Ministry of Health, Welfare and Sport

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Monograph Cannabis Flower

Cannabis Flos

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General note: The following monograph is elaborated by the Dutch Office of Medicinal Cannabis (OMC). The methods and specifications further described are elaborated conform the EDQM guideline "*Guide for the elaboration of monographs on herbal drugs and herbal drug preparations*" and where applicable refer to general Ph. Eur. chapters.

DEFINITION

Fragmented, dried flowering tops of *Cannabis sativa* L*,* harvested during flowering time*.*
This monograph is applicable for fragmented, dried flowering tops (*Flowers)* and fragmented, dried flowering tops that have been ground up (*Granulate*).

Content:
This monograph is applicable for Cannabis flowers with a stated content of constituents within the following ranges:

Type high THC: 10.0 – 30.0 per cent of total equivalence tetrahydrocannabinol expressed as THC ($C_{21}H_{30}O_2$; M_r 314.5), maximum 1.0 per cent of total equivalence cannabidiol expressed as CBD ($C_{21}H_{30}O_2$; M_r 314.5) (dried drug).

Type high CBD: 5.0 – 15.0 per cent of total equivalence cannabidiol expressed as CBD ($C_{21}H_{30}O_2$; M_r 314.5), maximum 1.0 per cent of total equivalence tetrahydrocannabinol expressed as THC ($C_{21}H_{30}O_2$; M_r 314.5) (dried drug).

Type THC/CBD: 5.0 – 15.0 per cent of total equivalence cannabidiol expressed as CBD ($C_{21}H_{30}O_2$; M_r 314.5), between 5.0 – 15.0 per cent of total equivalence tetrahydrocannabinol expressed as THC ($C_{21}H_{30}O_2$; M_r 314.5) (dried drug).

The measured content of total equivalence tetrahydrocannabinol does not deviate from the stated content by more than ± 20 per cent, unless declared as maximum limit.

The measured content of total equivalence cannabidiol does not deviate from the stated content by more than ± 20 per cent, unless declared as maximum limit.

CHARACTERS

The fragments have an odour characteristic for Cannabis flower.
The *Flowers* have leaves that shoot out no more than 20 per cent of the length of the flower and stalks are mostly cut off directly under the bottom site of the flowers.
Granulate does not contain stalks that are longer than 2.0 cm and only 20 per cent of the stalks are between 1.5 and 2.0 cm.

SUBSTANCE PREPARATION *FLOWERS*

Use for the following tests, except for foreign matter test and macroscopic botanical characters, freshly grinded flowers. Grind the flowers to be examined to about 4 to 6 mm in diameter.

IDENTIFICATION

A. Macroscopic botanical characters
Flowers. The fragments are brown, green clustered flowers of about 1.5 to 3.0 cm length.
Granulate. Brown, green granulates of flowers of about 4 to 6 mm in diameter.

B. Microscopic botanical characters
Examine under a microscope using chloral hydrate solution R. Under the microscope gland hairs are mainly observed.

C. Thin-layer chromatography (*2.2.27*)
Test solution. Use test solution (a) as prepared under ASSAY AND RELATED SUBSTANCES.
Reference solution (a). Dissolve 20.0 mg of CBD-A R and 20.0 mg of THC-A R in ethanol R and dilute to 20.0 mL with the same solvent.
Plate: Silicagel RP-18W / F_{254}.
Mobile phase: petroleum ether R, diethyl ether R (40:40 *V/V*).
Application: 10 µL as bands of 5 mm.
Development: 100 mm from the lower edge of the plate.
Drying: in air.
Detection: spray with 10 mg/mL solution of Fast Blue B Salt R in ethanol R, demi water R (50:50 *V/V*) and dry in an oven for 5 minutes at 80 °C.
System suitability: reference solution (a).
- The chromatogram shows 2 distinct separated zones in the lower third. The lower zone (CBD-A) shows an orange fluorescence and the upper zone (THC-A) shows a red fluorescence.

Results: see below the sequence of zones in the chromatograms obtained with reference solution (a) and the test solution. The related main zones in the test solution and reference solution (a) should have comparable retention times (comparable heights). Furthermore, in the chromatogram obtained with the test solution, other faint to very faint fluorescent zones, which may be orange or red-violet, may be present, especially above the red zones due to CBD and d9THC.

Top of the plate	
	Orange fluorescent zone (CBD) Red-violet fluorescent zone (d9THC)
Red fluorescent zone (THC-A) Orange fluorescent zone (CBD-A)	Red fluorescent zone (THC-A)[1, 2] Orange fluorescent zone (CBD-A)[2, 3]
Reference solution	Test solution

[1] Main zone for *Type high THC;*
[2] Main zone for *Type THC/CBD*;
[3] Main zone for *Type high CBD*.

TESTS

Loss on drying (2.2.32): maximum 10.0 per cent; determined on 500 mg (grinded) herbal drug in vacuo. The drying is carried out over about 100 g of phosphorus pentoxide for 24 h at a pressure between 1.5 and 2.5 kPa, at 40°C.

Foreign material: Free from insects and other vermin. Determine visually by inspection with an unaided eye on a suitable amount herbal drug in a beaker or watch glass.

Aflatoxins:
Test solution (a). Place 3.75 g (grinded) herbal drug in a 50 mL centrifuge tube. Add 10.0 mL purified water R and 7.3 mL acetonitrile R. Add 200 µL *Reference solution ISTD (TPhP)* to the mixture and mix for 1 minute at 2500 rpm. Add the content of 1 QuEChERS tube and mix for 1 minute at 2500 rpm. Centrifuge the mixture at 4600 rpm for 5 minutes. Take 4 mL of the upper layer and add to QuEChERS clean up tube 3. Mix for 1 minute at 2500 rpm. Centrifuge for 5 minutes at 4600 rpm. Filter the mixture over a 0.45 µm PFTE filter into a 15 mL centrifuge tube.

Reference solution AF (a). Dissolve 200 µL of AFB1, 200 µL of AFB2, 200 µL of AFG2 and 50 µL of AFG1 in a 5 mL volumetric flask in acetonitrile R.
Reference solution AF (b). Dilute 2.5 mL of *reference solution (a)* with acetonitrile R to 10 mL.
Reference solution AF (c). Dilute 500 µL of *reference solution (a)* with acetonitrile R to 10 mL.
Reference solution ISTD (TPhP). Dilute 125 µL QuEChERS standard with acetonitrile R to 10 mL.

Std Add 0. Add to a suitable LC-MS/MS vial 800 µL of *test solution (a)* and 200 µL acetonitrile R.
Std Add 1. Add to a suitable LC-MS/MS vial 200 µL of *Reference solution AF (c)*, 1000 µL of *test solution (a)*, 50 µL acetonitrile R (is equal to 1.60 ng/mL *of AF* in solution and 4.0 µg/kg *AF in product*).
Std Add 2. Add to a suitable LC-MS/MS vial 64 µL of *Reference solution AF (b)*, 800 µL of *test solution (a)*, 136 µL acetonitrile R (is equal to 3.20 ng/mL *AF in solution* and 8.0 µg/kg *AF* in product).

Column:
- *size*: *l* = 0.05 m, Ø = 2.1 mm;
- *stationary phase*: octadecylsilyl silica gel for chromatography R (1.8 µm);
- *temperature*: 35 °C.

Mobile phase:
- *mobile phase A*: 315 mg/L ammonium formate R in a solution of ultra-pure water LCMS and formic acid R (1.0 per cent; *V/V*)
- *mobile phase B*: methanol ULC.

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 0.7	90	10
0.7 – 1.0	90 – 80	10 – 20
1.0 – 2.3	80 – 76	20 – 24
2.3 – 3.5	76 – 70	24 – 30
3.5 – 4.5	70 – 40	30 – 60
4.5 – 5.0	40 – 30	60 – 70
5.0 – 8.0	30 – 25	70 – 75
8.0 – 8.1	25 – 5	75 – 95
8.1 – 12.1	5 – 90	95 – 10
12.1	90	10

Post run time: 1.50 min.
Flow rate: 0.3 mL/min.
Detection: MRM.
Injection: 4 µL.

System suitability:
- Sensitivity check solution: The S/N of all Aflatoxins should be ≥ 3;
- The correlation coefficient of the Standard addition should be $R^2 \geq 0.9$ for all samples.

Calculation:

$$FC\ AF = CC\ AF * \left(\frac{1000}{800}\right) * \left(\frac{7.5}{S\ weight}\right)$$

FC AF	=	Final concentration aflatoxin (µg/kg);
CC AF	=	Calculated concentration aflatoxin (ng/mL);
1000/800	=	Dilution factor;
7.5	=	Total volume acetonitrile R;
S weight	=	Sample weight (g).

Aflatoxin	Limit (µg/kg)
Aflatoxin B_1	≤ 2
Sum of aflatoxins B_1, B_2, G_1 and G_2	≤ 4

Pesticides (2.8.13): Limits according to EP 2.8.13; determined on 2 g (grinded) herbal drug.

Test solution (a). Depending on matrix effects choose one of the methods below:
Method 1: Place 2 g (grinded) herbal drug in a 50 mL centrifuge tube. Add 10.0 mL ISTD solution. Extract the sample in slight acidic conditions with analyte protector to prevent degradation and extract and clean-up the sample using QuEChERS.
Method 2: Place 2 g (grinded) herbal drug in a 50 mL centrifuge tube. Add 5.0 mL purified water R and allow to stand for 30 minutes. Add 10.0 mL ISTD solution. Extract the sample in slight acidic conditions with analyte protector to prevent degradation and extract the liquid phase with a SPE column filtration and purify sample using DSPE column.

Heavy metals (2.4.27)(2.2.58): Determined on 0.2 g (grinded) herbal drug.

Test solution. Add to the substance to be examined 10 mL nitric acid R. Destruct the mixture in the microwave and fill up to 50 mL with purified water R.

Lead: maximum 20.0 ppm;
Cadmium: maximum 0.5 ppm;
Mercury: maximum 0.5 ppm.

ASSAY AND RELATED SUBSTANCES (2.2.29)

Prepare test solution a and b in duplicate.

Test solution (a). Place 1000 mg (grinded) herbal drug and 40 mL ethanol R in a falcon tube. Mix well at ca. 300 rpm for 15 minutes. Take the clear upper layer, filtrate the extract over a 0.45 µm PTFE filter in transfer into a 100 mL volumetric flask.
Repeat the extraction step twice with 25 mL ethanol R and add the upper layer to the previous obtained extract in the 100 mL volumetric flask.
Dilute to 100 mL with ethanol R.
Dilute 1.0 mL of the filtrate with acetonitrile R to 10mL.
Test solution (b). Dilute 1mL of test solution (a) with acetonitrile to 10 mL.

Reference solution (a). Dissolve 50 mg TBA standard R in acetonitrile R in a 100 mL volumetric flask.
Reference solution (b). Dilute 0.4 mL with acetonitrile R to 100 mL.

Resolution Solution: Make a mixture containing Δ^9-THC and Δ^8-THC, wherein the ratio of Δ^9-THC / Δ^8-THC should be 0.04 mg/mL and 0.0025 mg/mL respectively. This solution can be prepared for example by the following method: Dilute 400 µL Δ^9-THC Stock Standard Solution (i.e. 1mg / mL Δ^9-THC in Ethanol) and 25 µL Δ^8-THC Stock Standard Solution (i.e. 1 mg / mL Δ^8-THC in Ethanol) with acetonitrile R in a volumetric flask of 10 mL.

Column:
- *size*: *l* = 0.100 m, Ø = 3 mm;
- *stationary phase*: octadecylsilyl silica gel for chromatography R (2.5 µm);
- *temperature*: 20 °C.

Tray temperature: 5 °C.

Mobile phase:
- *mobile phase A*: phosphoric acid R in water for chromatography R (0.1 per cent; *V/V*).
- *mobile phase B*: phosphoric acid R in acetonitrile R (0.1 per cent; *V/V*)

Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 – 1.0	35	65
1.0 – 17.5	35 – 30	65 – 70
17.5 – 27.35	30 – 0	70 – 100
27.35 – 27.45	0 – 35	100 – 65
27.45 – 32.5	35	65

Flow rate: 0.4 mL/min.
Detection: Spectrophotometer at 228 nm.
Equilibration: 30 min.
Injection: 3 µL.

Elution order:

Component	**Relative retention time (RRT)**	**Relative response factor (Rf)**
CBD-A	0.79	1.91
CBD	0.94	1.00
TBA	1.00	1.00
CBN	1.59	2.30
d9THC	1.97	0.91
CBN-A	2.15	1.50
THC-A	2.53	1.66

System suitability:
- The RSD of 6 injections of *Ref (b)* (peak area TBA) is not more than 2.0 per cent;
- The resolution between the peaks of Δ^9-THC and Δ^8-THC of the *resolution solution* is not less than 1.2;
- The TBA peak of the *Ref (b)* has a S/N (USP) value not less than 10;

Calculate the percentage content of the components using the following expression:

$$X\ (per\ cent) = 100 * \frac{Am * Vm * Ws * Vf}{Rf * As * Vs * Wm} * \frac{100}{100 - LOD}$$

X	=	*Component;*
Am	=	area of the test solution as indicated in the table below;
Vm	=	volume sample (mL);
Ws	=	weight of TBA reference (mg);
Vf	=	dilution factor (10 or 100);
Rf	=	response factor;
As	=	area of *Ref. (a);*
Vs	=	volume of TBA reference standard (mL);
Wm	=	sample (mg);
LOD	=	Loss on drying (per cent).

	Type high THC	***Type THC/CBD***	***Type high CBD***
CBDA	Test solution A	Test solution B	Test solution B
CBD	Test solution A	Test solution A	Test solution A
THCA	Test solution B	Test solution B	Test solution A
THC	Test solution A	Test solution A	Test solution A
CBNA	Test solution A	Test solution A	Test solution A
CBN	Test solution A	Test solution A	Test solution A

Calculation assay
Calculate the percentage of the total equivalents of the THC an CBD using the following expressions:

$$THC\ total\ euiqvalents\ (per\ cent) = per\ cent\ THCA * 0.877 + per\ cent\ d9THC$$
$$CBD\ total\ equivalents\ (per\ cent) = per\ cent\ CBDA * 0.877 + per\ cent\ CBD$$

Calculation related substances
CBN: maximum 1.0 per cent of total equivalence cannabinol expressed as CBN ($C_{21}H_{26}O_2$; M_r 310.4) (dried drug).

Calculate the percentage of the total equivalents cannabinol using the following expression:

$$CBN\ total\ equivalents\ (per\ cent) = per\ cent\ CBNA * 0.876 + per\ cent\ CBN$$

MICROBIOLOGICAL QUALITY

Microbiological quality will be analysed after gamma irradiation of the material.

Microbial contamination (2.6.12)(2.6.13):

Test solution (a): Add 290 mL neutralization solution (DE/NF) to 10 g (grinded) herbal drug.
TAMC: Add 1mL of test solution (a) to 9 mL sodium chloride peptone solution with 0.1 % Tween 80.
TYMC: Filter 5 mL of test solution (a) through a 5 µm filter.
Transfer a suitable amount of the prepared sample to the membrane filter.

Parameter	Acceptance criteria
Total aerobic microbial count (TAMC)	≤ 10^2 CFU/gram
Total combined yeasts/moulds count (TYMC)	≤ 10^1 CFU/gram
Staphylococcus aureus	Absent (1 gram)
Pseudomonas aeruginosa	Absent (1 gram)
Bile-tolerant gram-negative bacteria	Absent (1 gram)