Cannabis Species Inflorescence

Proposed For Comment Version 0.1

Briefing

Based on the comment received, *Cannabis* Species Inflorescence monograph has been revised as follows.

1. The Identification section has been revised to clarify that the requirements are met by complying with the *Identification A* for botanical characteristics (macroscopic and microscopic features), and one of the following: *Identification B* for HPTLC, *Identification C* for HPLC, or *Identification D* for GC.
2. A new method has been included for the *Loss on Drying*. The method is performed under vacuum at 40° for 24 h to avoid potential loss and other degradation not related to loss of water from decarboxylation of acidic cannabinoids and loss of terpenes. *Loss on Drying* is included as a *Specific Test* and serves as an independent quality parameter.
3. The proposal does not require "on the dried basis" for expression of cannabinoid content in the *Definition* and *Content of Cannabinoids* sections because the term "dried basis" is subject to interpretation differently by different groups, potentially leading to inflation of the labeled values of cannabinoids. This monograph proposes that the quantitative analysis in *Content of Cannabinoids* is performed on the "as is basis" at specified water activity level.
4. The specifications for microbial contaminants have been rearranged in the *Contaminants* section to provide *Option 1*, which specifies the limits for total aerobic counts, total yeast and mold, and specified microorganisms using USP general chapter <61> and general chapter <62>; and *Option 2*, which includes stringent limits for total aerobic counts, total yeast and mold, and specified microorganisms using general chapter <1111> and general chapter <62>. The latter represent more stringent requirements intended for products labeled to indicate to healthcare practitioners and patients that such products (particularly for inhalation products) have a reduced microbial load.
5. The *Content of Terpenes* section has been removed from *Specific Tests* (proposed in an earlier version) because there is insufficient information to support the use of terpenes for medical purposes.
6. Stakeholder comments and input are invited on the above revisions and other sections of the revised proposal.

Key Information

LATEST VERSION:
Proposed For Comment Version 0.1
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Visual Representation of Chromatographic Identification Procedures.pdf
View Comments and Responses
COMMENT END DATE:
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Cannabis Species Inflorescence[1]

DEFINITION

The article consists of the pistillate (female) inflorescence of *Cannabis sativa* L. (Fam. Cannabaceae) and its subspecies, varieties, and chemotypes or to all species of *Cannabis*, depending on how their taxonomy is interpreted[2]. The article is derived from homogeneous cultivars to contain NLT 80% and NMT 120% of the labeled amount (in mg/g) of the total tetrahydrocannabinol (THC) including Δ^9-THC and THCA; and NLT 80% and NMT 120% of the labeled amount (in mg/g) of the total cannabidiol (CBD) including CBD and CBDA. For the THC-dominant chemotype, the ratio of total THC content to total CBD content is NLT 5:1, and the chemotype contains NMT 10 mg/g total CBD and NLT 10 mg/g total THC. For the CBD-dominant chemotype, the ratio of total THC content to total CBD content is NMT 1:5, and the chemotype contains NMT 10 mg/g total THC and NLT 10 mg/g total CBD. For THC/CBD intermediate chemotype, the ratio of total THC content to total CBD content is NLT 0.2:1 and NMT 5:1, with NLT 10 mg/g total CBD and NLT 10 mg/g total THC.

CONSTITUENTS OF INTEREST

Cannabinoids: Delta-9-Tetrahydrocannabinol (Δ^9-THC), delta-8-tetrahydrocannabinol (Δ^8-THC), tetrahydrocannabinolic acid (THCA), tetrahydrocannabivarin (THCV), tetrahydrocannabivarinic acid (THCVA), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), cannabigerol (CBG), cannabinol (CBN), cannabichromene (CBC), cannabigerolic acid (CBGA)
Terpenes: β-Caryophyllene, ᴅ-limonene, β-myrcene, α-pinene, terpinolene

IDENTIFICATION

[Note—Perform *Identification A* and one of the following: *Identification B*, *Identification C*, or *Identification D*.]

• A. BOTANICAL CHARACTERISTICS

Macroscopic: *Cannabis* material of commerce consists of 1.5- to 15-cm or longer branches and branchlets, sometimes broken up, of the dried inflorescences of pistillate plants. The pistillate inflorescence is a small, obscure, congested, axillary spicate cyme of approximately 1–5 cm in length and in width, with slightly protruding bracts. The inflorescence segments, colloquially known as “buds”, are often closely trimmed by hand or machine, sometimes leaving portions of the leaf bases and stiff petioles. The segments are generally light to dark green, various shades of purple to dark purple, or greenish brown to brown and may include whole, or fragments of, reduced upper leaves, stems, bracts, bracteoles, rudimentary calyxes , immature ovules, styles, and glandular and nonglandular trichomes. When present, the upper leaves are light to dark green, sometimes purple or mottled brown, dried and shriveled, and sometimes clasping the inflorescence.

The bracts are light to dark green or brownish green, scabrous (rough) due to the presence of trichomes; numerous, alternate, and with overlapping edges, with narrow stipules at the base; simple and others tripartite, but in both cases the segments are lanceolate with an acuminate apex and a serrated or entire margin. Bracts subtending the spikes are often divided into five linear leaflets. Those subtending the individual flowers usually have three minute leaflets. The bracts enclose the female flower except for the exserted stigmas. Bracts and stipules both show a marked tendency to shrivel upon drying, and in some cases only the veins of the bracts remain intact. The bract’s proximal upper surface is densely covered by capitate, stalked, glandular trichomes, which are absent from the distal region. Numerous nonglandular trichomes are also seen. The perigonal bracts, also called “bracteoles” or “floral bracts”, are light to dark green or brownish green, sometimes purple to red and may be mottled or streaked and 4–8 mm long, formed in pairs in the axil of a bract. They are ovate with an acute apex; the entire margin, incurved and fused at the base to form a conical cuplike sheath that completely envelops the ovary and loosely encloses the mature fruit; and densely hispid or pilose. Numerous resinous glandular trichomes and nonglandular trichomes are observed on the abaxial (outer or lower) surface. In sinsemilla production where seeds do not develop due to prevention of pollination, the perigonal bracts remain quite small and are very densely covered with glandular trichomes. The very short pedicel bears a bract that subtends the perigonal bract.

A single flower approximately 5–10 mm long is formed in the axil of each perigonal bract, thus appearing essentially sessile. As the perigonal bracts are in pairs, the pistillate flowers also appear in pairs. The true perianth develops from the base of the ovary, initially divergent from the developing ovary but soon adhering closely to it and covering about two-thirds of the ovary at maturity with a hyaline membrane, simple, smooth, or slightly fringed along the margin, often marbled by patches of pigmented cells. The flower has a superior ovary with a short apical style with two caducous, long filiform stigmatic branches (styles are often three-branched in sinsemilla material) spreading at the apex and projecting well above the bracteole, densely covered with long club-shaped papillae. The style plus stigma is up to 1 cm in length and dark reddish-brown to orange. The fruit (often referred to as the seed) is an achene, but when grown under the sinsemilla cultivation technique, exposure to pollen is prevented so the pistillate flowers remain unfertilized throughout maturity and consequently do not develop achenes.

Microscopic: Transverse sections of the leaflets and bracts show a dorsiventral structure. The palisade consists of a single layer (rarely two layers) of cylindrical cells and the spongy tissue of 2–4 layers of rounded parenchyma; cluster crystals of calcium oxalate are present in all parts of the cortical parenchyma tissues of the mesophyll and palisade layer, but not in the trichomes, and may be seen scattered in powdered material. The upper epidermal cells bear unicellular, sharply pointed, curved, conical trichomes, approximately 150–220 µm long, with enlarged bases containing cystoliths of calcium carbonate; the lower epidermis bears conical trichomes, which are longer, approximately 340–500 µm, and slenderer, without cystoliths. Both upper and lower epidermises bear numerous glandular trichomes, which are especially abundant on the underside over the midrib. Both upper and lower epidermises in the midrib region are followed by a few layers of collenchyma. The vascular bundle is composed of phloem, made up of small cells, and xylem vessels arranged in radial rows. The lower epidermis displays numerous trichomes of three types: nonglandular, nonglandular cystolithic, and glandular. Bracteoles have an undifferentiated mesophyll of about four layers of cells, the lower hypodermal layer having a cluster crystal of calcium oxalate in almost every cell. The abaxial surface bears numerous bulbous, sessile, and stalked glandular trichomes as well as unicellular conical trichomes. These trichomes are most numerous where the bracteole curves in to enclose the flower or fruit.

Cannabis is characterized by the simultaneous presence of cystolithic trichomes on the upper surface and noncystolithic trichomes and sessile glandular trichomes on the lower surface of the leaflets.

Glandular trichomes are present mainly in three forms: 1) capitate sessile with a multicellular head but no visible stalk; 2) bulbous with a short stalk; and 3) capitate stalked with a multicellular head and multicellular stalk. Capitate sessile glandular trichomes are the most abundant type, occurring on all aerial epidermal surfaces of mature plants. They are especially abundant on the underside surface of leaves and bracts and on the abaxial surface of perigonal bracts. They are actually not sessile—the stalk is 1 cell high and 2–4 cells thick, but it is hidden beneath the glandular head, comprised of 8 (or more) secretory cells radially arranged into a disc, above which the resin chamber forms. The glandular head is typically 30–70 µm in diameter and approximately 15– 20 µm high, but two sizes can be distinguished: larger ones are found on the pistillate flowers, and smaller ones are found on pistillate flowers, leaves, and stems. The glandular head's resin and terpenoid-rich essential oil contents are clear during early stages of development but become opaque white with maturity and eventually age to become orange– brown. Detached capitate sessile glands can be seen in commercial samples. Bulbous glandular trichomes, approximately 10–20 µm in diameter and 15–30 µm high, are also widespread on all epidermal surfaces of the aerial parts, with the highest density on stems and the lowest on the bracts. Most have a 2-celled head and a stalk that appears to be 1 or 2 cells long and 1 or 2 cells thick, but the structure is variable. The glandular head may be simple and spherical or complex and multicompartmented, varying in size from 1–4 secretory cells. Capitate, multicellular, stalked, glandular trichomes are generally abundant, forming a pubescence on both upper and lower epidermal surfaces of the petioles, bracts, and mainly on the abaxial surface of the perigonal bracts. The stalks can be about 200 µm long. The secretory cell disk at the base of the glandular head, about 30 µm in diameter and 15 µm in height, forms a dome-shaped to eventually spherical resin chamber. The chamber is typically 50–70 µm in diameter but may be as large as 129 µm in some high-THC cultivars compared to 80 µm in industrial hemp cultivars. As the trichomes age, it is common for the resin head to become detached from the stalk at an abscission region, leaving the trichome with a tip that is reddish due to the presence of flavonoids. Fragments of multicellular glandular trichomes, including portions of the stalk, are also seen in commercial samples.

Nonglandular trichomes are unicellular, with major types distinguished by differences in size and location. Stigmas have pollen-trapping, club-shaped trichomes (papillae) approximately 90–180 µm long with rounded ends, which often become detached and are found scattered in the powder of commercial samples. *Cannabis* pollen may be present occasionally, adhering to the stigmas. Pollen grains are about 25-30 µm in diameter, spherical, the tectum bending vertically to form 3 sunken pores (maybe 2 or 4 pores in the smaller pollen grains of monoecious varieties) arranged at equal intervals around the equator, and have a smooth exine. The other two types of non-glandular trichomes are conical and highly silicified all over. The trichomes located on or near the major veins have a verrucose surface, i.e., with warts of cellulose and cutin, whereas those occurring between the veins have only a slightly warty or smooth surface. Simple, slender, unicellular noncystolithic trichomes (covering trichomes) approximately 250–370 µm long (some as long as 500 µm) are abundant on the stems and the abaxial surface of leaves, bracts, and, to a lesser extent, on the perigonal bracts forming a pubescence, much less abundant on the upper surface; they are lying almost flat and oriented toward the distal end. Those on the adaxial (inner or upper) surface of the perigonal bract have smooth surfaces and are completely flattened; some have a more cylindrical shape. On the abaxial surface, some are fairly short, rigid, verrucose, slightly enlarged at the base and abruptly tapering to the apex, such as those on the lower epidermis of the perigonal bract, while others, such as those on the veins and the edges of the perigonal bracts, are larger, more elongated, only some with an enlargement at the base, and gradually tapering to the apex. Some of the covering trichomes are bent and some have branches that join (anastomose) with neighboring trichomes. Detached fragments of covering trichomes are seen in commercial samples. Cystolithic trichomes, approximately 150–220 µm long, sharply pointed, not exceptionally enlarged at the base, often with a distinctly thickened, verrucose wall, are found mainly on the adaxial surface of the leaf, always pointing to the distal end, giving the surface a rough texture. Very short (approximately 50–125 µm) cystolithic trichomes with a much-enlarged base and with smooth or verrucose surfaces, are found on the adaxial surface of the bract; some are also found on the abaxial surface of the perigonal bracts. At the base of each cystolithic trichome is a cystolith, a well-defined concretion of calcium carbonate (sometimes described as grapelike in shape). Detached fragments of cystolithic trichomes with a warty surface are seen in commercial samples. The presence of silica on the outer wall surface, as well as cystoliths of calcium carbonate, which persist even after *Cannabis* material has been burned to ash, makes nonglandular trichomes useful in identification.

B. HPTLC for Articles of Botanical Origin <203>

Standard solution A: Neutralize by mixing USP Cannabinoid Acids Mixture RS with 98% formic acid (4:1).

Standard solution B: Use USP Cannabinoids Mixture RS.

Standard solution C: Prepare a 0.25-mg/mL solution of USP Delta-9-Tetrahydrocannabinol RS in acetonitrile.

Standard solution D: Prepare 1-mg/mL solution of USP Cannabidiol RS in methanol and dilute with acetonitrile to 0.25 mg/mL. Alternatively, prepare a 0.25-mg/mL solution of USP Cannabidiol Solution RS in acetonitrile.

Sample solution: Transfer 500 mg of *Cannabis* Species Inflorescence, finely powdered and homogenized, into a centrifuge tube and add 5 mL of a mixture of methanol and hexane (9:1, v/v). Vortex the solution for 10 s and sonicate for 15 min with intermittent vortex for 10 s every 5 min. Centrifuge and cool to room temperature. Transfer 1.0 mL of the supernatant into low-actinic vials.

Decarboxylated sample solution: Evaporate 1vial of *Sample solution* to dryness under a stream of nitrogen. Tightly close the vial and heat in an oven at 200° for 15 min. Cool to room temperature. Reconstitute the residue using 1 mL of a mixture of methanol and hexane (9:1, v/v), then vortex to mix.

Chromatographic system

(Use standard parameters as defined in HPTLC for Articles of Botanical Origin <203>, *Table 1*)

Stationary phase: Reversed phase C18 plate with an average particle size of 5 µm. [Note—Pre-wash the plate by developing in methanol then dry at 120° for 30 min before use.]

Application volume: 2 µL of each of the *Standard solutions* and of the *Sample solution*; as 8-mm bands, 8 mm from the bottom of the plate

Developing solvent system: Methanol, water, and glacial acetic acid (80:10:10)

Derivatization reagent: Vanillin–sulfuric acid prepared by dissolving 1 g of vanillin in a 100-mL mixture of 95% ethanol and 96% sulfuric acid (98:2)

Analysis

Samples: *Standard solutions A–D*, *Sample solution*, and *Decarboxylated sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop the chromatograms in a saturated chamber. Treat the plates with the *Derivatization reagent*, heat at 100° for 3 min, and examine under white light.

System suitability: *Standard solutions A–D* shows the cannabinoid bands as follows, in the order of increasing R_F:

tetrahydrocannabinolic acid (THCA), cannabichromene (CBC), tetrahydrocannabivarinic acid (THCVA), delta-8-tetrahydrocannabinol (Δ^8-THC), delta-9-tetrahydrocannabinol (Δ^9-THC), cannabinol (CBN), cannabigerolic acid (CBGA), tetrahydrocannabidivarin (THCV), cannabidiolic acid (CBDA), cannabidiol (CBD), cannabigerol (CBG), cannabidivarinic acid (CBDVA), and cannabidivarin (CBDV).

Acceptance criteria: The *Sample solution* of the *Cannabis* Species Inflorescence labeled as THC-dominant chemotypes shows the most-intense band corresponding to THCA in *Standard solution A* and the weak or absence of bands corresponding to CBD and CBDA. The *Sample solution* of *Cannabis* Species Inflorescence labeled as CBD-dominant chemotype shows the most-intense band corresponding to CBDA in *Standard solution A* and the weak or absence of bands corresponding to THC and THCA. The *Sample solution* of *Cannabis* Species Inflorescence labeled as a THC/CBD intermediate chemotype shows intense bands corresponding to Δ^9-THC/THCA and CBD/CBDA of similar intensity. The *Decarboxylated sample solution* of THC-dominant and CBD-dominant chemotypes shows bands corresponding to the THC or CBD, respectively, and a complete absence of bands corresponding to THCA and CBDA, respectively. The *Decarboxylated sample solution* of a THC/CBD intermediate chemotype shows bands corresponding to the THC and CBD and the complete absence of THCA and CBDA bands.

C. HPLC

Analysis: Proceed as directed in the *Content of Cannabinoids, Procedure 1*.

Acceptance criteria: The chromatographic pattern for a THC-dominant chemotype exhibits the principal peak for THCA corresponding in retention time to the peak for the compound in *Standard solution C*. The chromatographic pattern for a CBD-dominant chemotype exhibits the principal peak for CBDA corresponding in retention time to the peak for the compound in *Standard solution C*. The chromatographic pattern for a THC/CBD intermediate chemotype shows two principal peaks: one for THCA and one for CBDA, corresponding in retention times to the peaks for the compounds in *Standard solution C*.

D. Gas Chromatography

Analysis: Proceed as directed in the *Content of Cannabinoids, Procedure 2*.

Acceptance criteria: The chromatographic pattern for a THC-dominant chemotype exhibits the principal peak for THCA corresponding in retention time to the peak for the compound in *Standard solution C*. The chromatographic pattern for a CBD-dominant chemotype exhibits the principal peak for CBDA corresponding in retention time to the peak for the compound in *Standard solution C*. The chromatographic pattern for a THC/CBD intermediate chemotype shows two principal peaks: one for THCA and one for CBDA, corresponding in retention times to the peaks for the compounds in *Standard solution C*.

ASSAY

• **Content of Cannabinoids:** Perform either *Procedure 1* or *Procedure 2*. [Note—*Procedure 1* correlates with *Identification C* and *Procedure 2* correlates with *Identification D*.]

Procedure 1

Solution A: 0.1% formic acid (98%) in water

Solution B: 0.1% formic acid (98%) in acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	26	74
3.5	26	74
6.5	15	85
7.0	15	85
7.01	26	74
8.5	26	74

Standard solution A: Prepare a 0.1-mg/mL solution of USP Delta-9-Tetrahydrocannabinol RS by diluting with methanol (1:10).

Standard solution B: Prepare a 0.1-mg/mL solution of USP Cannabidiol Solution RS by diluting with methanol (1:10). [Alternatively, use a 1-mg/mL solution of USP Cannabidiol RS in methanol and dilute with methanol (1:10).]

Neutralized cannabinoid acids mixture: Mix USP Cannabinoid Acids Mixture RS with 98% formic acid (4:1).

Standard solution C: Combine equal amounts of USP Cannabinoids Mixture RS and *Neutralized cannabinoid acids mixture*.

Sample solution: Transfer 0.5 g of *Cannabis* Species Inflorescence, finely powdered and accurately weighed, into a 50-mL conical vial containing an 11-mm stainless steel ball bearing. Add 20 mL of methanol and place the sealed conical vial in a high-throughput homogenizer for 1 min at 1500 rpm. Allow the conical vial to cool to room temperature. Dilute 1 in 20 with methanol and mix well. Filter through a submicron pore filter if necessary.

To compensate for dilution and solution transference errors, a 1-mg/mL solution of butyl-4-hydroxybenzoate in methanol can be used as an internal standard instead of methanol to dissolve the Reference Standards and as an extraction solvent for the inflorescence. In such case, the calculation would need to include the peak response ratio of the internal standard (the reference peak) divided by the response factor of the analyte (response factor = peak area/concentration).

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 222 nm

Column: 4.6-mm × 15-cm, 2.7-µm C18, hard core with superficially porous shell, L1 (similar to Restek Raptor ARC-18)

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 5 µL

System suitability

Samples: *Standard solution A* and *Standard solution C*

Suitability requirements

Verification of UV max: The maximum of absorbance of the UV spectrum at the apex of the peak for CBDA is 222 ± 2 nm, *Standard solution C*.

Resolution: NLT 1.0 between CBG and CBD, and NLT 1.0 between Δ^9-THC and Δ^8-THC, *Standard solution C*

Tailing factor: NMT 2.0 for Δ^9-THC, *Standard solution A*

Relative standard deviation: NMT 2.0% for Δ^9-THC in repeated injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution C* is similar to the chromatograms provided with the lots of USP Cannabinoid Acids Mixture RS and USP Cannabinoids Mixture RS being used.

Analysis

Samples: *Standard solutions A–C* and *Sample solution*

Using the chromatograms of *Standard solutions A–C*, identify the retention times of the peaks corresponding to each of the cannabinoids in the *Sample solution* chromatogram. See *Table 2* for the relative retention times and conversion factors against CBD.

Table 2

Analyte	Relative Retention Time	Conversion Factor
CBDVA	0.62	0.68
CBDV	0.68	0.94
CBDA	0.85	0.70
CBGA	0.89	0.69
CBG	0.94	0.99
CBD	1.00	1.00
THCV	1.06	1.03
THCVA	1.35	0.68
CBN	1.43	0.52
Δ^9-THC	1.77	1.03
Δ^8-THC	1.82	1.21
CBC	2.04	0.67
THCA	2.17	0.73

[Note—1) As variability in relative response can occur between HPLC instruments, laboratories should verify the system suitability with regard to accuracy of wavelength for maximum absorbance and establish response factors for each instrument. 2) The conversion factors in *Table 2* can be used as a guide. Conversion factors are derived by dividing the response factor of CBD (the reference peak) by the response factor of the analyte.]

Calculate the amount of each cannabinoid, in mg/g, in the portion of *Cannabis* Species Inflorescence taken:

Result = $(r_U/r_S) \times C_S \times (V/W) \times F$

r_U = peak response of the cannabinoid from the *Sample solution*

r_S = peak response of CBD from *Standard solution B*

C_S = concentration of CBD in *Standard solution B* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of *Cannabis* Species Inflorescence taken to prepare the *Sample solution* (g)

F = conversion factor for the analyte (see *Table 2*)

Calculate total THC, in mg/g, as follows:

Result = (THCA × 0.877) + Δ^9-THC

Calculate total CBD, in mg/g, as follows:

Result = (CBDA × 0.877) + CBD

Calculate the percentage of the labeled amount of cannabinoids in the portion of *Cannabis* Species Inflorescence taken:

Result = $(P/L) \times 100$

P = content of total THC or total CBD as determined previously (mg/g)

L = labeled amount of cannabinoids (mg/g)

Acceptance criteria

THC-dominant chemotype

- It contains NLT 80% and NMT 120% of the labeled amount of the total THC (mg/g).
- The ratio of the total THC content to total CBD content is NLT 5:1, containing NMT 10 mg/g of total CBD and NLT 10 mg/g of total THC.
- It contains NLT 80% and NMT 120% of the labeled amount of all other cannabinoids measured (mg/g). *Cannabis* Species Inflorescence must be labeled with the name and amount of any cannabinoid present in an amount of 10 mg/g or more.
- The content of CBN is NMT 2% of the total content of THC. No unidentified peak in the *Sample solution* chromatogram exceeds the area of the CBN peak.

CBD-dominant chemotype

- It contains NLT 80% and NMT 120% of the labeled amount of the total CBD (mg/g).
- The ratio of the total THC content to total CBD content is NMT 1:5, containing NMT10 mg/g of total THC, and NLT 10 mg/g of total CBD.
- It contains NLT 80% and NMT 120% of the labeled amount of all cannabinoids measured (mg/g). *Cannabis* Species Inflorescence must be labeled with the name and amount of any cannabinoid present in an amount of 10 mg/g or more.

THC/CBD intermediate chemotype

- It contains NLT 80% and NMT 120% of the labeled amount of the total THC and total CBD (mg/g).
- The ratio of the total THC content to total CBD content is NLT 0.2:1 and NMT 5:1, containing NLT 10 mg/g of total THC and NLT 10 mg/g of total CBD.
- It contains NLT 80% and NMT 120% of the labeled amount of all cannabinoids measured (mg/g). *Cannabis* Species Inflorescence must be labeled with the name and amount of any cannabinoid present in an amount of 10 mg/g or more.

- The content of CBN is NMT 2% of the total content of THC. No unidentified peak in the *Sample solution* chromatogram exceeds the area of the CBN peak.

B. Procedure 2

Extraction solution: Acetonitrile and methanol (8:2)

Internal standard solution: 50 µg/mL of 4-androstene-3,17-dione in *Extraction solution*

DMAP solution: Prepare a 2% dimethylaminopyridine (DMAP) solution by dissolving 2 g of DMAP in 100 mL of methanol.

Standard solution A: Dilute USP Delta-9-Tetrahydrocannabinol RS with methanol to 20 µg/mL.

Standard solution B: Prepare a 20-µg/mL soluton of cannabidiol in methanol from either USP Cannabidiol Solution RS or USP Cannabidiol RS.

Standard solution C: Combine 400 µL of USP Cannabinoid Acids Mixture RS and 400 µL of USP Cannabinoids Mixture RS in a 4-dram vial.

Sample solution: Transfer 300 mg of *Cannabis* Species Inflorescence, finely powdered, to a centrifuge tube and add 2.5 mL of *Extraction solution*. Sonicate for 20 min and centrifuge for 5 min at 4000 rpm. The extraction is repeated four times and all supernatants are combined into a 10-mL volumetric flask. Add *Extraction solution* to volume.

Derivatization solutions: Prepare derivatization solutions for *Standard solutions A–C* and for the *Sample solution* as follows:

1. For *Standard solution A* and *Standard solution B*, add 10 µL of *DMAP solution* and 50 µL of *Internal standard solution*.
2. For *Standard solution C*, transfer 8 µL to a derivatization vial, add 100 µL of *DMAP solution* and 500 µL of I*nternal standard solution*.
3. For the *Sample solution*, transfer 10 µL to a derivatization vial and add 10 µL of *DMAP solution* and 50 µL of *Internal standard solution*.

Vortex each derivatization solution and evaporate to dryness under stream of nitrogen at 50º. Add 100 µL of *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) to the residues and heat for 30 min at 70º. Cool the vial to room temperature before injection.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: FID

Column: 0.25-mm × 15-m fused silica capillary; 0.25-µm film of G2 phase coating (Agilent DB-1ms or similar)

Temperatures

Injector: 275º

Detector: 300º

Column: See *Table 3*.

Table 3

Initial Temperature (º)	Temperature Ramp (º/min)	Final Temperature (º)	Hold Time at Final Temperature (min)
190	—	190	1
190	30	230	2
230	5	250	1
250	20	300	2.75
190	—	190	2.95

Carrier gas: Helium

Flow rate: 0.8 mL/min

Injection volume: 3 µL

Mode: Split injection, 20:1

System suitability

Samples: *Standard solutions A–C*

Suitability requirements

Resolution: NLT 1.0 between any two cannabinoid peaks, *Standard solution C*

Relative standard deviation: NMT 2.0% for the Δ^9-THC and CBD peaks, *Standard solution A* and *Standard solution B*

Chromatogram similarity: The chromatogram of *Standard solution C* is similar to the reference chromatogram provided with the lots of USP Cannabinoid Acids Mixture RS and USP Cannabinoids Mixture RS being used.

Analysis

Samples: *Standard solutions A–C* and *Sample solution*

Using the chromatogram of *Standard solutions A–C,* identify the retention times of the cannabinoid peaks corresponding to each cannabinoid in the *Sample solution* chromatogram. See *Table 4* for the relative retention times against the internal standard.

Table 4

Analyte	Relative Retention Time
CBDV	0.43
THCV	0.48
CBD	0.63
CBC	0.69
Δ^8-THC	0.71
Δ^9-THC	0.74
CBDVA	0.77
CBG	0.81
CBN	0.83
THCVA	0.90
CBDA	0.94
Internal standard	1.00
THCA	1.12
CBGA	1.19

[Note—Conversion factors may be used. In such case, conversion factors are derived by dividing the response factor of CBD (the reference peak) by the response factor of the analyte at the same concentration levels.]

Calculate the amount of each cannabinoid, in mg/g, in the portion of *Cannabis* Species Inflorescence taken:

Result = $(R_U/R_S) \times C_S \times (V/W) \times F$

R_U = peak response ratio of the cannabinoid relative to the internal standard from the *Sample solution*

R_S = peak response ratio of CBD relative to the internal standard from *Standard solution B*

C_S = concentration of CBD in *Standard solution B* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of *Cannabis* Species Inflorescence taken to prepare the *Sample solution* (g)

F = conversion factor for the analyte

Calculate total THC, in mg/g, as follows:

Result = (THCA × 0.877) + Δ^9-THC

Calculate total CBD, in mg/g, as follows:

Result = (CBDA × 0.877) + CBD

Calculate the percentage of the labeled amount of cannabinoid in the portion of *Cannabis* Species Inflorescence taken:

Result = (P/L) × 100

P = content of total THC or total CBD as determined previously (mg/g)

L = labeled amount of cannabinoid (mg/g)

Acceptance criteria

THC-dominant chemotype

- It contains NLT 80% and NMT 120% of the labeled amount of the total THC (mg/g).
- The ratio of the total THC content to total CBD content is NLT 5:1, containing NMT 10 mg/g of total CBD, and NLT 10 mg/g of total THC.
- It contains NLT 80% and NMT 120% of the labeled amount of all other cannabinoids measured (mg/g). *Cannabis* Species Inflorescence must be labeled with the name and amount of any cannabinoid present in an amount of 10 mg/g or more.
- The content of CBN is NMT 2% of the total content of THC. No unidentified peak in the *Sample solution* chromatogram exceeds the area of the CBN peak.

CBD-dominant chemotype

- It contains NLT 80% and NMT 120% of the labeled amount of the total CBD (mg/g).
- The ratio of the total THC content to total CBD content is NMT 1:5, containing NMT 10 mg/g of total THC, and NLT 10 mg/g of total CBD.
- It contains NLT 80% and NMT 120% of the labeled amount of all cannabinoids measured (in mg/g). *Cannabis* Species Inflorescence must be labeled with the name and amount of any cannabinoid present in an amount of 10 mg/g or more.

THC/CBD intermediate chemotype

- It contains NLT 80% and NMT 120% of the labeled amount of the total THC and total CBD (mg/g).
- The ratio of the total THC content to total CBD content is NLT 0.2:1 and NMT 5:1, containing NLT 10 mg/g of total THC and NLT 10 mg/g of total CBD.
- It contains NLT 80% and NMT 120% of the labeled amount of all cannabinoids measured (mg/g). *Cannabis* Species Inflorescence must be labeled with the name and amount of any cannabinoid present in an amount of 10 mg/g or more.
- The content of CBN is NMT 2% of the total content of THC. No unidentified peak in the *Sample solution* chromatogram exceeds the area of the CBN peak.

CONTAMINANTS

• Elemental Impurities—Procedures <233>

Acceptance criteria

Arsenic: NMT 0.2 µg/g

Cadmium: NMT 0.3 µg/g

Lead: NMT 0.5 µg/g

Mercury: NMT 0.1 µg/g

• Pesticide Residues[3]: Conform with the relevant regulatory body requirements with regard to the authorized or unauthorized use of pesticides, as applicable. The limits for other pesticides that are detected may be determined using the following formula and the subsequent limit requirements for the calculated value:

$V = A \times M / 1000B$

V = calculated value of the pesticide residue (mg/kg)

A = ADI, as published by FAO-WHO (in mg/kg of body weight)

M = body weight in kg (60 kg)

B = daily dose of the article (kg)

The calculated value is used to determine limits based on the requirements that the general maximum residue limit is 0.1 ppm if the calculated value is more than 0.1 ppm, and 0.01 ppm if the calculated value is less than 0.1 ppm.

Microbial Contaminants—Option 1

• Microbial Enumeration Tests <61>: The total aerobic bacterial count is NMT 10^5 cfu/g and the total combined molds and yeasts count is NMT 10^4 cfu/g.

• Tests for Specified Microorganisms <62>: The total bile-tolerant Gram-negative bacteria does not exceed 10^3 cfu/g. Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

• Articles of Botanical Origin <561>, *Aflatoxins*: Use *Method 2* or *Method 3*. Meets the requirements

Microbial Contaminants—Option 2

• Microbial Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use <1111>: The total aerobic bacterial count is NMT 10^2 cfu/g and the total combined molds and yeasts count is NMT 10^1 cfu/g. It meets the requirements of the tests for absence of total bile-tolerant Gram-negative bacteria, *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

• **Tests for Specified Microorganisms <62>:** The total bile-tolerant Gram-negative bacteria does not exceed 10^3 cfu/g. Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

• **Articles of Botanical Origin <561>, *Aflatoxins*:** Use *Method 2* or *Method 3*. Meets the requirements

SPECIFIC TESTS

• **Articles of Botanical Origin <561>, *Methods of Analysis, Foreign Organic Matter*:** Foreign organic matter includes *Cannabis* plant parts that are not part of the inflorescence as well as other organic matter. NMT 5% of the sample consists of *Cannabis* Species Inflorescence stems that are 3 mm or more in diameter. NMT 2% of the sample consists of any other foreign organic matter.

• **Articles of Botanical Origin <561>, *Methods of Analysis, Total Ash*:** NMT 20.0%

• **Articles of Botanical Origin <561>, *Methods of Analysis, Acid-Insoluble Ash*:** NMT 4.0%

• **Water Activity <922>:** 0.60 ± 0.05

• **Loss on Drying <731>**

Analysis: Dry 1.0 g of *Cannabis* Species Inflorescence, finely powdered, under vacuum at 40° for 24 h.

Acceptance criteria: NMT 10.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage[3]:** *Cannabis* Species Inflorescence should be stored in a cool and dry place in well-closed containers and protected from light and moisture. Water activity during storage should be maintained at 0.60 ± 0.05. Packaging and Storage Requirements <659> defines *cool conditions* as “any temperature between 8° and 15 ° (46°F and 59°F)” and a *dry place* as “a place that does not exceed 40% average relative humidity at 20° (68°F) or the equivalent water vapor pressure at other temperatures".

• **Labeling[3]:** The label states the name of the article as *Cannabis* Species Inflorescence and the scientific Latin binomial as *Cannabis sativa* L. The label states the amount of the total tetrahydrocannabinol-related constituents (THCA, Δ^9-THC) and the amount of the total cannabidiol-related constituents (CBD, CBDA) in mg/g. The label also indicates whether the cultivar is THC-dominant type, CBD-dominant type, or THC/CBD intermediate type. The label states the method used for *Content of Cannabinoids* if *Procedure 1* is not used. The label states any other cannabinoids present above 10 mg/g. The label indicates the dominant and codominant terpene. The label indicates whether the article has been treated to reduce the microbial load and the method used. In cases where the product conforms to limits for inhaled use found in Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use <1111>, and in order to aid at-risk populations in choosing lower-risk products, the label should identify the product as having a reduced microbial load.

• **USP Reference Standards <11>**

USP Cannabidiol RS

USP Cannabidiol Solution RS

USP Cannabinoid Acids Mixture RS

USP Cannabinoids Mixture RS

USP Delta-9-Tetrahydrocannabinol RS

[1] *Cannabis* containing more than 0.3% Δ^9-THC is a Schedule I substance in accordance with the Controlled Substances Act. For detailed explanations of cannabis quality attributes see the USP article "*Cannabis* Inflorescence for Medical Purposes: USP Considerations for Quality Attributes" (*Journal of Natural Products.* 2020;83(4):1334–1351. *doi:10.1021/acs.jnatprod.9b01200*). The *HMC* monograph provides updated information where available.

[2] The assignment of a botanical name to *Cannabis* species plants is a matter of taxonomic debate. Some authorities recognize *Cannabis* as a single highly variable species, designated as *Cannabis sativa* L. with two subspecies: *C. sativa* subsp. *sativa* and *C. sativa* subsp. *indica* (Lam.) E.Small & Cronquist. Variation has also been described at the taxonomic level of varieties: *C. sativa* var. *sativa*, *C. sativa* var. *indica* (Lam.) Wehmer, and *C. sativa* var. *ruderalis* Janisch.) S.Z.Liou. Other authorities consider the aforementioned three varieties to be distinct species: *C. sativa*, *C. indica* Lam., and *C. ruderalis* Janisch. (This latter species is considered by some to be extinct). Other *Cannabis* species, subspecies, varieties, and formas have also been described in the literature. For the purpose of this monograph, USP subscribes to the opinion that *Cannabis* consists of one highly variable species and the subspecies or varieties as stated above. This definition may be modified in the future if and when a more definitive taxonomic consensus has been reached. The quality standards set out in this monograph are broadly applicable to the *Cannabis* inflorescence subspecies, varieties, and cultivars currently in commerce. In order to achieve the level of standardization in this monograph, the article should be derived from homogeneous cultivars grown under controlled conditions.

[3] Sarma ND, Waye A, ElSohly MA, Brown PN, Elzinga S, Johnson HE, Marles RJ, Melanson JE, Russo E, Deyton L, Hudalla C, Vrdoljak GA, Wurzer JH, Khan IA, Kim N-C, Giancaspro GI. *Cannabis* Inflorescence for Medical Purposes: USP Considerations for Quality Attributes. *Journal of Natural Products.* 2020;83(4):1334–1351. *doi:10.1021/acs.jnatprod.9b01200*.

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