography, octadecylsilyl, polar-embedded, solid core, end-capped. *1222100.*

Silica gel with spherical silica particles containing a non-porous solid silica core surrounded by a thin outer porous silica coating with polar-embedded octadecylsilyl groups. To minimise any interaction with basic compounds it is carefully end-capped to cover most of the remaining silanol groups.