American Herbal Pharmacopoeia®

Cannabis Inflorescence
Cannabis spp.

Standards of Identity, Analysis, and Quality Control

Revision 2014

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Cover Photograph

Cannabis cultivated under the Compassionate Investigational New Drug program at the University of Mississippi administered by the National Institute on Drug Abuse (NIDA). Photograph courtesy of: University of Mississippi.

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Legal Notification

The following Standards of Identity, Analysis, and Quality Control of *Cannabis* are intended to provide scientifically valid methods for the analysis of cannabis and its preparations that can be used to comply with state and federal regulations and policies. The analytical methods were obtained from peer reviewed literature, have been used as part of international or federal monitoring programs for cannabis, and have been verified for their scientific validity. Methods other than those presented in this monograph may be scientifically valid and provide reliable results. However, all methods must be verified as being scientifically valid prior to use for regulatory compliance.

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Nomenclature

Botanical Nomenclature

Cannabis L. (includes *Cannabis sativa*, *C. indica*)

Botanical Family

Cannabaceae

Pharmacopoeial Nomenclature

Cannabis Inflorescentia

Pharmacopoeial Definition

Cannabis consists of the dried inflorescences and remains of subtending leaves of pistillate *Cannabis* species plants.

Common Names

Cannabis, ganja, grass, hemp, marijuana (alternatively spelled marihuana), pot, weed, sinsemilla.

Identification

Botanical Identification

Taxonomic Discussion

The taxonomic classification of *Cannabis* has been the subject of considerable debate in scientific and legal forums for decades and is driven by classical botanical taxonomy, chemotaxonomy, and molecular sequencing. Opinions regarding *Cannabis* have been split between polytypic (multiple-species) and monotypic (single-species) views of the genus. Both views usually segregate plant populations by their relative concentrations of Δ^9-tetrahydrocannabinol (Δ^9-THC; hereafter referred to as THC) and cannabidiol (CBD). For a detailed account of the taxonomic history of *Cannabis* see Hillig (2005), Russo (2004), Schultes et al. (1974), and Small and Cronquist (1976).

Following the formal description of *C. sativa* by Linnaeus in 1753, Lamarck (1785) published a description of what he considered a different species, *C. indica*, based on plant specimens collected in Asia. The *C. indica* plants were relatively shorter, had smaller leaves, narrower leaflets, smaller fruit, and, as described by Lamarck, poorer fiber quality than *C. sativa*, but greater utility as an inebriant. Since then, the name *C. indica* has been applied to variants with high levels of psychoactive THC, while the name *C. sativa* has generally been applied to plants selected for their yield of bast (phloem) fibers in the stem and relatively high CBD to THC ratio. Wild-type plants growing in southeast Europe, possibly descending from the ancestor of C. sativa, were named *C. sativa*. var. *spontanea* Vav. and *C. ruderalis* Janisch. Vavilov encountered unique, broad-leafleted plants in Afghanistan. After some equivocation, he named them *C. indica* var. *kafiristanica* (a wild-type plant) and *C. indica* var. *afghanica* (plants with traits of domestication). Numerous other botanical names have appeared in the literature (e.g., see Schultes et al. 1974 for discussion and Tropicos.org for a nearly complete list).

Schultes et al. (1974) and Anderson (1980) recognized 3 entities: *Cannabis sativa* L. (tall, branched plants, used mainly for fiber and seed and also for drugs), *C. indica* Lam. (short, densely branched plants with firm stem, broad leaflets, and high content of psychoactive THC), and *C. ruderalis* Janisch. (short, often unbranched "roadside" plants usually yielding a high CBD to THC ratio). The taxonomic treatments by Schultes and Anderson departed from the concepts of Linneaeus, Lamarck, and Janischevsky: These authors treat *C. sativa* as a source of psychoactive drugs; Lamarck's *C. indica* designates plants from India, which are relatively tall, laxly branched, with narrow leaflets; they apply *C. ruderalis* to plants from Central Asia, whose morphology departs from Janischevsky's description of European plants with moderate height, strong branching, and long, narrow leaves.

Small and Cronquist (1976) analyzed 350 world-wide accessions in a common garden experiment. These authors argued that due to the absence of reproductive barriers and the morphological discontinuities of the plant, only one polymorphic species, *C. sativa*, currently exists. They further suggested that the current gene pool of *Cannabis* was heavily influenced by human agronomic selection and proposed the recognition of subspecies *sativa* (low content of THC, grown primarily for fiber and seed use) and *indica* (high content of THC, grown primarily for intoxicant use) within the single species, *C. sativa*.

Conversly, Hillig argues that the split between *sativa* and *indica* may have pre-dated human intervention. He analyzed 157 accessions of known geographic origin in a common garden experiment, using genetic evidence (Hillig 2005), cannabinoid profiles (Hillig and Mahlberg 2004), terpenoid variation (Hillig 2004), and host-parasite data (McPartland and Hillig 2006). He recognized a *sativa* gene pool included hemp fiber and seed landraces from Europe and Central Asia and Eastern European ruderal (roadside) accessions. The *indica* gene pool comprised narrow-leaflet drug strains from Southern Asia, Africa, and South America, wide-leaflet drug strains from Afghanistan and Pakistan, Far Eastern fiber and seed landraces, and feral populations from Nepal and India. A putative third gene pool was formed by ruderal accessions from Central Asia. This classification and nomenclature was adopted and expanded by Clarke and Merlin (2013).

A vernacular taxonomy of "Sativa" and "Indica" has arisen, which conflicts with the formal botanical taxonomy of Linneaus and Lamarck, as noted by Small (2007). The 2 names have been commonly used to refer to, narrow- and wide-leafleted drug varieties, respectively (Hillig 2004). However, due to the widespread interbreeding of the species, the application of these terms to narrow and broad leafleted specimens is botanically imprecise. Recent floristic treatments of *Cannabis* recognize only one (*C. sativa*) or, rarely, 3 species, noting the confused state of taxonomic understanding: *Flora of China* and *Flora of the USSR* lists 2

Figure 1 **Morphological characteristics of *Cannabis***

A. Inflorescence of male (staminate) plant.
B. Fruiting female (pistillate).
a. Staminate flower.
b. Stamen (anther and short filament).
c. Stamen.
d. Pollen grains.
e. Pistillate flower with bract.
f. Pistillate flower without bract.
g. Pistillate flower showing ovary (longitudinal section).
h. Seed (achene) with bract.
i. Seed without bract.
j. Seed (side view).
k. Seed (cross section).
l. Seed (longitudinal section).
m. Seed without pericarp (peeled).

Source: Köhler, *Medizinal-Pflanzen in naturgetreuen Abbildungen und kurz erläuterndem Texte* (1887).

2a.

2b.

2c.

2d.

Figure 2 Botanical characteristics of cannabis inflorescences

2a. Full view of mature high-THC-producing female (pistillate) plant.
2b. Full view of mature high-CBD-producing female (pistillate) plant.
2c. Long dense raceme of a high THC-producing female plant bearing pistillate (female) flowers.
2d. Inflorescence of a high CBD-producing (CBD:THC ratio 30:1) female plant (note long slender leaves).

2e.

2f.

2g.

2h.

Figure 2 (continued) Botanical characteristics of cannabis inflorescences

2e. Maturing female inflorescence showing young yellow styles and stigmas (often referred to as "pistils").

2f. Close-up of maturing female inflorescence showing young yellow styles and stigmas senescing brown and shriveling and an abundance of glandular trichomes.

2g. Female inflorescence with senesced reddish-brown styles and stigmas, an indicator of inflorescence maturity.

2h. Close-up of female inflorescence with senesced reddish-brown styles and stigmas.

(*C. sativa* L. and *C. ruderalis* Janisch.), *Flora of Pakistan* lists one, *Flora of Missouri* lists one, *Flora of North America* lists one, and *Flora of Taiwan Checklist* lists one.

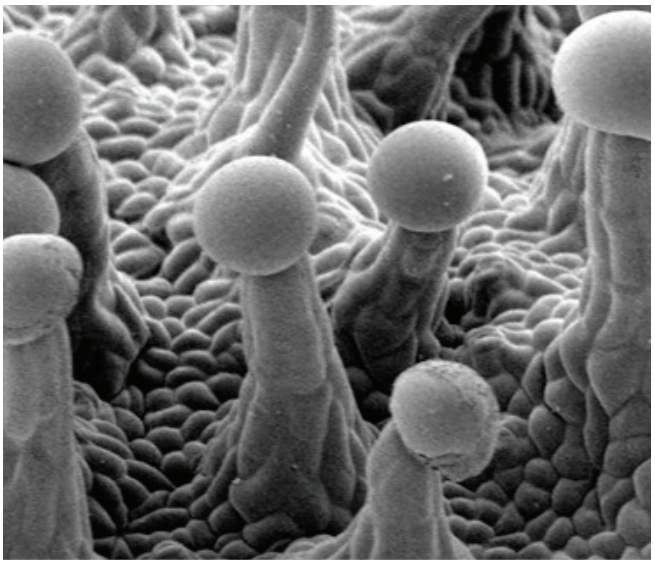
Extensive co-cultivation and crossbreeding practices have effectively crossed the boundaries between the various taxonomic categories within *Cannabis*. Although outside of the strictly botanical classification, nomenclature of cultivated plants, governed by the International Code of Nomenclature for Cultivated Plants (Brickell et al. 2009), may be more applicable to further differentiation of *Cannabis* plants cultivated today. Such nomenclature is not taxonomic, but cultonomic, and recognizes cultivars and groups based on economically important characteristics, without an appeal to the phylogenetic hierarchy. With this approach, *Cannabis* plants that satisfy selected criteria might be assigned to any number of groups, depending on the use emphasized, e.g., THC-drug group, CBD-drug group, mixed THC-CBD-drug group, fiber-hemp group, seed-oil group, etc.

Cannabis is a member of the *Cannabaceae* family, together with another well-known member of the family, hops (*Humulus*). The family has recently been expanded to contain 9 other genera (Stevens 2001). The following

2i.

2j.

2k.



2l.

Figure 2 (continued) Botanical characteristics of cannabis inflorescences

2i. Trichomes along anther scale.
2j. Trichomes along the pedicel of a male flower.
2k. Close-up of glandular trichomes.
2l. Magnification of multicellular glandular trichomes with electron microscopy.

Photographs courtesy of: (2a–b, e, f) The Wo/Men's Alliance for Medical Marijuana (WAMM), Santa Cruz, CA; (2c, h) Gianpaolo Grassi, CRA-CIN, Industrial Crop Research Center, Rovigo, Italy; (2d, g, i–k) © David J Potter, Salisbury, UK; (2l) University of Mississippi, University, MS.

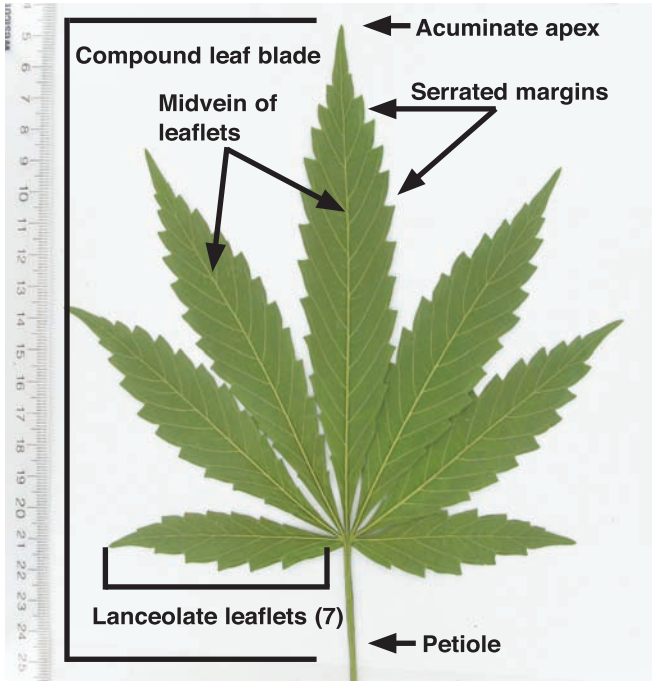
describes the published range of morphological diversity within plants recognized as *Cannabis* spp.

Morphological Characterization of *Cannabis* L.

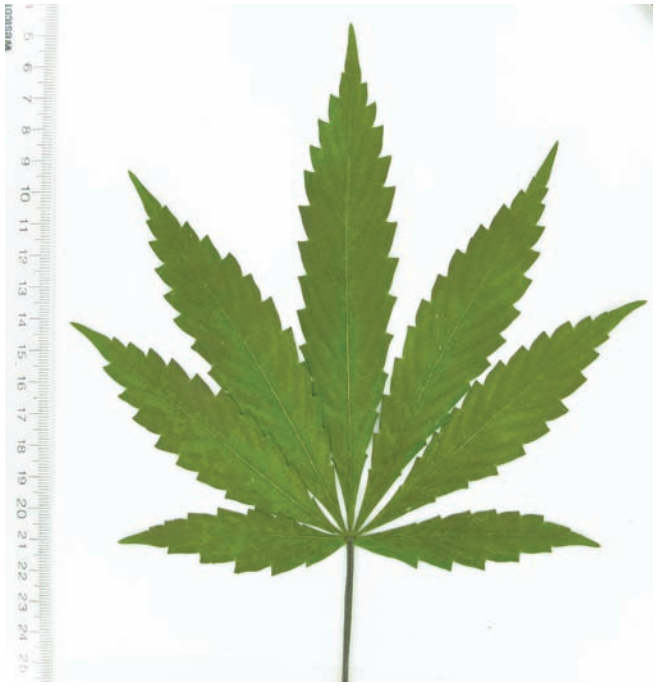
Herbaceous annual, taprooted (taproot not developed on vegetatively propagated/cloned plants). Plants dioecious (male and female flowers occur on separate plants) and rarely monoecious (male and female flowers occur on the same plant). Monoecious plants are often referred to as "hermaphrodites." True hermaphrodites bear bisexual flowers and are less common, whereas monoecious plants bear unisexual male and female flowers at different locations on the plant. Staminate (male) plants tend to be taller but less robust than pistillate (female) plants. Height and degree of branching depends on both genetic and environmental factors (UNODC 2009). **Root:** A laterally branched taproot, generally 30–40 cm deep in loose soil, and up to 2.5 m deep; the horizontal spread of lateral roots also depends on the soil type, up to 80 cm in width. **Stem:** Erect, furrowed, round to obtusely hexagonal in cross-section often hollow, 0.2–6 m (usually 1–3 m) tall, simple to well branched; branchlets densely pubescent; staminate (male) plants usually taller and less robust, compared with pistillate (female) plants (Raman 1998); stipules linear, lateral, acute, persistent, 2–5 mm. **Leaves:** Alternate or opposite basally on

3a.

3b.



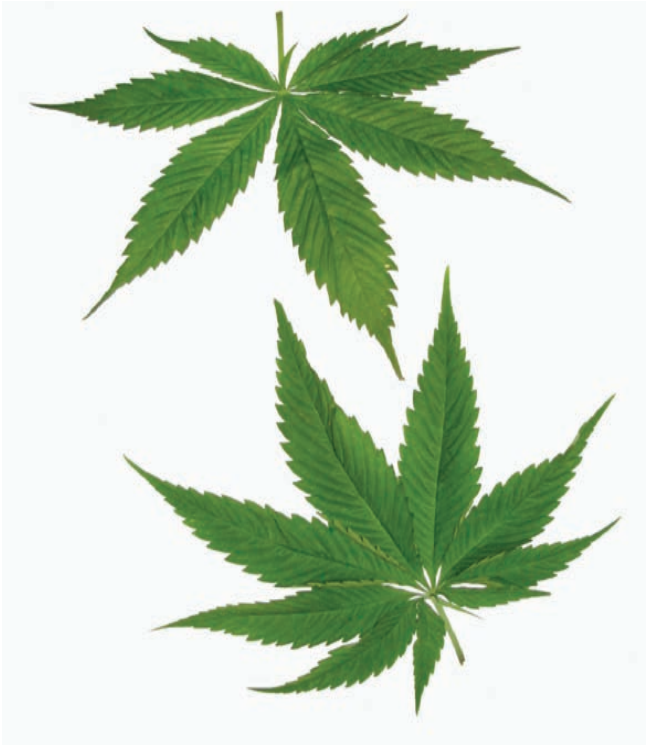
3c.



3d.

Figure 3 Botanical characteristics of cannabis leaf

3a. Adaxial (upper) surface of a typical cannabis leaf (9 leaflets).
3b. Adaxial (upper) surface of a typical cannabis leaf (5 leaflets).
3c. Abaxial (lower) surface of a typical cannabis leaf.
3d. Adaxial (upper) surface of a typical cannabis leaf.



3e.

3f.

Figure 3 (continued) Botanical characteristics of cannabis leaf

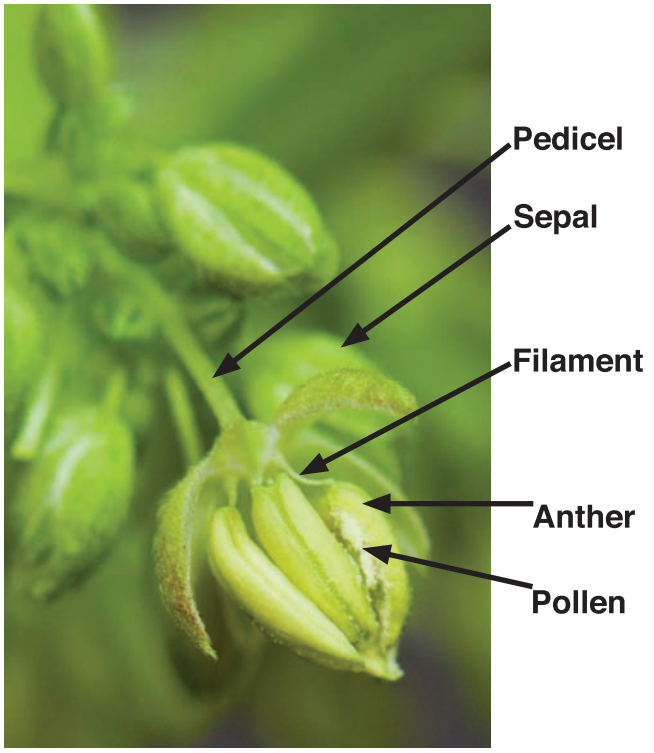
3e. Upper and lower surface of broad-leaf strain ("indica" type).
3f. Upper and lower surface of narrow-leaf strain ("sativa" type).
Photographs courtesy of: (3a–d) WAMM, Santa Cruz, CA; (3e–f) Gianpaolo Grassi, CRA-CIN, Industrial Crop Research Center, Rovigo, Italy.

stem, with the longest in the middle, palmately compound, basally with (3)5–11(13) leaflets, apically with 1–3 leaflets. **Leaflet:** Usually lanceolate, sometimes oblanceolate to linear, uneven in size, (3)7–15 x (0.2)0.5–1.5(2) cm, ; margin serrate, with fine, very acute to coarser, almost blunt serrations; apex acuminate; petiole 2–7 cm; leaf blade abaxially whitish-green, strigose, and rarely whitish-clear to opaque to brownish glandular trichomes, adaxially dark green with cystolithic trichomes. Blade surfaces abaxially sparsely to densely pubescent. **Staminate (male) inflorescences:** Axillary or terminal, erect, up to ca. 25 cm, a lax panicle or a compound cyme. **Male flowers:** Yellowish green, nodding; pedicel 2–4 mm, thin; sepals imbricate, ovate to lanceolate, 2.5–4 mm, membranous, with sparse prostrate trichomes; petals absent; filament 0.5–1 mm, straight in bud; anthers oblong; rudimentary pistil small. **Pistillate (female) inflorescences:** Pseudospikes, congested, erect to spreading, among leaf-like bracts and bracteoles. **Female flowers:** Green, sometimes purple to red and/or mottled or streaked, sessile; bract (subtending floral leaves) proximal upper surfaces are densely covered by capitate stalked trichomes, with serrate or entire margins (Potter 2009); bracteole (alternately called a calyx, perigonium, or perigonal bract) usually refers to a small (4–8 mm long), fused, conically-shaped sheath that completely envelopes the ovary and loosely encloses mature fruit, densely hispid or pilose, covered with resinous glandular trichomes; perianth thin, papery, undivided, closely appressed to the ovary and mature fruit, often reduced or absent in cultivated forms, often marbled with light and dark areas; ovary superior, sessile, subglobose, one-locular with one pendulous anatropous ovule; styles 2, long-linear, caduceus, emerging from the apex of the bracteole. **Achenes (fruits):** Solitary, usually green-brown but also white or gray, with a pale, fine reticulation pattern on the smooth surface (in cultivated forms), or with brown or purple mottling (in strains retaining wild-type morphology and a persistent perianth), ovoid to oblong in outline, somewhat compressed (lenticular) in cross section, 2–5 mm; endosperm fleshy and oily; embryo strongly curved; cotyledon fleshy.

The upper leaves, unfertilized (female) flower heads, and flower bracts of the female plant are the primary source of cannabinoids in *Cannabis*. The cannabinoids are enclosed in tiny (just visible to the eye) glandular trichomes occurring in several different forms: sessile glands (trichomes without a stalk); small bulbous glandular trichomes with one-celled stalks; and long, multicellular-stalked glandular trichomes mainly present on bracts and bracteoles surrounding female flowers (Hammond and Mahlberg 1977; Raman 1998; Starks 1990; see Table 2). Numerous unicellular non-glandular trichomes are located on both surfaces of the leaves, bracts, and bracteoles. Those on the upper (adaxial) epidermal surface frequently bear calcium carbonate crystals (cystoliths) at the base. The presence and distribution of the rigid, curved cystolithic non-glandular trichomes on the upper leaf surfaces and of the fine, slender non-cystolithic non-glandular trichomes on both upper and lower surfaces are characteristic of *Cannabis* and enable positive identification of even fragmented material (UNODC 2009).

Although some selections of cannabis are day-neutral (flower under any day-length; sometimes referred to as "autoflowering"), most are short-day-length plants (needing

4a.



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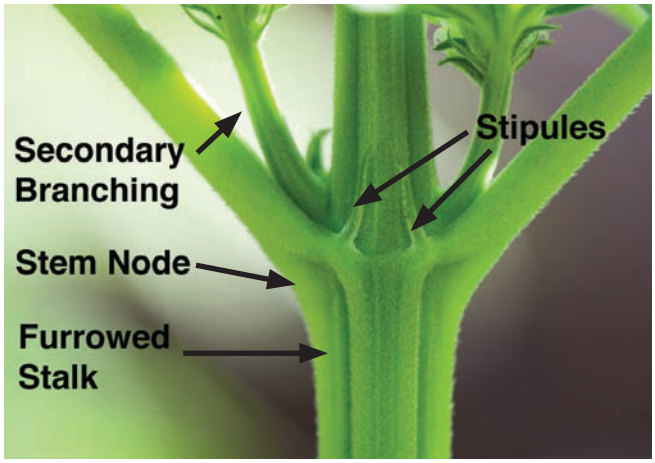


4c.

4d.

Figure 4 Botanical characteristics of cannabis staminate (male) flowers and stem

4a. Male (staminate) flowers.
4b. Close-up of male flowers showing primary floral characteristics.
4c. Vegetative stem of mature plant showing node, furrows, stipules, and axillary branching.
4d. Stalk of purple variety.
Photographs courtesy of: WAMM, Santa Cruz, CA.

a long, usually ≥ 14 hours dark period) and shift from vegetative to reproductive growth upon exposure to short day-length conditions. With the change to reproductive growth, the leaf pair arrangement changes from opposite to an alternate, spiral arrangement (Potter 2004). Distinguishing male and female plants during vegetative growth is difficult, although the female plant tends to be stockier and to flower later than the male plant (Raman 1998). Occasionally, one or few individual flowers are produced in lower leaf axils to allow the determination of the plant's sex during the vegetative phase of growth.

Distribution: Humans have dispersed cannabis worldwide over the past 10,000 years from probable origins in Central Asia, the Northwestern Himalayas, and China to a variety of habitats throughout the temperate and tropical regions of the world. Within the purported native range, the plant occurs in open, disturbed habitats, such as along riverbanks, bottomlands, and hillsides. In North America, C. *sativa* subsp. *sativa* is reported naturalized or ruderal in most all states and provinces across the Northeast, Midwest, and Eastern Plains, occurring at altitudes 0–2000 m (USDA-NRCS 2014). The plant can be observed in fertile, moist farmlands, in open habitat, in waste areas, and, occasionally, in fallow fields and open woodlands (Small 1997). In addition to the habitats in which the

5a.

5b.

5c.

Figure 5 Botanical characteristics of cannabis seed (achene)

5a. Developing seed (fruit; formally known as "achene").

5b. Seed emerging from and surrounded by the sac-like fused calyx.

5c. Mature seeds.

Photographs courtesy of: Gianpaolo Grassi, CRA-CIN, Industrial Crop Research Center, Rovigo, Italy.

plants would otherwise be naturalized if growth were not actively curtailed, cannabis is widely cultivated outdoors and indoors for both recreational and medicinal purposes.

Macroscopic Identification

Cannabis raw material is most often supplied as variously sized, 1.5–15 cm or longer, branches and branchlets, sometimes broken up, of the dried inflorescences of pistillate plants. These 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 from green-brown to brown and may include whole, or fragments of, reduced upper leaves, stems, bracts, bracteoles, rudimentary calyx, immature ovules, styles, and glandular and non-glandular trichomes. Cannabis selections vary to the extent of the length of the internodes within the inflorescence. Those of short length have a denser cluster of flowers so that the segment pieces appear more rounded; those of a longer length have a greater distance between individual flowers. Variation exists between selections in the size and prominence of the various parts. Morphological characteristics and variation in color of cannabis products are influenced by the variety as well as environmental factors including light, water, nutrients, and methods of cultivation, harvesting, handling, and curing. For macroscopic examination of material that is stuck together, soak the material in strong alcohol (70%) to dissolve the resin, pour off the alcohol, and then soak in water. The leaves, stems, bracts, flowers, and fruit can then be separated. However, material prepared in this manner should not be used for quantitative analysis due to constituent loss.

Stems: Light brown, pale green, or variously mottled or entirely purple in color. Stems within inflorescences are

6a.

6b.

6c.

6d.

6e.

6f.

Figure 6 Macroscopic characteristics of cannabis inflorescence

6a. Dried, untrimmed pistillate inflorescences of morphological type "sativa."
6b. Dried pistillate inflorescences of morphological type "sativa" (bottom – untrimmed; top – trimmed).
6c. Storage effects on color of cannabis material (left – 1-year-old; right – new harvest).
6d. Dried pistillate inflorescences of morphological type "indica" (bottom – untrimmed; middle and top – trimmed).
6e. Close-up of a dried pistillate inflorescence (note the visible glandular trichomes).
6f. Powdered dry cannabis material (leaves and pistillate inflorescences).
Photographs courtesy of: (6a–e) WAMM, Santa Cruz, CA; (6f) University of Mississippi, University, MS.

often cut just below the node. Stems branch freely and repeatedly but the extent of branching is dependent on environmental and hereditary factors, and the method of cultivation. Nodes and internodes are distinct, with alternate branches, and can be of varying length. Stem texture is fibrous and the surface is longitudinally furrowed with short stiff hairs. The cortex and wood are thin with the pith white and porous. Larger diameter (≥ 3 mm) branch pieces are often sourced from terminal shoots. Material with thinner stems is most often from lateral inflorescence branches or from side branches cut from terminal inflorescences.

Upper leaves: Rarely present in cultivated plants as these are often removed through mechanical or hand trimming. When present, the upper leaves are light to dark green, sometimes purple or mottled purple in color, or brown, dried and shriveled, and sometimes clasping the inflorescence. After trimming, only the base of the petioles is typically left as stiff remnants at the nodes.

Bracts: Light to dark green or brownish-green. Numerous, alternate, with narrow stipules at the base; some are simple and others tri-partite, but in both cases the segments are lanceolate with an entire margin. Bracts subtending the spikes are often divided into 5 linear leaflets. Those subtending the individual flowers usually have 3 minute leaflets. Bracts and stipules both show a marked tendency to shrivel upon drying, and in some cases only the veins of the bracts remain intact. With magnification (10x) numerous glandular and non-glandular trichomes are seen.

Bracteoles: Light to dark green or brownish-green; formed in pairs in the axil of a bract. Ovate with an acute apex and incurved at the base to enclose the flower or fruit. With magnification (10x) numerous glandular and non-glandular trichomes are observed.

Flowers: A single flower is formed in the axil of each bracteole. Calyx is light to dark green or brownish, pubescent, and somewhat folded around the ovary or fruit. Ovary is single-chambered containing a single campylotropous ovule, surrounded by the thin hairy perianth. Attached to the flower are 2 slender, long, pubescent styles and stigmas, spreading at the apex, and of a dark reddish-brown to orange color. Plants are dioecious. Male flowers have stamens; female flowers do not.

Fruit: The fruit of cannabis is an achene and, together with the enclosed seed, is commonly referred to as the "seed." Unless specifically desired, seeds should be lacking from properly harvested material. Achenes separate easily from dry samples. The achene is 2–5 mm in diameter and enclosed within an enlarged persistent perianth surrounded by bracts; solitary, somewhat compressed (lenticular) ovoid, glossy, off-white, green, brown-green, or yellowish-green often mottled in purple. The thin wall of the ovary tightly covers the shell of the seed. The pericarp is dry and brittle and finely reticulate. The endosperm and cotyledons are fleshy. The embryo is curved.

Trichomes: Two primary categories of trichomes are present; glandular, cannabinoid-producing trichomes, and non-

Table 1 Microscopic characteristics of cannabis inflorescence powder

Bracts	Polygonal upper epidermis cells with faintly striated cuticle and few trichomes; sinuous-walled lower epidermis cells with anomocytic stomata and abundant trichomes; small clusters of calcium oxalate in the mesophyll cells.
Bracteoles	Polygonal upper epidermis cells with beaded walls; sinuous lower epidermis cells with slightly beade walls, anomocytic stomata, and tapering, unicellular covering trichomes; mesophyll similar to that of bracts, containing calcium oxalate clusters.
Trichomes and glands	Glandular and non-glandular trichomes.
Stigma	Fragments occurring as epidermal cells with reddish-brown papillae.
Seed	Fragments mainly visible as thick-walled sclereids of the epicarp.
Stem	Occurring as epidermis with large cystolith trichomes, parenchyma containing clusters of calcium oxalate, fibers, which are normally unlignified, vessels lignified with reticulate or annular thickening, and lactiferous tissue containing red-brown content.
Bracts	Polygonal upper epidermis cells with faintly striated cuticle and few trichomes; sinuous-walled lower epidermis cells with anomocytic stomata and abundant trichomes; small clusters of calcium oxalate in the mesophyll cells.
Leaflet	Upper epidermis cells wavy-walled with striated cuticle, stomata absent, sessile glandular trichomes and cystolith trichomes abundant; lower epidermis wavy-walled cells with anomocytic stomata and all trichomes characteristic of cannabis.

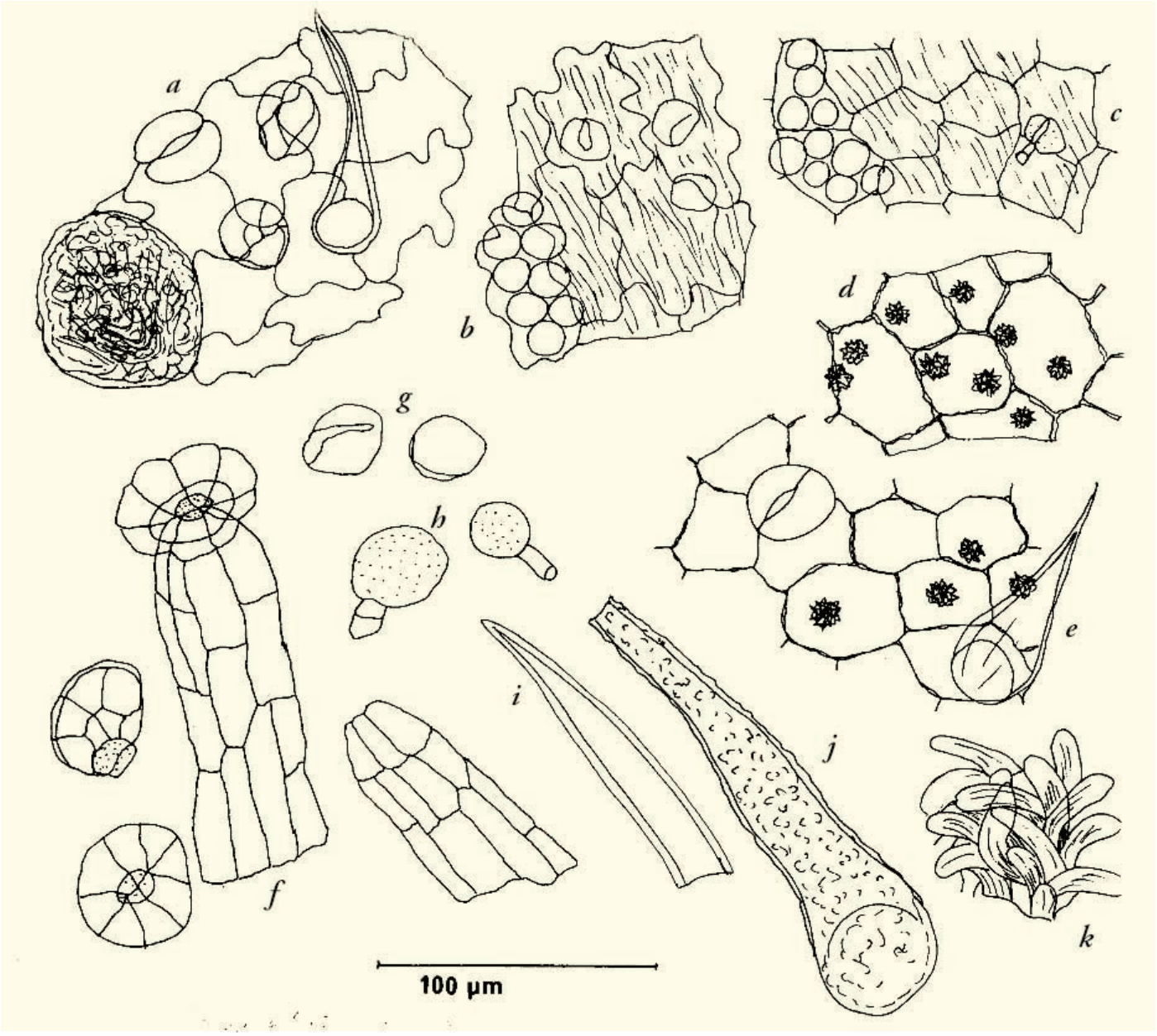


Figure 7 Microscopic characteristics of cannabis (illustrations)

7a. Lower epidermis of leaf showing cystolithic trichome.
7b. Upper epidermis of leaf with underlying palisade.
7c. Upper epidermis of bract with underlying palisade.
7d. Upper epidermis of bracteole with underlying calcium oxalate.
7e. Lower epidermis of bracteole with stoma and underlying calcium oxalate crystal.
7f. Fragments of multicellular glandular trichomes.
7g. Detached sessile glands.
7h. Small glandular trichomes.
7i. Part of a covering trichome.
7j. Part of large warty covering trichome from stem.
7k. Fragment of stigma.
Microscopic images courtesy of Elizabeth Williamson, University of Reading, Reading, UK.

glandular, non-cannabinoid-producing trichomes. Both can be observed with 10–20x magnification (see Table 2).

Powder: Dull light to dark green, to brown; sometimes purplish. When viewing coarsely ground material under 20x magnification, fragments of lower epidermis of leaves contain wavy vertical walls and oval stomata, while upper epidermis pieces have straight vertical walls and no stomata (see Table 1). Most of these characters require higher magnification if viewing finely ground powder.

Organoleptic Characterization

Aroma: Historically, the aroma of cannabis was described as agreeably aromatic, strong and heavy, peculiar, and narcotic. In recent decades, breeding and selection have produced a wide variety of aromas within cannabis strains. Commercial marketing of cannabis has led to the use of numerous comparative terms to describe the aromas of can-

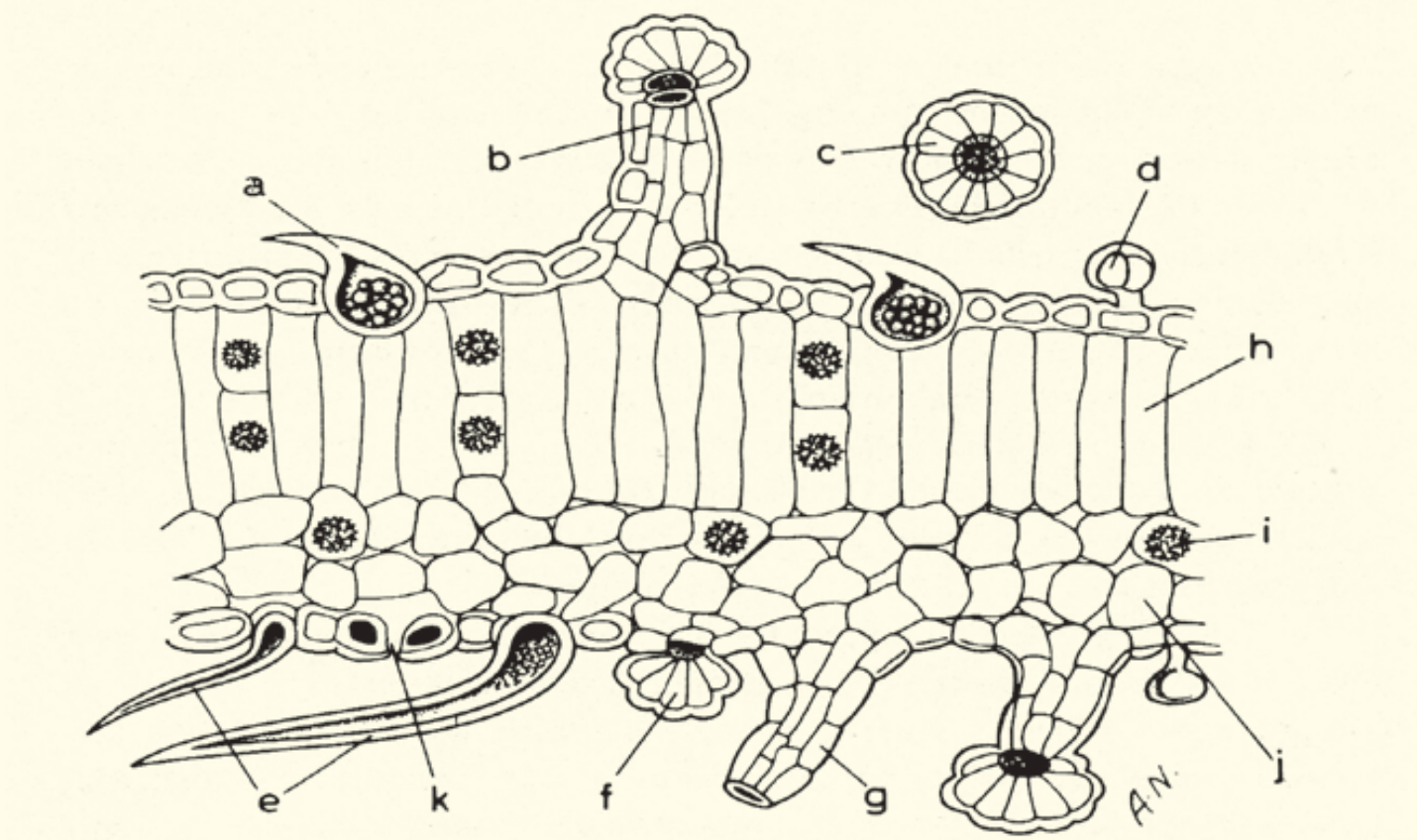


Figure 8 Microscopic characteristics of cannabis; cross section of a fruiting bract

8a. Cystolithic trichome.
8b. Glandular multicellular trichome.
8c. Surface view of large glandular trichome head.
8d. Glandular trichome with bicellular head and unicellular stalk.
8e. Thick-walled conical trichome.
8f. Developing glandular trichome.
8g. Stalk of a glandular trichome.
8h. Palisade cell.
8i. Cluster crystal of calcium oxalate.
8j. Parenchymal cell.
8k. Stoma.
Source: *The botany and chemistry of cannabis*. Joyce CRB and Curry SH (eds.) (1970) J & A Churchill, London.

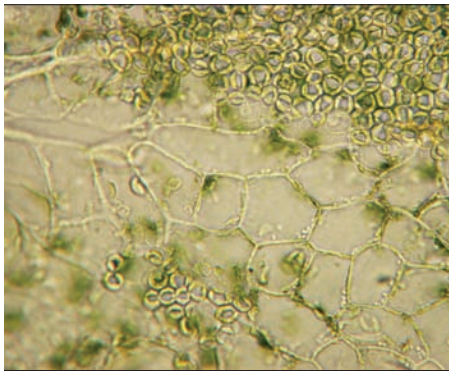
nabis strains. The aromas as described in modern advertising include: peculiar, narcotic, strong, sweet to sour, fruity to pungent, agreeable, aromatic, fresh and sweet, euphoric, spicy citrusy, musty, skunky, acrid, juniper, floral, sour, diesel, vanilla, complex, blueberry, pineapple, perfumed, piney, sandalwood, mango, skunky-cheese, and more.

Color: Color is influenced by variety and mode of cultivation, handling, harvest, and curing. Pistillate inflorescence parts vary in color from bright, light green to deeper, dark green through dark purple to light yellow-gold to brown, sometimes with flowers having long reddish-orange to brown styles and stigmas. Indoor grown material is often lighter green to bright purple, while material cultivated outdoors tends to be darker green to green-brown to dark purple. The color should be consistent throughout each sample and should not show signs of gray or black, which are indicators of fungal infection. Inflorescence parts with a high density of glandular and non-glandular trichomes can appear bright whitish and crystalline.

Taste and mouth feel: Bitter, somewhat acrid, resinous, sticky, and pungent.

Microscopic Identification

Bracts and Leaves: Microscopically, transverse sections of the leaflets and bracts show a dorsiventral structure. The palisade consists of a single layer (rarely 2 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 mesophyll. The upper epidermis 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 more slender, but without cystoliths. Both upper and lower epidermises bear numerous glandular trichomes, and on the underside glandular trichomes are especially abundant over the midrib. The glandular trichomes are of 3 types: (1) a long multicellular stalk and a multicellular head with approximately 8 radiating club-shaped cells; (2) a short unicellular stalk and a bicellular, rarely 4-cell, head; (3) sessile (without stalk) with a multicellular head. 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 3 types: non-glandular, non-glandular cystolithic,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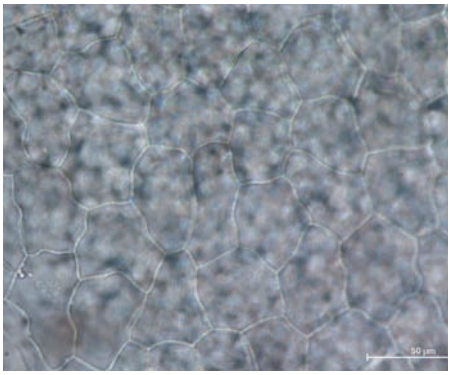
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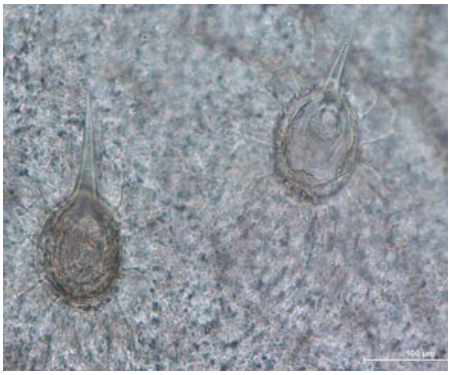
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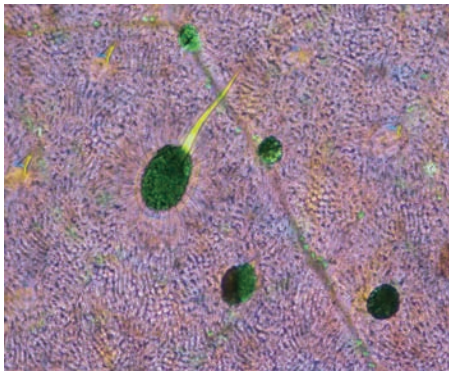
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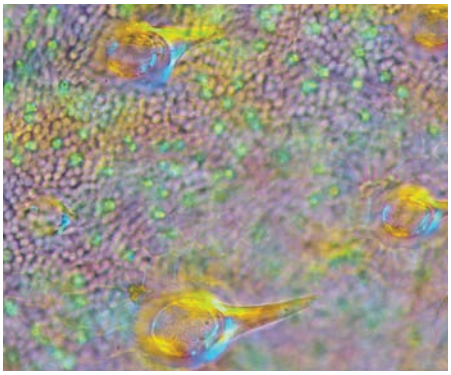
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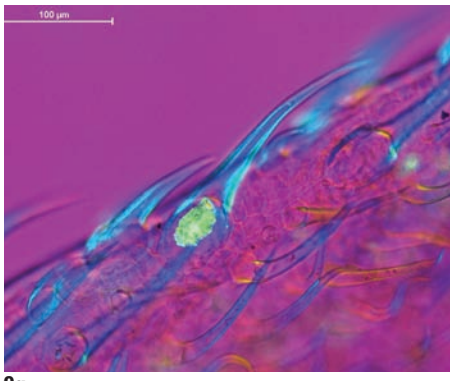
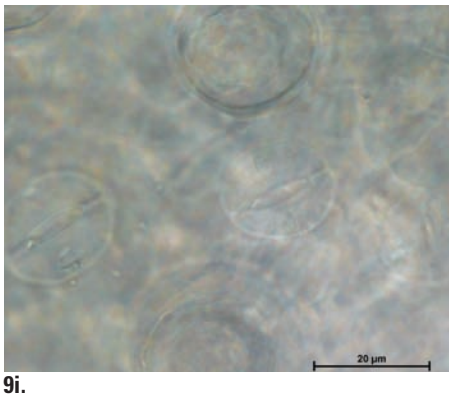


9g.


9h.

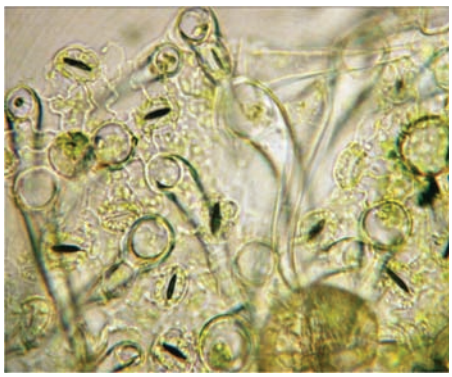


9i.

Figure 9 Microscopic characteristics of cannabis

9a. Upper (adaxial) leaflet surface showing epidermal cells with anticlinal walls.
9b. Upper (adaxial) leaflet epidermis showing curved anticlinal walls.
9c. Cystolithic trichomes on the upper surface of the leaflet (surface view).
9d. Cystolithic trichomes on the upper surface of the leaflet (surface view; polarized light).
9e. Cystolithic trichomes on the upper surface of female flower bract (surface view; polarized light).
9f. Cystolithic trichome (lateral view; polarized light).
9g. Cystolithic trichomes on the leaflet margin (lateral view; polarized light).
9h. Transverse section at the leaflet midrib.
9i. Stomata on the lower (abaxial) surface of the leaflet (surface view).
9j. Lower (abaxial) leaflet surface showing long unicellular non-cystolithic trichomes

Microscopic images courtesy of: (9a-e; g-l) University of Mississippi, University, MS; (9f, m) Reinhard Länger, AGES PharmMed, Vienna, Austria; (9n) ©2013 David J. Potter, Salisbury, UK; (9o–u) Elan Sudberg, Costa Mesa, CA.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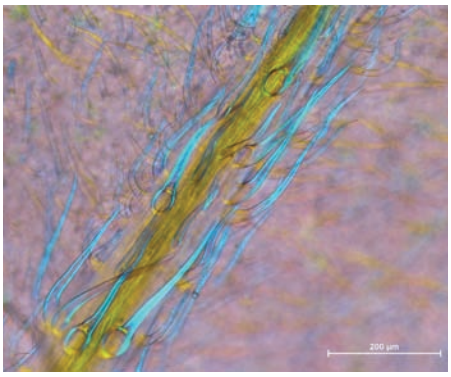
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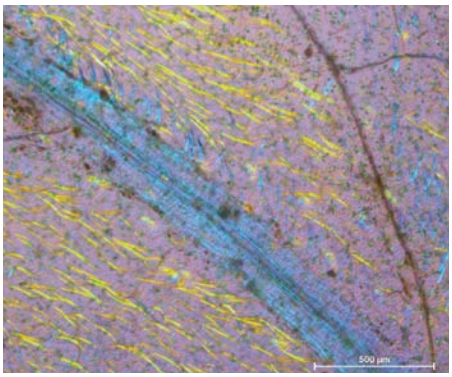
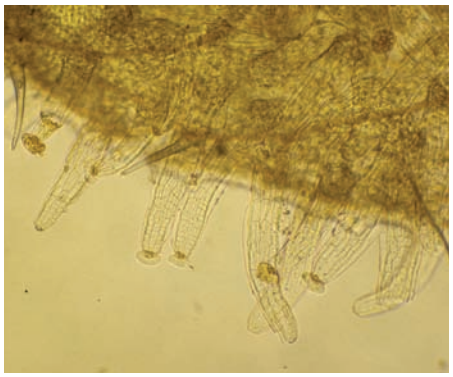

9l.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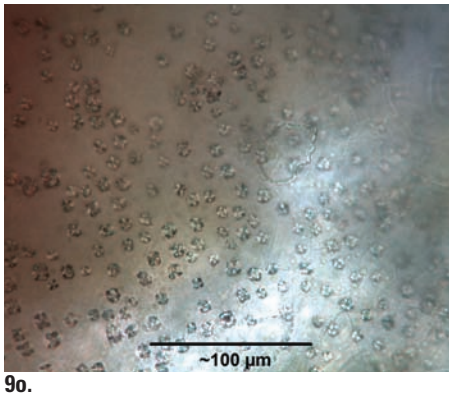

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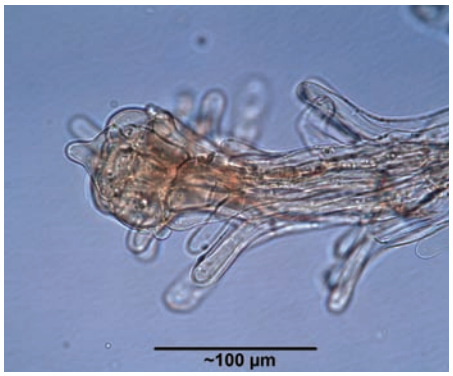

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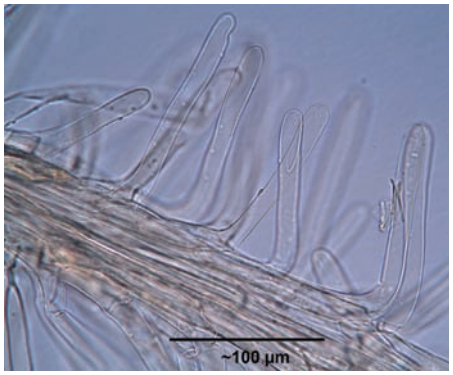

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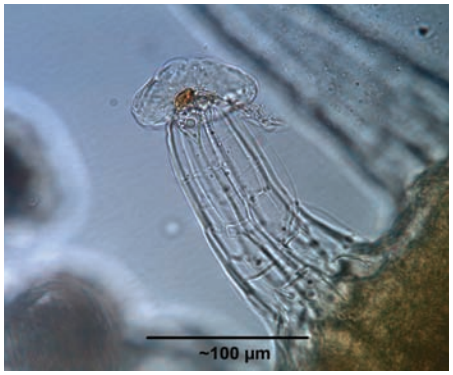
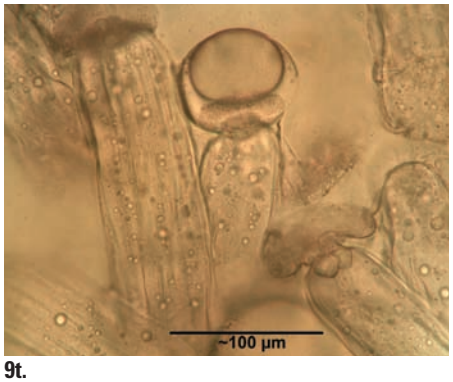
9r.



9s.



9t.

9u.

Figure 9 (continued) Microscopic characteristics of cannabis

9k. Non-cystolithic trichomes on the lower leaflet surface (polarized light).

9l. Non-cystolithic trichomes on the lower surface of the bract (polarized light).

9m. Multicellular-stalked glandular trichomes on bract.

9n. Multicellular-stalked glandular (left) and non-glandular cystolithic trichomes (right).

9o. Upper epidermis of bracteole showing underlying calcium oxalate cluster crystals in the mesophyll.

9p. Terminal end of a senesced stigma.

9q. Mid-section of a senesced stigma showing unicellular trichomes with rounded ends.

9r. Multicellular glandular trichomes.

9s. Multicellular glandular trichome showing orange-brown resin-oil deposits.

9t. Glandular trichome showing orange-brown resin-oil deposits.

9u. Sessile glandular trichome showing orange-brown resin-oil deposits either exuding or retracting through the stalk.

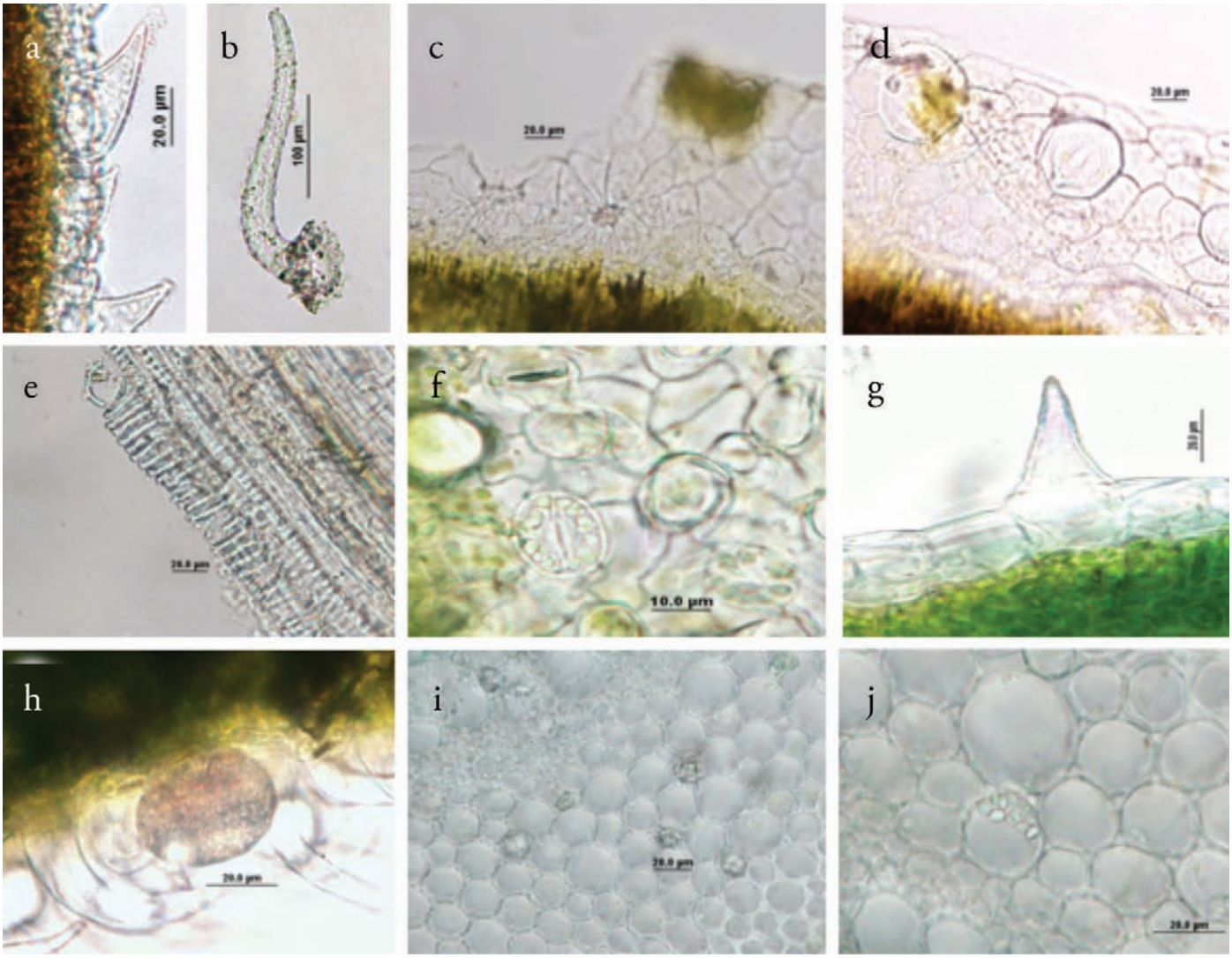
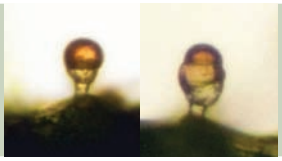


Figure 10 **Microscopic characteristics of cannabis inflorescence powder**

10a. Non-glandular conical trichomes on the upper epidermis of leaflet.
10b. Cystolithic trichome with warty cuticle.
10c. Head of glandular trichome showing cells radiating from basal cells.
10d. Surface view of epidermis showing trichomes and cystoliths.
10e. Fragments of vessel elements showing spiral wall thickenings.
10f. Lower epidermis showing anomocytic stomata.
10g. Non-glandular conical trichome with cystolith.
10h. Head of a glandular trichome covered with cuticle.
10i. Cortical parenchyma showing crystals of calcium oxalate.
10j. Cortical parenchyma showing simple starch grains.
Photographs courtesy of: University of Mississippi, University, MS.

Table 2 **Types and distribution of cannabis trichomes**

Trichome type		Cannabinoid-producing	Distribution	Image
Glandular	**Sessile (unstalked) glands**	Yes	All aerial epidermal tissues. Especially abundant on the underside (abaxial) surface of leaves and bracts and outer surface of bracteoles.	
	Bulbous (one-cell-stalked) trichomes	Possibly	All aerial epidermal tissues.	
	Multicellular-stalked trichomes	Yes	Bracts, bracteoles, and (rarely) on uppermost leaves; both surfaces.	
Non-glandular	**Cystolithic trichomes**	No	Leaves, bracts; mostly upper epidermis only.	
	Non-cystolithic trichomes	No	All aerial epidermal surfaces.	

Photographs © 2013 David J. Potter, Salisbury, UK.

and glandular. Clusters of calcium oxalate crystals are scattered in the ground tissue. The simultaneous presence of cystolithic trichomes on the upper surface and non-cystolithic trichomes and sessile glandular trichomes on the lower surface of the leaflets is characteristic of cannabis (UNODC 2009).

Bracteoles: Bracteoles have an undifferentiated mesophyll of about 4 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 ovary or fruit.

Flowers: In the stigmatic epidermis, nearly every cell has an extended papilla about 90–180 µm long with a rounded apex.

Stem: The stem epidermis bears very few trichomes similar to those of the leaves. In cross-section of the stem, large, unbranched laticiferous tubes can be seen in the phloem. Well-developed bundles of pericyclic fibers are present to the interior of the phloem. Both pith and cortex contain calcium oxalate cluster crystals, about 25–30 µm in diameter.

For microscopic examination, leaves, bracts, and twigs can be mounted in alcohol, water, or chloral hydrate solution. Some compounds may be diluted or lost when prepared in this manner so these samples should not be used for quantitative analysis

Commercial Sources and Handling

In commerce, cannabis generally refers to the dried inflorescences and subtending leaves and stems of the female plant, commonly referred to as the bud. Considerable efforts in breeding and selection have produced cannabis strains that are uniquely suited for either fiber (hemp; rich in bast phloem cells in the stem) or drug production (cannabinoid-

containing resin secreted by epidermal glands) (Small and Marcus 2002).

The most important cannabinoid in this context is the psychoactive molecule THC. Fiber types are economically important in China, Europe, Canada and many other territories, and grown in subtropical and temperate climates. Drug types however are more typically acclimatized to semi-tropical zones. In Canada, most Western US states, and Northern Europe, the climate is not optimal for most drug strains, encouraging indoor or greenhouse cultivation. Since the 1970s, in the US and Canada, a law enforcement crackdown and large-scale eradication efforts may have inadvertently encouraged more indoor growers. Breeding for high THC strains (predominantly for recreational purposes) has occurred historically and very aggressively over the past 40 years, with growers in California, the Pacific Northwest and British Columbia, and Holland crossing plants of Afghan, Columbian, and Mexican origin in order to increase THC yields well above 10% THC. Potency is especially high when only female plants are grown. Unfertilized female cannabis plants, (widely known as sinsemilla, a Spanish term meaning without seeds) utilized no energy in seed production and diverted more to total THC biosynthesis. Later changes obtained through breeding and controlled indoor growing conditions led to strains with increased total THC potency.

In the 1960s and 1970s, the average percentage of THC in herbal cannabis was less than 1%, although anomalous samples reaching 9.5% were reported (reviewed in Mikuriya and Aldrich 1988). In 1980 (Turner 1983) average total THC concentrations were less than 1.5%, but rose to approximately 3.3% in 1983 and 1984, fluctuated around 3% until 1992, and increased to 4.7% (average) in 1997. Since 1997, due to the increasing prevalence of strains grown using a variety of techniques, samples have been found to contain a mean of 8.8% and anomalous samples have contained as high as 29% total THC. In the same time period, other cannabinoid concentrations (e.g., CBD) remained relatively stable (ElSohly et al. 2000; Mehmedic et al. 2010).

In the European Union (EU) as a whole, total THC potency of crude cannabis has not had the same steady upward trend as in the US. For example, between 1998 and 2002 EU supplies ranged from a low of 1.1% [Hungary 2002], a high of 16.9% [Italy 1998], and a mean of approximately 7.7% total THC (EMCDDA 2004). In most European countries the current upper legal limit for cultivated cannabis for industrial purposes is 0.2% THC (for comparison Canada: 0.3%) with a ratio of CBD to THC greater than one (UNODC 2009). There are currently no minimum or maximum THC-CBD concentrations legally mandated.

Comparison of total THC values, as well as interpretation of trends in most countries, should be taken as relative numbers due to intraspecies differences, inconsistent sampling, and variance in analytical techniques, among other factors affecting total THC concentration and yields. Reported US values can be taken as more, but still relatively, consistent, as they are predominantly based on analyses through NIDA's Marijuana Potency Monitoring Program.

In comparison with THC-predominant strains, fiber strains contain < 1% total THC and have a very low level of psychoactivity (De Backer et al. 2009; Galal et al. 2009). Additionally, due to putative therapeutic effects of CBD, CBD-predominant strains are being developed both domestically and internationally.

Sourcing

Cannabis is cultivated in at least 172 countries (EMCDDA 2008). North America is the largest self-supplying market for herbal cannabis. Europe is the largest consumer market for cannabis resin, which is predominantly supplied by Morocco (EMCDDA 2012).

There are 3 primary sources of indoor and outdoor cultivated cannabis in the United States: 1. Federally legal material; 2. Material that is regulated by select states; 3. Material that is traded illegally according to state or federal law. Sources in other countries vary, with some (e.g., the Netherlands) exerting national controls on the production of cannabis. Despite such national controls, illegal supplies still exist. Sources in the US are briefly described below.

Federally Legal Cannabis: Because cannabis is classified as a Schedule 1 controlled substance, its growth, transport, possession, and use are stringently restricted. The Coy W. Waller Laboratory Complex of the University of Mississippi is the only source of cannabis for research and medicinal purposes that is legally approved federally. Since 1968, the National Institute on Drug Abuse, and its predecessor agency, has contracted with the University of Mississippi (UM) to grow, harvest, and process cannabis and to provide material to licensed facilities across the country for federally approved research purposes. UM also receives and collects samples of cannabis seized by law enforcement to determine the potency of confiscated samples and to document national drug trends. The federal government continues to legally provide cannabis grown by UM for medicinal use to the few remaining patients in the Compassionate Investigational New Drug program started in 1978.

According to federal regulations, transfer of cannabis requires that material originate from a Drug Enforcement Administration (DEA)-registered facility and be sent directly to another DEA-registered facility. DEA-registered facilities that receive or transfer cannabis from or to a non-registered source, risk loss of their DEA registration and criminal penalties.

State-Regulated Cannabis: Numerous states have adopted initiatives allowing the medicinal use of cannabis and provide provisions for growing, accessing, possessing, and using

cannabis. Additionally, Colorado and Washington approved the non-medical use of cannabis in 2014. Regarding medical use, state regulations vary greatly, often varying between counties and municipalities, and often changing. Sometimes, cannabis may be grown by a patient who, based on a physician's recommendation, has been given approval to use cannabis medicinally. In other cases, designated caregivers cultivate cannabis and supply products to individual patients, or to members of a collective. Often, cannabis products are made available to patients through dispensaries. In all cases, the amount that can be grown or possessed is limited, with a variety of restrictions. Federal regulators have formally stated they will only take action against those not complying with state regulations governing the medicinal use of cannabis (Cole 2013), while maintaining their authority to respond when actions are deemed outside of compliance with state regulations. Additionally, Federal policies contend that states do not have the legal right to regulate cannabis. Thus, current exercise of federal policy is inconsistent with state policy and also inconsistently enforced.

Illegally Traded Cannabis: By far the overwhelming majority of cannabis used and traded in the United States is from illegal sources. Most of this material is traded for recreational purposes and lesser amounts are used for medical purposes, either with some basis of legal sanction or for unapproved medicinal use. Federal regulators actively work to curtail the illicit trade of cannabis.

In the United States, it is estimated that 17% of the domestic cultivation of cannabis occurs indoors under controlled conditions (Gettman 2006). Cannabis is grown in substantial quantities in every state within the US. Illicit imports predominantly originate in Mexico and, to a lesser extent, in Canada. This illegal supply primarily fulfills the illicit recreational market, but may find its way into medicinal use (UNODC 2011).

State-regulated or illegally traded cannabis is supplied from material produced either outdoors (in temperate, subtropic, or tropical zones) or indoors throughout all climates. Indoor production of cannabis is concentrated in developed countries, such as in North America, Europe, and Oceania.

Cultivation

There is a plethora of information regarding the cultivation of cannabis. The following information provided is specifically relevant to the development of material to be used medicinally. This information does not take into consideration any of the production methods specifically used to enhance total THC content for recreational purposes, the large number of strains that are bred, or practices employed for fulfilling various recreational desires (e.g. differing organoleptic profiles).

The Ministry of Health, Welfare, and Sport in the Netherlands developed a set of guidelines for the cultivation of cannabis specifically for purposes of medicinal use, all of which is grown indoors. The key guidelines are relevant to outside growing as well and are provided below along with additional information that can contribute to making a quality medicinal product. There are advantages and disadvantages with both indoor and outdoor growing.

Seed and Clone Selection

Selection of seed and clones is based upon both the strain desired and growing environment. Growing from seed results in a portion of the crop being male plants. This can be avoided by starting with clones. Cross breeding of *indica* and *sativa* strains has resulted in the hybrid commonly known as "skunk" which is reportedly 75% *sativa* and 25% *indica* and combines the high THC concentration of *C. sativa* with the growth and yield of *C. indica* (UNODC 2009).

Plant Selection

All material to be propagated, whether from seed or clone, must be identified to genus, species, variety, and chemotype. Plants should be traceable to origin and be free of pests and disease as is practically attainable to ensure healthy growth. Cuttings of female plants are typically used as propagation material for the production of cannabis in order to avoid male plants. Restricting male plants prevents seed fertilization, which allows the female plants to produce more flowers and increased production of resin and cannabinoids. Additionally, plants showing an anomalous concentration of yellow coloring, reflecting a lack of chlorophyll, will not be robust. This can result in misshapen leaves that can curl and turn into each other, and interfere with the growth of the plant. During the entire production process (cultivation, harvest, drying, packaging), the presence of male plants as well as different species, strains, or different plant parts must be monitored and removed if present.

Soil and Fertilization

Cannabis prefers neutral to alkaline loamy and sandy soils, with good water-holding capacity that is not subject to water logging, and an optimum pH of 6.5–7.2. In hydroponic growing, the nutrient solution is best at 5.8–6.0 (Cervantes 2006). Growing mediums for medicinal cannabis should be free of contaminants, such as those introduced from sludge, metals, pesticides, and waste products not required for appropriate growing. If manure is used, it should be thoroughly composted and must be devoid of human feces. Fertilizers should be used in such a way that leaching is kept to a minimum.

Irrigation

Irrigation should be controlled and only applied according to the actual needs of the cannabis plant to prevent over watering. The water used must contain as few contaminants as possible, such as fecal contamination, metals, herbicides,

pesticides and toxicologically hazardous substances (see Limit Tests).

Sexing

Under outdoor growing conditions, plants display all sexual characteristics at approximately 8 weeks, and to maturation from seed. These early, fully formed and receptive pre-flowers are used to determine the sex of the plants, to select seed parents for breeding and for culling if desired. Flowering can occur as early as 4 weeks and is dependent on strain and environmental conditions (UNODC 2009).

Male plants are generally culled, because of the relatively low total THC content of the leaves compared to the inflorescences of the female plant and to prevent pollination of all plants (Chandra et al. 2013). Male plants can be tested for their concentrations of specific cannabinoids (e.g., high or low-yielding THC or CBD strains) and those plants used for breeding depend on the class of cannabinoids desired.

Outdoor Cultivation, Planting, and Maturity

In the Northern Hemisphere, outdoor cultivation of seeds normally begins late March to early April (depending on environment). Full maturity of the plant is typically reached by 6–8 months (depending on variety). The THCA content increases as the plant matures, typically reaching its maximum at full budding stage, maintaining maximum levels for 2–3 weeks after budding, and declining with the onset of senescence. When grown from seeds outdoors, it is difficult to maintain a constant chemical profile due to changing environmental conditions (Chandra et al. 2013), and so some growers (e.g., the Netherlands) only produce approved medicinal products from material cultivated indoors where all conditions can be controlled. Autoflowering strains mature from seed to harvest in approximately 75 days.

There are numerous advantages to outdoor cultivation. Cannabis is relatively resistant to pests so pesticides are seldom needed (McPartland et al. 2000). Growing plants in well cared for soil allows for a more natural growing environment, provides stresses that the plant would experience in a natural environment, allows for natural light cycles, does not require the intensive investment in equipment needed for indoor cultivation, and, when done properly, is more ecologically sound. The primary disadvantages of cultivating cannabis outdoors is the inability to control all growing conditions, many of which affect the chemical profile, purity, and quality, potential for mold, and logistics of harvest and processing. For example, changes in weather may make it unfavorable to harvest when the plant material has reached desired maturity level and cannabinoid profile desired (Potter 2009), or may introduce moisture from rain or fog that could result in damage to the plants when harvest is anticipated. According to one report, cannabinoid and terpenoid profiles of outdoor and indoor cultivated plants were similar if the crops were harvested at the same stage of maturity, as denoted by complete style and stigma senescence. However, as outdoor cultivation requires a longer growing season than plants cultivated indoors, there is greater chance for fungal development (e.g., *Botrytis* spp.), especially in regions with autumn rain or fog (McPartland et al 2000; Potter 2009).

Indoor Cultivation

Indoor cultivation occurs in a variety of locations (basements, warehouses, converted grow houses, etc). The primary advantage of indoor cultivation is that it allows for control of environmental conditions that would otherwise influence cannabinoid profile. However, there are numerous disadvantages to indoor growing. Due to lack of insect predators normally abundant in outdoor growing environments, cannabis grown indoors can be subject to insect infestation, primarily spider mites. This leads to growers utilizing a host of pesticides that can contaminate the medicinal material. Soil composition and nutrient content and distribution in purchased commercial soil mixes may have significantly varied nutrient density that can lead to nutrient deficiencies or excesses that negatively affect the plant. With indoor growing, artificial lighting conditions may also cause burning of the plant. The following parameters are considered critical for indoor cultivation (Chandra et al. 2013).

Light: Cannabis requires high photosynthetic photon flux density (PPFD) for photosynthesis and growth. Because photosynthesis prefers certain wavelengths, PPFD is a more accurate metric than simple irradiance (measured in W/m^2) or light intensity (measured in Lux or Lumens). Chandra et al. 2008 report photosynthesis leveling off at 1500 $\mu mol/m^2/s$ PPFD. Different light sources can be used for indoor propagation, namely, fluorescent light bulbs for juvenile cuttings, and metal halide (MH) and/or high pressure sodium (HPS) bulbs for established plants. MH bulbs impart less PPFD than HPS bulbs per watt. Separate ballasts are required to regulate MH and HPS bulbs. MH and HPS bulbs should be placed 3–4 feet from the plants to avoid overexposure. Photoperiods of 12 and 18 hours are optimum for initiation of flowering and vegetative growth, respectively. Ultraviolet (UV_B) light increases THC yields, although Potter and Duncombe (2012) conclude that the small increase does not warrant human exposure to UVB.

Humidity and moisture: Humidity plays a crucial role in plant growth, starting from seed germination or vegetative propagation/reproduction through budding and harvesting. Juvenile plants require high humidity (ca. 75%), vegetative cuttings require a regular water spray on the leaves to maintain a high humidity in the microclimate until the plants are well rooted, while the active vegetative and flowering stages require 55–60% humidity (Chandra et al. 2013).

Temperature: The optimal temperature for growing any given plant depends on its genetic origin and original growth habitat. However, the photosynthetic maximum for strains of tropical origin is 25–30 °C with a lower maximum of 25 °C for plants of temperate origin (Bazzaz et al. 1975; Chandra et al. 2008; 2011a).

Carbon dioxide: Increased (doubled) ambient carbon dioxide levels stimulate both photosynthesis (50%) and water use efficiency (111%) in cannabis, resulting in increased growth (Chandra et al. 2008; Chandra et al. 2011b).CO_2 enrichment has been used in cannabis glasshouses for more than 35 years.

Irrigation: The optimal amount and frequency of watering needed depends on a variety of factors including environment, variety, and growth stage. Soil should be kept evenly moistened during the early seedling and vegetative stage. In established plants the top layer of soil should be allowed to dry out before watering (Chandra et al. 2013).

Air circulation: Regulation of gas and water vapor exchange affects thermal conductance and energy budget of the leaf and overall growth and physiology of the plants. Electric fans can be used to facilitate the circulation of air (Chandra et al. 2013). Plants exposed to oscillating fans produce stronger stems, which lessens lodging in varieties with heavy apical colas.

Seed Propagation

Seeds are typically planted in moist aerated soil. Germination usually begins after 4 days with all seeds generally germinating within 15 days. For enhanced winter germination, seedling heating mats can be placed under pots. A photoperiod of 18 h of cool fluorescent lights should be used for seedlings. When transferred to larger pots, cool fluorescent lights should be exchanged for full spectrum lights. At the end of the vegetative growth, the photoperiod can be reduced to 12 h to initiate flowering. Flowers should emerge within 3 weeks (Chandra et al. 2013).

Soil Propagation Through Vegetative Cuttings

Cuttings from the lower branches of select female plants can be used for vegetative propagation using a fresh segment of branch (6–10 cm long) that contains at least 3 nodal segments and planted in soil, a liquid hydroponic medium, or for in vitro micropropagation (Chandra et al. 2013).

For soil propagation, cut a soft apical branch at a 45° angle immediately below a node, immediately dip in distilled water to avoid any air bubble formation in the stem, then dip in rooting hormone (e.g., Green Light, US), and plant in pots of a coco natural growth medium with equal parts of sterile potting soil and fertilome (e.g., Canna Continental, US). Cover at least one of the nodes with soil. Irrigate regularly; rooting occurs in 2–3 weeks; after 6 weeks, transplant into larger pots. These can be maintained in a constant vegetative state with 18 h light exposure (Chandra et al. 2013; Potter 2009).

Hydroponics

A small branch consisting of a growing tip with 2 or 3 leaves is cut and immediately dipped in distilled water. Prior to dipping the cutting in a rooting compound, a fresh cut is made just above the first cut. The cuttings are inserted one inch deep into a rockwool cube or a hydroton clay ball supporting medium. Plants are supplied with vegetative fertilizer formula (e.g., Advanced Nutrients, Canada) and exposed to a diffused light: dark cycle (18:6) for vegetative growth. Rooting initiates in 2–3 weeks, followed by transplantation to a larger hydroponic system.

Micropropagation

Seed raised plants are highly heterozygous due to the allogamous nature of cannabis, while vegetative propagation of a selected mother plant can only produce a certain number of cuttings at a time, thus presenting difficulties when large scale cultivation of cannabis is needed. Micropropagation and tissue culture techniques have tremendous potential to overcome these problems. Direct organogenesis using nodal segments and axillary buds is the most reliable method for clonal propagation since it upholds genetic uniformity among progenies (Hartsel el al. 1983; Mandolino and Ranalli 1999; Slusarkiewicz-Jarzina et al. 2005). An efficient micropropagation protocol for mass propagation of drug-type strains using apical nodal segments containing axillary buds has been reported (Lata et al. 2009a; 2009b) as well as the micropropagation of hemp using shoot tips (Wang et al. 2009). Somaclonal variation produced by formation of calli is a fundamental step for the genetic manipulation and improvement in crops (Lata et al. 2002). Micropropagation of cannabis through callus production has been reported, including production of roots through cannabis calli (Fisse et al. 1981), occasional shoot regeneration (Mandolino and Ranalli 1999), and high frequency plant regeneration from leaf tissue derived calli (Lata et al. 2010).

Genetic Integrity

Micropropagation of shoot tips, axillary buds, and nodal cuttings generally maintain their genetic fidelity. However, use of plant growth regulators and prolonged cultivation of the plant can result in somaclonal variation (Chandra et al. 2013).

Diseases and Pests Associated with Cannabis Cultivation

There are a host of pests, bacteria, and fungi associated with both indoor and outdoor cultivation of cannabis. Generally speaking, plants cultivated outdoors in a healthy environment are relatively resistant to pests, so commercial pesticides are often not needed (EMCDDA 2012; McPartland et al 2000), and with indoor cultivation, most conditions can be controlled.

In outdoor cultivation, small animals, such as birds and rabbits can eat sown seeds and emerging greenery. Insects and nematodes are not a significant problem in healthy growing environments that maintain healthy populations

11a.

11c.

11b.

11d.

Figure 11 Cultivation of cannabis at the University of Mississippi

11a–b. Micropropagated cannabis plants.
11c. Rooted plant.
11d. Field-grown cannabis.
Photographs courtesy of: University of Mississippi, University, MS.

11e.

11f.

Figure 11 (continued) Cultivation of cannabis at the University of Mississippi

11e. Partially grown plants.

11f. Fully grown plants.

Photographs courtesy of: University of Mississippi, University, MS.

Figure 12 Common fungal contamination of cannabis

Photographs courtesy of: WAMM, Santa Cruz, CA

of natural predators. The primary concern with outdoor growing, in addition to security and weather, are mold and fungi, which have been addressed in detail by McPartland (1996), among others.

In indoor cultivation systems, the primary pests of concern are spider mites, thrips, aphids and white flies. Growers can use a host of natural (e.g., copper or sulfer sprays, garlic [*Allium sativum*] and neem [*Azadirachta indica*] solutions) or synthetic pest controls, while some companies growing for medicinal preparations (e.g., GW Pharmaceuticals, UK) control indoor pests with natural predators. Application of any treatment has to be timed in a manner that allows the treatment agent to be cleared prior to harvest, as use of commercial pesticides at time of harvest can pose a health risk to consumers, and all treatments can affect the organoleptic profile of the material.

Outbreaks of hepatitis associated with cannabis use have been reported in Germany (Cates and Warren 1975) and Mexico (Alexander 1987), where human excrement was used as a fertilizer.

Harvest

Pre-harvest Considerations

Cannabinoids and terpenoids are predominantly biosynthesized and stored in the trichomes of the plant (Mahlberg et al. 1984; Malingre et al. 1975; Turner et al. 1980a), which are at their highest densities on mature inflorescences. Timing of harvest can be determined based on chemical analysis of the inflorescences, specifically for those compounds that are

Physical Evaluation for Determining Optimal Harvest Time

1. When the largest percentage of styles and stigmas turn-reddish brown and shrivel (senesce) (Chandra et al. 2013; Potter 2009); the higher the percentage of senesced and stigmas and styles, the greater the maturity. Appropriate harvest times based on percent of senesced stigmas and styles are given as 75% (UNODC 2009) and 95% (Clarke 1981), varying according to the variety and the grower's personal preference.
2. Firmness of the inflorescence, which should display a relatively firm resistance when pressed.
3. Some growers suggest that the ideal harvest timing is indicated by the color of the glandular trichomes. The resin head on newly formed trichomes is crystal clear, but eventually turns more cloudy and then almost opaque white before finally turning brown with age. In some cases, trichomes turn brown without experiencing a white phase. Although peak potency and harvest timing is often associated with the clouding of the trichome, research has shown that peak potency is achieved in plants exhibiting clear trichomes (UMiss 2013 personal communication to AHP, unreferenced).
4. Organoleptic profile: At maturity, the aromatic terpenoid composition of the inflorescence matures to the pungent, often unique, strain-specific aroma. Over-maturity can be observed as the inflorescences begin to develop leaves (Corral 2012, personal communication to AHP, unreferenced). The timing of harvest affects the total cannabinoid content (potency), its psychoactive effects, and medicinal benefits.

13a.

13b.

13c.

Figure 13 Maturation of cannabis inflorescences

13a. Maturing female inflorescence showing no styles and stigmas.
13b. Semi-mature female inflorescence showing light-colored styles and stigmas.
13c. Matured female inflorescence showing shriveled reddish-brown styles and stigmas.
Photographs courtesy of: (13a) WAMM, Santa Cruz, CA; (13b & c) © 2013 David J Potter, Salisbury, UK.

most desired (e.g., tetrahydrocannabinolic acid [THCA] and cannabidiolic acid [CBDA]) or more usually through observation and organoleptic evaluation. Cannabinoid ratios of a particular strain of cannabis are genetically determined (de Meijer et al. 2003), while cannabinoid levels (potency), which are determined by biosynthetic pathways, are subject to the influence of age and environmental factors, particularly temperature, light, and humidity. In general, cannabinoid content reaches a maximum when inflorescences are fully ripe and remain at this level until the onset of plant senescence (Chandra et al. 2013; Potter 2009).

When using analytical techniques for determining optimal harvest times, high performance liquid chromatography (HPLC) or gas chromatography (GC) are the most appropriate tools for quantitation of desired compounds. These 2 methodologies can give different quantitative values for the same plant, so consistent baselines with either method should be established to determine the time of maximal potency. Thin layer chromatography (TLC), promoted by commercial testing laboratories, provides a qualitative comparison of cannabinoids, but the method is not quantitative. High performance TLC (HPTLC), can provide more accurate quantitative data than standard TLC, but remains secondary to more accurate methodologies.

All stages of maturity are often present within an inflorescence with mature flowers occurring at the base of the inflorescences and younger, less mature flowers at the apices or tips. Towards the end of the flowering process, plant growth slows and fewer new florets are formed within the inflorescence.

Time of cannabis harvest depends upon which class of compounds is desired (Potter 2009). Total THC content varies widely with the particular strain and part. Analyses by the United Nations (UNODC 2009) report total THC values as highest in the inflorescences (10–12%) followed

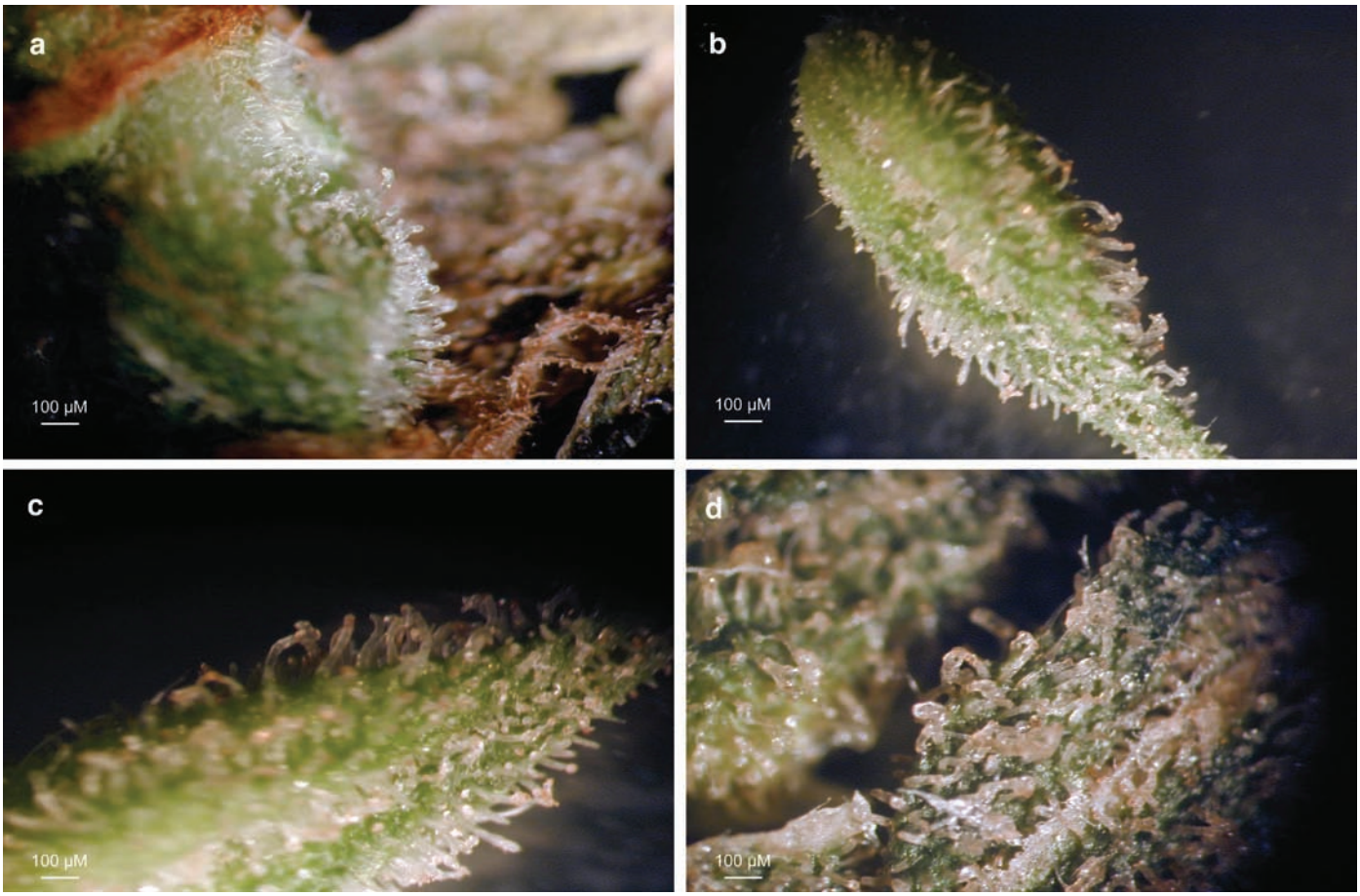


Figure 14 Glandular trichomes of *Cannabis sativa* showing THC-containing ducts at various stages of flowering
14a. 2 weeks.
14b. 4 weeks.
14c. 6 weeks.
14d. 8 weeks post flowering.
Photographs courtesy of: University of Mississippi, University, MS.

15a.

15b.

15c.

15d.

Figure 15 Mechanical trimming of freshly harvested cannabis inflorescences

15a. Hopper feeding freshly harvested inflorescences into the trimmer.

15b. Leaves extend through the openings and are trimmed by blades below the spinning chamber, removing ~90% of subtending leaves.

15c. Trimmed inflorescences awaiting visual inspection before final trimming by hand.

15d. Trimmed inflorescences tumble into catch basin.

Photographs courtesy of: WAMM, Santa Cruz, CA.

by the leaves (1–2%), stalks (0.1–0.3%), and roots (< 0.03%). Cannabinoids are almost completely absent in clean seeds.

The ratio of THCA and CBDA is under strict genetic control. Research suggests that the production of THCA or CBDA, from the common precursor cannabigerolic acid (CBGA), is closely controlled by 2 co-dominant alleles at a single locus (de Meijer et al. 2003). As a result, cannabis plants can be identified as belonging to any one of 3 chemotypes. These can be THCA dominant (homozygous for the THCA synthase allele), CBDA dominant (homozygous for the CBDA synthase allele), or contain an approximately equal mixture of the 2 (heterozygous condition). Cannabis today is almost entirely derived from the THCA dominant chemotype. The majority of seeds sold commercially for the cultivation of recreational cannabis in Europe have been found to be of the homozygous THCA chemotype, with a small minority being the heterozygous mixed profile THCA+CBDA chemotype (EMCDDA 2012).

Over the past 3 decades worldwide, optimization of growing techniques, domestic production versus imported material, and selective breeding and cloning, among other parameters, have focused on the development of increasingly potent THC-yielding strains. Production of high cannabinoid CBDA strains has been of more limited interest, but breeding of CBDA-rich strains has been achieved (de Meijer et al. 2003). An increasing number of heterozygous mixed THCA/CBDA strains are being produced to provide users with material with different pharmacological activity than the pure THCA type; however, this is an exception not a rule in both legal and illegal cannabis production.

Optimal Harvest Times

The optimal harvest time depends on the level of constituents desired and environmental conditions of the crop. Some growers (e.g., University of Mississippi) perform analyses of their raw material daily to determine the optimal time of harvest for peak THCA concentrations. Generally, optimal harvest time is when the inflorescences reach full maturity (Chandra et al. 2013). Optimal harvest time can also be determined visually when at least ~75% of the stigmas turn brown and shrivel (senesce) (UNODC 2009). With higher degrees of maturity, higher concentrations of THC will be produced. However, when resin heads shift from a clear or cloudy color to brown, this indicates the conversion of THC to CBN (Potter 2009)

There are 2 primary ways to harvest inflorescences: harvesting individual buds or branches as they ripen, and harvesting the entire plant. When harvesting individual buds or branches, the mature upper buds are harvested first, usually by cutting approximately 38 cm (15-inch) long branch sections, while the lower branches are given more time to develop (Chandra et al. 2013). Collecting when buds ripen allows other buds hidden in the canopy to ripen, a process that takes approximately 10 days. Buds closest to the outer edges, capturing the most light, typically ripen first.

Harvest is done in 4 primary steps: clipping a bud-filled stem from a plant; clipping the bud from the stem; removing large leaves from the bud; removing small leaves from the bud. Alternatively to harvesting individual ripened inflorescences or branches, whole plants can be harvested and hung upside down in a drying room. The large leaves are removed while the plant is hung and is followed by a manicuring as described below. Drying or storage in unclean barns and other such areas can lead to significant microbial contamination.

Post-harvest Handling

Directly after harvesting, plant material must be processed in a manner that protects it from pests and contaminants, packaged in a manner that prevents damage, dried as soon as possible to prevent chemical degradation, and protected from excess exposure to light and humidity.

Manicuring (trimming): After harvesting the inflorescences, the leaves immediately subtending the buds as well as any dead leaves or stems are trimmed or removed. Manicuring is best accomplished when the inflorescences are fresh for maximum preservation of the trichomes, which when fresh, are pliable rather than brittle: dry trichomes break off easily. Manicuring can be accomplished by hand trimming, machine trimming, or a combination of both.

Budding branches (rather than the entire plant) are harvested and the buds are removed manually and the subtending leaves removed either by hand or with a trimming machine. Machine trimming removes approximately 90% of subtending leaves. If desired, the rest can be removed by hand after the buds are fully dry.

At UM, buds are carefully rubbed through different sized screens (e.g., mesh of ~100 strands per square inch) to separate small stems and seeds. Automated plant processing machines can also be used to separate large stems from the useable biomass.

Manicuring is sometimes done by working over a screen (mesh of ~100 strands per square inch) to allow for collection of the trichomes that fall off in the manicuring process, a technique also used in the processing of hops. The loose trichomes (commonly known as "kief"), have very little vegetation, contain high concentrations of cannabinoids, and can be used in a variety of medicinal preparations. The multi-fingered leaves surrounding the inflorescence are often removed (commonly referred to as "trim"), have more glands than larger lower leaves, and yield a higher concentration of cannabinoids. This post-harvest processing should be conducted in cool temperatures with good air circulation to prevent molding.

Drying

When drying medicinal plants, great care must be taken in the drying process (Chen and Mujumdar 2006), both for preservation of putative medicinally relevant compounds

16a.

16b.

16c.

16d.

Figure 16 Drying cannabis

16a. Freshly harvested inflorescences drying on screens.

16b. Drying leaves to be used in tincture and edible preparations.

16c. Moldy leaves.

16d. Hang-drying whole plants.

Photographs courtesy of: WAMM, Santa Cruz, CA.

and to reduce the risk of molding. Drying is usually done by either cutting the flowering tops from the plant or by hanging the entire plant upside down in shaded areas.

In the production of medicinal cannabis at UM, a commercial tobacco drying barn (e.g., BulkTobac, Gas-Fired Products, Inc., US), is used and material is dried at 40 °C for 12–15 h. Prior to drying, larger leaves and stems are removed from the mature buds. The buds can be dried whole or halved or quartered for quicker drying. The material is fully dried when the central stem of the floral cluster snaps, when bent, rather than remaining pliable (Chandra et al. 2013).

Varying drying practices are employed by state-approved growing facilities. These practices are predominantly designed to preserve maximum cannabinoid content and a myriad of organoleptic characteristics. Numerous references (e.g., Cervantes 2006; Clarke 1981; Rosenthal 2010; among others) describe a multi-step process of curing and drying in much the same way that tobacco leaves are prepared. When drying by hanging, drying is complete when the leaves next to the flowering tops are brittle and the central stem snaps. This takes from 24–72 hours, depending on temperature and humidity. The moisture content of such plants is usually 8–13% (UNODC 2009). Many growers use fans and or heaters to maintain some control over the drying environment. Following are commonly employed drying practices.

Initial Drying: After the inflorescences are harvested and initially processed (trimmed), they are typically placed in single layers in boxes, on breathable trays, or screens that allow for steady airflow in a well-ventilated area. The initial drying is done for approximately 3 days at a temperature of approximately 15–21 °C and a humidity of approximately 35% until the inflorescences reach 25% of their original weight. Heaters are typically required to maintain a consistent temperature, fans are typically used to maintain a constant airflow, and sometimes dehumidifiers are used to remove moisture. Buds that are dried too quickly retain a greater amount of chlorophyll, which changes the qualitative organoleptic characteristics of the material (Corral 2012, personal communication to AHP, unreferenced). Excess humidity encourages molding.

Reports from state-approved markets indicate a preference for all but the tiniest leaves to be completely removed. However, some state-approved growers (e.g., in California) consider it advantageous to keep the surrounding leaves intact until the material is to be used. This creates a protective covering that shields the trichomes from damage in storage (Corral 2013, personal communication, unreferenced).

Final Drying: After the initial drying process, the inflorescences are often placed in plastic bags or glass containers, are initially closed and then opened every 12–24 h for 1–2 weeks until the material is completely dried. This allows the moisture that remains inside the buds to evaporate. Drying is sufficient when the small stem attached to the inflorescence snaps easily. If the stem bends, too much moisture remains. When completely dried, the inflorescences contain approximately 10% moisture (Clarke 1981). If not properly dried, mold can form over a several month period and is evident by smell, graying of color, a slippery feel, and loss of firmness. If dried completely in papsmither bags or on open trays, the outside of the inflorescence turns brittle, while the inside remains moist, increasing the potential to mold.

When drying, plants should be protected from light and should be minimally handled as the inflorescences bruise easily during handling. Bruised tissue will turn dark green or brown upon drying (Clarke 1981). At 45–55% humidity, buds will dry gradually over 1–2 weeks depending on inflorescence size. Humidity can be lowered to 20–40% to hasten drying times. Proper drying maintains the terpenoids, which give cannabis its characteristic organoleptic qualities. Improper drying, such as at high temperatures, dramatically alters the organoleptic profile.

Over time, decarboxylation of the cannabinoid acids occurs in dried flowers. The process is expedited by heat. During this period, cannabinoid acids decarboxylate into the psychoactive cannabinoids, and terpenoid isomerize to create new polyterpenes with tastes and aromas different from fresh floral clusters (Clarke 1981).

According to the Dutch Office of Medicinal Cannabis (OMC 2003), the moisture content of cannabis prior to packaging must be between 5–10%. Dutch consumers have reported a more pleasant flavor when the moisture content of buds is approximately 8%.

In the UK, in one investigation, plants were spread evenly on the floor of a well-ventilated drying room at a depth of approximately 15 cm. Gas burners maintained a constant temperature of 40 °C to a moisture content of approximately 15% (+/- 2%) and took 24 h. In another experiment, plants were hung from wires in the same drying room at 30, 40, and 50 °C. Mean times to achieve a finished moisture content of 15% were approximately 36, 18, and 11 h, respectively. Alternatively, the same cultivars dried in a glasshouse crop drying facility at 25 °C, but with different ventilation, took 4.5–5 days to reach the same moisture level. These latter prolonged drying conditions resulted in fungal and bacterial growth. Additionally, plants initially showing preliminary signs of fungal or bacterial damage further deteriorated under these conditions (Potter 2009).

Packaging

In the Netherlands, packaging of medicinal materials is done according to the European Pharmacopoeia Chapter 5.1.4 Microbiological Quality of Pharmaceutical Preparations and Substances For Pharmaceutical Use. These guidelines are specific to medicinal preparations used for inhalation, specifically to prevent microbial exposure. To reduce microbial loads, Netherlands cannabis may be subjected to gamma irradiation (dose < 10 kGy). Use of irradiation for ingredients in the US requires specific approval. Ungerleider et al. (1982), demonstrated that 15-20 kGy killed bacterial contaminants (*Klebsiella*, *Enterobacter*, and *Enterococcus*

spp.) in NIDA-sourced cannabis. In comparison, packaged meat and poultry may be irradiated with 70 kGy.

Treatment with irradiation of other medicinal plants (e.g., *Digitalis*, *Ephedra*, etc.) has been shown to negatively effect constituent profiles (Samuelsson 1992) and in other plant material to specifically lower terpenoid levels (e.g., cilantro, oranges) (Fan and Gates 2001; Fan and Sokarai 2002). Thus irradiation may similarly negatively affect the general composition and specific terpenoid profile of cannabis.

Storage

Once cannabis is properly dried, degradation of the primary cannabinoids is negligible, if protected from air and light and the material can remain active for many years. The UM produced material is stored in FDA approved polyethylene bags placed in sealable fiber drums. If stored for short periods of time, a storage temperature of 18–20 °C is used; for long-term storage a temperature of -20 °C is used. However, some sources (eg., Clarke 1981) suggest that freezing damages trichomes.

THC is especially sensitive to degradation by oxygen and light (Chandra et al. 2013) and decarboxylation of THCA to the active THC occurs in storage (Hazekamp 2007). Over time, the concentration of THC in cannabis products decreases slowly, while the concentration of CBN increases (Chandra et al. 2013; Ross and ElSohly 1999). In one experiment, approximately 90% of the THC content of dried plant material was still present after storage for 1 year at room temperature in the dark (Fairbairn et al. 1976). According to the same experiment, storage temperatures of up to 20 °C had little effect on stability of THC. Further evidence of cannabinoid stability was provided in an analysis of 3 dried samples from the turn of the 20th century, which were stored at room temperature with some possible exposure to light. The analysis detected trace amounts of THC, THC acid (1.39-1.79%), traces of other cannabinoids, and significant amounts of CBN (17.26–44.51%) and CBN acid (7.19–10.95%) (Harvey 1990).

A number of popular sources (e.g., Clarke 1981) recommend against freezing, which can cause the trichomes to become brittle and break off with handling. For the same reason, handling of dried material should be kept to a minimum. Additionally, according to Fairbairn et al. (1976), excessive handling of the inflorescences causes them to rupture exposing the cannabinoids to oxidation even when protected from light.

The stability of a 140-year-old ethanolic cannabis extract has been investigated (Harvey 1985). Using gas chromatography, it was shown that, while traces of THC, CBD, and CBC were present, most of the THC had decomposed to CBN. Additionally, cannabitriols were also present.

Natural Contaminants and Adulterants

Due to its widespread cultivation, there is little concern regarding adulteration of the plant itself. However, the large economic potential and illicit aspect of cannabis has given rise to a number of reported potentially hazardous natural contaminants or artificial adulterants in crude cannabis and cannabis preparations.

Natural contaminants: Several plant species have morphological characteristics comparable to *Cannabis* species, e.g., *Hibiscus cannabinus* (kenaf), *Acer palmatum* (Japanese maple), *Urtica cannabina* (an Asian species of nettle), *Dizygotheca elegantissima* (false aralia), *Potentilla recta* (sulphur cinquefoil, rough-fruited cinquefoil), and *Datisca cannabina* (false hemp), leading to occasional contamination of cannabis internationally (UNODC 2009). However, these plants can be readily differentiated from cannabis by inspection of their macroscopic and microscopic characteristics. More commonly, natural contaminants consist of degradation products, microbial (fungi and bacteria) contamination, and heavy metals. These contaminants are usually introduced during cultivation and storage (McLaren et al. 2008; McPartland 2002).

Adulterants: Growth enhancers and pest control chemicals, introduced during cultivation and storage, are possible risks to the producer and the consumer (McPartland and Pruitt 1999). There are anecdotal reports of the use of banned substances such as daminozide (Alar), the degradation product of which is the highly toxic hydrazine. Cannabis can also be contaminated for marketing purposes. This usually entails adding substances, e.g., tiny glass beads, to increase the weight of the cannabis product, or adding psychotropic substances, e.g., tobacco, calamus (*Acorus calamus*), and other cholinergic compounds, to enhance the efficacy of low-quality cannabis or to alleviate the side effects of cannabis (McPartland et al. 2008; McPartland 2008).

In the Netherlands, chalk and sand have been used to make cannabis appear to be of higher quality, the sand giving the appearance of trichomes. In the UK, similar adulterations have been made by adding glass beads with a similar diameter to trichome resin heads to cannabis (Randerson 2007). In Germany, lead has intentionally been added to street cannabis to increase its weight. Lead is readily absorbed upon inhalation and this adulteration resulted in lead intoxication in at least 29 users (Busse et al. 2008). Additionally, in the Netherlands, 2 chemical analogs of sildenafil (Viagra) were found in cannabis samples. In the UK, other contaminants including turpentine, tranquilizers, boot polish, and henna, have been reported (Newcombe 2006).

In recent years, various products laced with synthetic cannabinoids have appeared on the market. These are believed to mimic the effects of cannabis. These products are known by various names (e.g., “Spice” and “K2”) and might be sold as “incense” or “natural smoking blends”.

Like cannabis, some of these synthetic cannabinoids are Schedule 1 restricted substances. The Spice blend is reported to contain synthetic cannabinoids with a mixture of otherwise legal, safe, and non-psychotropic herbal dietary supplement ingredients including: damiana (*Turnera diffusa*), Chinese motherwort (*Leonurus japonicus*), and water lily (*Nymphaea* spp.). According to the National Institute on Drug Abuse (NIDA 2012), those using some of these various blends have been admitted to Poison Control Centers and report "rapid heart rate, vomiting, agitation, confusion, and hallucinations. Spice can also raise blood pressure and cause reduced blood supply to the heart (myocardial ischemia), and in a few cases it has been associated with heart attacks. Regular users may experience withdrawal and addiction symptoms."

Qualitative Differentiation

Cannabis used for medicinal purposes should be as free from foreign matter as practically possible (see Limit Tests). Medicinal material should be free of molds and bacteria that have a high likelihood of pathogenicity (e.g. *Aspergillus* spp., *E. coli* (O157:H7). Visible mold should be absent, material should be free of stems greater than 1.5 cm, only subtending leaves should be present, material should be free of metals to the degree allowed by a naturally occurring growing substrate, and free of pesticides and fungicides that could present a health hazard to the consumer. Microbial standards should be adopted based on those required for non-sterile pharmaceutical preparations for use by inhalation (see European Pharmacopoeia 8.0: section 5.1.4). Color should be consistent throughout each sample and should not show signs of gray or black, which are indicators of fungal infection.

For medical users of cannabis, there is a balance sought between organoleptic qualities (taste and aroma) and medicinal effect, as well as a balance between THC- and CBD-yielding cultivars. Many cultivators select, breed, and process for these varying qualities. For medicinal purposes an optimal ratio between total THC, Δ^9-THC, and/or CBD has not been definitively determined. Different health conditions may respond differently to plants containing different ratios of the 2 primary cannabinoids. For example, evidence indicates that CBD is responsible for some of the putative anxiolytic and anti-schizoprenic effects of the plant (Mechoulam et al. 2002; Zuardi et al. 2002) while THC has been associated with appetite stimulation (Dejesus et al. 2007; Nelson et al. 1994). The process of trimming is done both for yielding higher concentrations of THC and for yielding more desirable, organoleptic qualities, since the leaves possess a sharp and bitter organoleptic characteristic. A better organoleptic profile may enhance compliance.

Dispensaries should maintain strict quality control practices to ensure the purity and quality of their material by contracting for testing with independent labs that apply independently verified testing methodologies and transparent testing standards. Individual growers and care givers producing medical cannabis for personal use should employ good agricultural practices (GAPs) to the extent possible in all aspects of growing, harvesting, drying, and storage.

Documentation Guidelines (OMC 2003)
a. Location of cultivation and the name of the supervising cultivator.
b. Details on crops previously grown at that location.
c. Nature, origin and quantity of the herbal starting materials.
d. Chemicals and other substances used during cultivation, such as fertilizers, pesticides, and herbicides.
e. Standard cultivation conditions, if applicable.
f. Particular circumstances which occurred during cultivation, harvesting, and production that may affect the chemical composition, such as plant diseases or temporary departure from standard cultivation conditions, particularly during the harvesting period
g. Nature and quantity of the yield.
h. Date or dates and time or times of day when harvesting occurred.
i. Drying conditions.
j. Measures for pest control.

Sustainability and Environmental Impact

As all cannabis is derived from cultivated sources, there is little risk of the plant becoming environmentally threatened unless aggressive eradication programs are implemented worldwide. However, without development, implementation, and enforcement of Good Agricultural Practices (GAPs), both indoor and outdoor cannabis cultivation can have significant negative environmental and social impacts (Montford and Small 1999). Environmentally, the illegal diversion of water, clear cutting of trees, dumping of chemicals, misappropriation of state and federal lands, and disruption of sensitive ecosystems are associated with outdoor cultivation, while high carbon emissions are associated with indoor production. In North America, especially with crops grown indoors, part of this environmental impact is driven by the illegality of cannabis cultivation that requires growers to hide crops. Others may choose indoor growing for greater control over crops and higher yields. The high-energy intensive processes associated with controlling all aspects of the indoors growing environment has been estimated to consume 1% of the national electricity use (Mills 2011). Whether by regulation or choice, growers should apply GAPs to cannabis cultivation.

In addition to the impacts of cannabis cultivation, the manufacture of butane extracts poses significant risks. A number of explosions and fires associated with home cannabis extract production have been reported, some that have included injury. Industrial grade butane contains

Table 3 Cannabis plant groups and typical Δ⁹-THC/CBD concentration and ratios

		THC	CBD	THC:CBD Ratio
I	Drug	0.5–15%	0.01–0.16%	50:1
II	Intermediate	0.5–5%	0.9–7.3%	0.25/~ 2
III	Fiber	0.05–0.70%	1.0–13.6%	< 1:5
IV	CBG	< 0.05%	< 0.5%	-
V	Non-cannabinoid	0	0	-

Source: Modified from Galal et al. (2009). Note: THCA-predominant strains can yield in excess of 25% Δ⁹-THC; specially selected CBDA clones can yield up to 20% CBD.

compounds that may not be desirable in finished products. Extraction with CO_2 (sub- or super-critical) is preferred by some and is one environmentally safe extracting option.

Documentation of Supply

For cannabis that is to be used in medicinal preparations, every aspect of cultivation, harvest, processing, and storage should be documented to the fullest extent possible. Various county and state ordinances require adherence to specific regulations that differ between locations for trade of cannabis among growers, dispensaries, and collectives. The Dutch OMC provides the following guidelines for documentation as follows (also see inset page 32).

Security (modified from OMC 2003)

The buildings in which cannabis is cultivated, processed, packaged and stored must be sufficiently secured, only allowing authorized personnel access to the buildings. Personnel involved in the production process of cannabis must be authorized for that purpose by the employer. Waste must be stored in such a way that the potential for theft is minimized.

Suppliers and Dispensaries

Cannabis products supplied by dispensaries should be as fully characterized as possible with traceability and a verifiable chain of custody to type of material, whether the plants were cultivated conventionally or organically, or were indoor or outdoor cultivated. Procedures should be implemented to ensure the absence of pesticides and raw material and finished product should be characterized as to its basic chemical profile (e.g., THC and/or CBD content). This information should be made available to patients upon request. Dispensary personnel should be appropriately trained in how to process and handle cannabis to ensure purity, maintain quality, and to morphologically identify material. The cannabis committee of the American Herbal Products Association (AHPA) has developed a set of draft guidelines outlining recommended practices for dispensaries and cultivators to follow (AHPA 2013a), and Americans for Safe Access (ASA) has developed an industry certification program for dispensaries and cultivators (ASA 2013).

Constituents

To date, more than 750 different constituents have been identified in cannabis. The diversity of constituents encompasses numerous phytochemical classes, notably, cannabinoids, and a host of other secondary metabolites. These other compound classes include terpenoids, non-cannabinoid phenols, nitrogenous compounds, as well as other more common plant compounds, all of which are non-psychotropic. Cannabinoids are the most studied and well-known chemical constituents of cannabis. Of these, THC has received the most attention, since it is the principal psychoactive component of the plant. Cannabinoid acids lack psychoactivity. Therapeutic activity is not limited to cannabinoids. Emerging research suggests that other minor compounds (e.g., terpenoids) may also play a role in the complex pharmacology of this botanical, either directly or through modulation of cannabinoid responses (reviewed in Russo 2011) (see Table 6). Additionally, research on the non-psychoactive acid cannabinoids has been limited due to the overriding interest in decarboxylated THC (Mechoulam 2013, personal communication, unreferenced).

Cannabinoids

Cannabinoids (CBs) are a class of more than a hundred terpenophenolic compounds, most commonly associated with the pharmacological activity of cannabis. Several main structures are distinguished (Tabel 6). The term "phytocannabinoids" (Pate 1994) has been used to designate naturally occurring cannabinoids in cannabis; however, the discovery of compounds from other plants (e.g., *Echinacea* spp.) also have CB-receptor activity and, thus, can be named "phytocannabinoids." A synonymous term "exocannabinoids" is used to distinguish phytocannabinoids from endocannabinoids, the endogenous ligands to cannabinoid receptors. "Classical" and "non-classical" cannabinoids refer to synthetic cannabinoid receptor agonists (Makriyannis et al. 2005) and indicate the relative degree of structural similarity with phytocannabinoids.

Cannabinoids mainly exist in the plant as carboxylic acids and are converted to neutral analogs by light and heat while in storage (Veress et al. 1990) or when combusted. The alkyl group at the third carbon atom (C-3) is considered an important site in substrate-receptor interactions (Loewe

1944; Pertwee et al. 2010). This group is typically a pentyl—for example, in Δ^9-THC, cannabigerol (CBG), cannabidiol (CBD), and cannabinol (CBN)—but can also be a propyl, in which case the compounds are named by attaching the suffix -varin to the name of the pentylated analog, e.g., tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabigerovarin (CBGV), and cannabivarin (CBV).

Cannabis plants typically exhibit one of 3 main distinctly different chemotypes based on the absolute and relative concentrations of Δ^9-THCA and CBDA (after conversion from the respective acids). Small and Beckstead (1973) refer to these as drug-type, intermediate type, and fiber-type plants. Plants with more rare chemical profiles have been identified, including those predominant in cannabigerol (CBG) (de Meijer and Hammond 2005) or tetrahydrocannabivarin (THCV), and those lacking any cannabinoids (de Meijer et al. 2009), for a total of 5 general types (Table 3).

The cannabinoid profile is affected most by the plant's sex, genotype (de Meijer et al. 1992; 2003), and maturity (Small et al. 1976), followed by environmental and other factors, such as light intensity, light cycle (Valle et al. 1978), temperature (Chandra et al. 2008), and fertilization (Bocsa et al. 1997). Cannabinoids are produced in glandular trichomes distributed across all epidermal surfaces of the plant's aerial parts in varying degrees. The distribution of glandular trichomes and, hence, phytocannabinoids varies widely, from the lowest concentrations found in stems to increasing amounts in large leaves, subtending leaves of the inflorescences, the inflorescences, and to the highest concentrations found in female flower bracts.

Cannabinoids are highly lipophilic, permeate cell membranes, and have the ability to cross the blood-brain barrier both when inhaled and ingested.

Following is a review of major and minor cannabinoids primarily associated with the psychoactive and pharmacological effects of cannabis. Not all compounds will be found in every plant sample, and the ratios of the compounds will vary. THC is generally the most abundant cannabinoid in contemporary horticulture of cannabis, due to the focus of growers on high THC yielding strains, specifically for enhanced psychoactive effects.

Cannabinoid Acids

Cannabinoids occur in living plants mainly in carboxylated form. Cannabigerolic acid (CBGA), derived from olivetolic acid and geranyl pyrophosphate (Fellermeier and Zenk 1998), is the precursor of all other major cannabinoid acids—THCA, CBDA, and cannabichromic acid (CBCA)—as well as their analogs and biogenic derivatives (Yamauchi et al. 1968). Two THC acids are present in cannabis and differ in the position of the carboxyl group THC acid-A (Korte et al. 1965) and THC acid-B (Mechoulam et al. 1969). Both are non-psychotropic and their pharmacology is almost unknown (Mechoulam 2013, personal communication, unreferenced). In fresh, unheated plant material, virtually no neutral (non-carboxylated) compounds have been found (Verhoeckx et al. 2006), despite cannabinoid acids being readily thermo- and photolabile (Hazekamp 2007; Johnson et al. 1984). The THCA-Δ^9-THC ratio in leaves and flowers of the female plants has been reported to be from 2:1 (these days rarely) to 20:1 and higher, depending on the variety (strain) tested (e.g., Brenneisen 1984; Pitts et al. 1992, among others). Higher THCA-Δ^9-THC ratios are more typical and are often found even in dried, one-year-old plant material (Wurzer and Dixon 2013, personal communication, unreferenced). Heating for 5 minutes (min) at 200–210 °C has been reported to be effective for conversion to occur, but slow decarboxylation occurs also at room temperature (Brenneisen 1984). An aqueous decoction of cannabis (simmered for 15 min) retained a large THCA-Δ^9-THC ratio (Hazekamp 2007).

Cannabinoid acids, including THCA, are devoid of psychotropic effects (Burstein 1999; Dewey 1986). Medical users report health benefits from modes of cannabis consumption that do not use combustion or high temperatures (certain kinds of foods, capsules, infusions, juices), thus preserving most of the cannabinoids in their acid forms. Little specific pharmacological investigation of THCA has been published to date, but immunomodulatory activity of THCA has been reported (Verhoeckx et al. 2006).

Major Cannabinoids

Δ^9-Tetrahydrocannabinol (Δ^9-THC) Type Phytocannabinoids

Δ^9-THC was isolated in 1964 (Gaoni and Mechoulam 1964a), and additional THCs were identified by 1980 (e.g., reviewed in Suurkuusk 2010, among others), followed by almost 3 decades before a series of 8 Δ^9-THCA terpenoid esters were isolated from high-potency cannabis (Ahmed et al. 2008b). THCA, commonly the primary cannabinoid of this group existing in the plant, is synthesized from CBGA by THCA synthase, which is abundantly present in glandular trichomes. Δ^9-THC is a product of THCA decarboxylation, usually formed via degradation (such as during storage) or heating (vaporization or combustion). THCA is typically the predominant cannabinoid in cannabis strains that exhibit psychoactivity, but it also occurs in low levels in fiber-type plants (Table 3).

THC has a high affinity for cannabinoid receptors of both type 1 (CB_1) and type 2 (CB_2), and is thought to behave as their partial agonist, similar to the endocannabinoid anandamide (Howlett et al. 2010; Pertwee 2008). The primary natural isomer, (−)-trans-Δ^9-THC, displays a higher potency compared to the other isomers (e.g., (+)-trans-) or enantiomers (e.g., (3R,4R)-Δ^1-THC) (Mechoulam et al. 1990) and is used preferentially in clinical trials. THC has been used as an antiemetic in chemotherapy-associated nausea and emesis; as an appetite promoter, especially for AIDS and cancer patients who are prone to severe weight loss due to anorexia and anorexia-cachexia, respectively; as an analgesic, e.g., for cancer, damaged nerves, migraine, spinal cord injury, postoperative, dental, and phantom limb pain; for treatment and symptom management of neurological disorders such as

multiple sclerosis (Fox and Zajicek 2002; Rog et al. 2005). Its utility for treating glaucoma is limited by the high dosage needed to lower intraocular pressure, and its short duration of action in this condition (Buys and Rafuse 2010).

Tetrahydrocannabivarin (THCV) is the propyl homolog of Δ^9-THC and usually occurs in cannabis in minor amounts, although THCV-rich strains (up to about 16% dry weight in selected inflorescences) have been developed (de Meijer 2013, personal communication to AHP, unreferenced). This cannabinoid is a CB_1 neutral antagonist at low doses (Gill 1971; Thomas et al. 2005) and agonist at both CB_1 and CB_2 receptors at high doses (Bolognini et al. 2010; Thomas et al. 2005). Anticonvulsant, anti-inflammatory, and analgesic properties have been reported for THCV (Bolognini et al. 2010; Hill et al. 2010). A recent study reported antioxidant and potential neuroprotective effects of THCV in an experimental Parkinson's disease model in mice, suggesting utility in the amelioration of Parkinsonian symptoms, in part via activation of CB_2 (Garcia et al. 2011).

Cannabidiol (CBD) Type Phytocannabinoids

Cannabidiol (CBD) and cannabidiolic acid (CBDA) are the main non-psychotropic cannabinoids in cannabis and are the most abundant cannabinoids in European hemp. Cannabidiol was isolated in 1940 (Adams et al. 1940b), with its structure determined in later studies (Mechoulam and Shvo 1963; Mechoulam and Gaoni 1967; Petrzilka et al. 1969). Cannabidiolic acid, cannabigerolic acid, and cannabinolic acid were first isolated by Mechoulam and Gaoni (1965). To date, 8 CBD-type phytocannabinoids have been identified (Shoyama et al. 1972a; Sirikantaramas et al. 2007).

Cannabidiol lacks the cognitive and psychoactive properties of THC and displays a very low affinity for cannabinoid receptors (Thomas et al. 2007). Research has focused on identifying CB_1- and CB_2-independent mechanisms of CBD action. Cannabidiol is known to be an agonist at serotonin (5-HT1A) receptors (Mishima et al. 2005; Russo et al. 2005) and transient receptor potential vanilloid type 1 (TRPV1) receptors (Bisogno et al. 2001; McHugh et al. 2010). Cannabidiol can also enhance adenosine receptor signaling by inhibiting adenosine inactivation, suggesting a potential therapeutic role in pain and inflammation (Carrier et al. 2006). Some of the pharmacological actions of CBD include anticonvulsive, anti-inflammatory, antioxidant, antipsychotic, hypnotic, and sedative (at very high doses). The antioxidant and anti-inflammatory properties account for the neuroprotective actions of CBD (Scuderi et al. 2009), which could potentially be utilized for the treatment and symptom relief of a number of neurological disorders, e.g., epilepsy and seizures (Hofmann and Frazier 2013; Jones et al. 2010), psychosis (Zuardi et al. 2006), anxiety (Bergamaschi et al. 2011), movement disorders (e.g., Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis) (de Lago and Fernandez 2007; Iuvone et al. 2009), Alzheimer's disease (Martin-Moreno et al. 2011), and multiple sclerosis (Lakhan and Rowland 2009). Cannabidiol has demonstrated an exceptional tolerability in humans, making it a potential candidate for clinical application or as a lead compound for the development of cannabimimetic drugs (Mechoulam and Hanus 2002).

Cannabigerol (CBG) Type Phytocannabinoids

Cannabigerolic acid (CBGA) is a direct precursor to THCA, CBDA, and cannabichromenic acid (CBCA) (Gaoni and Mechoulam 1964b, 1966; Taura et al. 1995a, 1995b, 1996). It is typically present in cannabis only in minute amounts, though in some cannabis this class of cannabinoids may be dominant (de Meijer et al. 1992), and cannabis plants that produce CBG as the primary cannabinoid have been cultivated (de Meijer and Hammond 2005). To date, 16 CBG-type cannabis constituents have been identified (DeBacker et al. 2009; ElSohly and Slade 2005; Turner et al. 1980b), including cannabigivarin (CBGV). CBGV is the biosynthetic precursor of THCV and is reputedly found in higher concentration in some feral accessions from India (Hillig and Mahlberg 2004; Vollner et al. 1969). While there is little research to date on CBGV, there are indications of anti-inflammatory action associated with THCV (Tubaro et al. 2010) and activation of CB_2 receptors on mesenchymal cells (Izzo et al. 2009).

CBG-type cannabinoids are non-psychoactive cannabinoids that generally act as weak ligands at both CB_1 and CB_2 receptors (Costa 2007; Fisar 2009; Eisenstein et al. 2007; Gaoni and Mechoulam 1964b; Pollastro et al. 2011). Cannabigerol is a GABA uptake inhibitor with more potent effects than THC or CBD (Banerjee et al. 1975). It is a potent alpha$_2$-adrenocorticotropic receptor agonist (Cascio et al. 2010), a potent antagonist of transient receptor potential cation channel subfamily M member 8 (TRPM8) (De Petrocellis et al. 2008), and has been shown to have some uptake-inhibitory activity at 5-HT1A receptors (Banerjee et al. 1975; Rock et al. 2010). This latter action is responsible for countering the anti-emetic effects of CBD (Rock et al. 2010). Additionally, this cannabinoid has demonstrated antimicrobial activity (Appendino et al. 2008), inhibited proliferation of keratinocytes (Wilkinson and Williamson 2007) and cancer cells (Ligresti et al. 2006), and was shown to have greater analgesic activity than THC (Cascio et al. 2010; Evans 1991). These actions suggest that CBG may have a therapeutic potential, e.g., as an antidepressant or for the treatment of psoriasis (Wilkinson and Williamson 2007). The presence of CBG has also been found in *Helichrysum umbraculigerum* (Woelkart et al. 2008).

Minor Cannabinoids

Δ^8-Tetrahydrocannabinol (Δ^8-THC) Type Phytocannabinoids

This group has only 2 compounds, namely, (–)-Δ^8-THC and (–)-Δ^8-tetrahydrocannabinolic acid A ((–)-Δ^8-THCA A). Δ^8-THC is stable in air, and is less psychotropic than Δ^9-THC, making it a viable option as a therapeutic alternative to Δ^9-THC. At low doses, Δ^8-THC (0.001 mg/kg po) is capable of inducing appetite stimulation without psy-

chotropic effects such as alterations in cognitive function (Avraham et al. 2004).

Cannabielsoin (CBE) Type Phytocannabinoids

To date, 5 compounds of this type have been identified, including cannabielsoin (Bercht et al. 1973), cannabielsoic acids (CBEA) A and B (Shani and Mechoulam 1970, 1974), and 2 additional isomers (Hartsel et al. 1983). Cannabielsoic acids and CBE are not always found in natural sources and can be obtained by photooxidation or pyrolysis of naturally occurring CBDAs and CBDs (Kueppers et al. 1973). Rather, these compounds are found in processed cannabis products such as hashish and may be artifacts of other naturally occurring phytocannabinoids (Bercht et al. 1973; Grote and Spiteller 1978a; Kueppers et al. 1973; Shani and Mechoulam 1974). Cannabielsoin is found in mammals as a metabolite of CBD (Yamamoto et al. 1988).

Cannabitriol (CBT) Type Phytocannabinoids

(–)-Cannabitriol was isolated from cannabis grown in Japan (Obata and Ishikawa 1966). Other related cannabitriols (e.g., 6α,7,10α-trihydroxytetrahydrocannabinol, 9,10-epoxycannabitriol) were identified in pollen grains (Ross et al. 2005). Cannabidiolate, 9-O-CBT, was isolated from hashish (Von Spulak et al. 1968).

Cannabichromene (CBC) Type Phytocannabinoids

In the 1970s, CBC was reported to be the second most abundant cannabinoid in some strains of cannabis growing in the United States (Holley et al. 1975), but this may be attributed to past difficulties distinguishing CBC from CBD. To date, a total of 8 CBC-type phytocannabinoids have been identified (Radwan et al. 2009). Usually CBC is present in minor amounts due to its biosynthetic enzyme being produced by a recessive gene (de Meijer and Hammond 2005), although high CBC plants have been selectively bred. This compound is also present in a higher concentration in juvenile cannabis plants, and may be concentrated into an "enriched trichome product" (Potter 2009). More recently, cannabinoids of this type were isolated from high-potency cannabis (Radwan et al. 2009) (concentrations not reported).

Cannabichromene interacts with TRPV channels, having a strong affinity for TRPV1, but has poor affinity for the CB_1 receptor (Booker et al. 2009; DeLong et al. 2011; De Petrocellis et al. 2011). The compound is known to produce anti-nociceptive and anti-inflammatory effects in rodents (Davis and Hatoum 1983; Turner and ElSohly 1981; Wirth et al. 1980). Three cannabinoids of this type have been reported to have antimicrobial and moderate anti-leishmanial activities, while lacking cytotoxicity against African green monkey kidney fibroblast cell line Vero (Radwan et al. 2009).

Table 4 Content ranges of major and minor cannabinoids in cannabis and their degradation products

Compound	% dry weight
Δ^9-THC	0.1–25
CBD	0.1–7.98
CBN	0.0–1.6
THCV	0.0–1.36
CBG	0.03–1.15
CBC	0.0–0.65
Δ^8-THC	0.0–0.1

Source: Modified from McPartland and Russo (2001) with additional data from Fischedick et al. (2010); Fournier et al. (1987); Pitts et al. (1992); Small (1979); and Veszki et al. (1980).

Degradation Products and Artifacts

Cannabinol (CBN) Type Phytocannabinoids

Cannabinoids of this type are fully aromatized derivatives of THC, and, although they have been isolated from different cannabis extracts (Bercht et al. 1973; Harvey 1976; Mechoulam and Gaoni 1965; Wood et al. 1896), they are believed to be artifacts (Bowd et al. 1975) obtained by non-enzymatic oxidation of THC. Some of the reported levels in dry plant material are summarized in Table 4. There are 10 known CBN-type cannabinoids (Adams et al. 1940a; Cahn 1932; Ghosh et al. 1940). The concentration of CBN in cannabis products (marijuana, hashish, and hash oil) has been reported to increase during storage, while the THC concentration decreases, but at a different rate (Ross and ElSohly 1999).

Cannabicyclol (CBL) Type Phytocannabinoids

This group has 3 known compounds: cannabicyclol (Claussen et al. 1968; Korte and Sieper 1964a, 1964b), cannabicyclolic acid A (CBLA) (Shoyama et al. 1972b), and cannabicyclovarin (CBLV) (Claussen et al. 1968; Vree et al. 1972). The photochemical conversion of CBC into CBL has been demonstrated (Crombie et al. 1968). Larger amounts of CBLA were observed in cannabis harvested earlier, during the vegetative phase, and stored for prolonged periods of time, compared with that harvested later, in the reproductive phase (Shoyama et al. 1968). These observations prompted the conclusion that CBL and CBLA are not genuine cannabinoids but artifacts produced by natural irradiation of CBC and CBCA during storage (Shoyama et al. 1972b).

Cannabinodiol (CBND) Type Phytocannabinoids

Cannabinoids of the CBND type are the fully aromatized derivatives of CBDs (Lousberg et al. 1977; Van Ginneken et al. 1972).

Table 5 Content of major terpenoids in the volatile oil freshly extracted from cannabis inflorescences, as determined by GC-MS by various research groups

Compound	Content, % oil
Monoterpenoids	
α-**Pinene**	1.11–31.0
β-**Pinene**	0.6–7.95
β-**Myrcene**	8.23–67.11
Limonene	0.2–16.38
Terpinolene	0.12-23.8
***cis*-Ocimene**	0.04–10.28
Linalool	0.09–2.8
Sesquiterpenoids	
β-**Caryophyllene**	1.33–28.02
Humulene	0.28–12.61
β-**Eudesmol**	0.02-1.56
Caryophyllene oxide	0.3–11.3
***trans*-Nerolidol**	0.09–1.72

Source: Compiled from Bertoli et al. (2010); Mediavilla and Steinemann (1997); and Ross and ElSohly (1996).

Benzoquinone Type and Other Phytocannabinoids

Two geranyl-*n*-pentyl-1,4-benzoquinones were isolated from high-potency cannabis (Radwan et al. 2008b, 2009). Cannabicitran was first synthesized (Crombie and Ponsford 1971) and subsequently isolated from Lebanese hashish (Bercht and Paris 1974). Its structure was described by Bercht et al. (1974). The isolation and identification of cannabichromanone, dehydrocannabifuran, cannabifuran, and 10-oxo-$\Delta^{6a(10a)}$-THC from hashish (Friedrich-Fiechtl and Spiteller 1975) was followed by the isolation of cannabichromanone-C3 (Grote and Spiteller 1978a) and cannabicoumaronone (Grote and Spiteller 1978b). *cis*-Δ^{9}-THC was found in samples of confiscated cannabis (Smith and Kempfert 1977). Cannabiripsol was isolated from South African-grown cannabis (Boeren et al. 1979). Cannabis grown in Thailand (Meao strain) provided (±)-*cis*-Δ^{7}-isotetrahydrocannabivarin (Shoyama et al. 1981). Cannabiglendol was isolated from an Indian cannabis variety grown in Mississippi (Turner et al. 1981). A polyhydroxylated cannabinoid, cannabitetrol, was was also isolated from natural sources and identified (ElSohly et al. 1984). The GC-MS analysis of hash oil (Morita and Ando 1984) led to the identification of *trans*-(1*R*,3*R*,6*R*)-Δ^{7}-*iso*-THCV and *trans*-(1*R*,3*R*,6*R*)-Δ^{7}-*iso*-THC. Three cannabichromanone derivatives (Ahmed et al. 2008a) and cannabicoumarononic acid A (Radwan et al. 2009) were isolated from high-potency cannabis.

Terpenoids

The essential oil (volatile oil) of cannabis is a blend of terpenoids, a term that encompasses terpenes and modified terpenes (where the methyl group has been moved or removed, or oxygen atoms added). Approximately 200 terpenoids have been extracted from cannabis, primarily monoterpenoids ($C_{10}H_{16}$ template) and sesquiterpenoids ($C_{15}H_{24}$ template), as well as di-, and triterpenoids, megastigmanes, and apocarotenoids. No terpenoids are unique to cannabis, but various types of cannabis biosynthesize unique terpenoid profiles (Brenneisen and ElSohly 1988; Hillig 2004; Mediavilla and Steinemann 1997). The qualitative and quantitative profile of terpenoids may vary between different batches of the same seed source (Fischedick et al. 2010).

Ester conjugates of terpenoid alcohols with cannabinoid acids have been reported as minor constituents in cannabis extracts (Ahmed et al. 2008b). The biological profile of these compounds is currently unknown, despite their potential to act as pro-drugs of pre-cannabinoids.

Terepenoids are primarily respnsible for the aroma of cannabis, while cannabinoids, despite their terpenoid origins, are odorless.

Terpenoids produce a wide range of biological activity, possibly including modulation of the effects of THC via their own anxiolytic, sedative, analgesic, antinociceptive, and anti-depressant effects (reviewed in McPartland and Pruitt 1999; Russo 2011). Other actions of terpenoids include anti-inflammatory, acetylcholinesterase (AChE) inhibition, antioxidant, antibiotic, and anti-mutagenic (Maffei et al. 2011).

Terpenoids, together with cannabinoids, alkanes, and other compounds, are synthesized inside glandular trichomes via a common precursor, geranyl pyrophosphate. Yields of cannabis essential oil obtained from fresh plants through steam distillation range from 0.05–0.29% v/w and may represent 10% of trichome content, varying greatly with growing, drying, and harvest conditions (Hazekamp 2008–2009; McPartland and Mediavilla 2001; Potter 2009). Ross and ElSohly (1996) demonstrated the ephemeral nature of terpenoids in stored flowering tops. Freshly-collected material yielded 0.29% v/w essential oil; 1-week-old material air-dried at room temperature and stored in a paper bag yielded 0.20%, a loss of 31%; 1-month-old cannabis yielded 0.16%, a loss of 45%; 3-month-old cannabis yielded 0.13%, a loss of 55%.

Monoterpenoids

Monoterpenoids typically predominates in cannabis, comprising 47.9–92.48% of essential oil extracted from fresh plant material (Mediavilla and Steinemann 1997; Ross and ElSohly 1996 (see Table 5). β-myrcene usually dominates the monoterpenoid fraction in all types of cannabis. Limonene or terpinolene predominate in some drug-type plants (Fischedick et al. 2010, terpinolene and α-pinene predominate in some European fiber-type plants (Bertoli et al. 2010), and α-inene predominates in some Chinese fiber-type plants (Hillig 2004). Other common monoterpenoids

Table 6 Structure and activity of primary phytocannabinoids

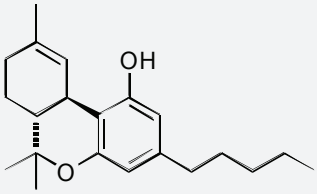
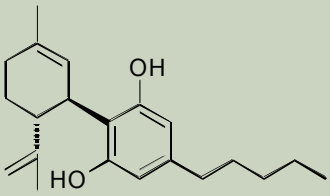
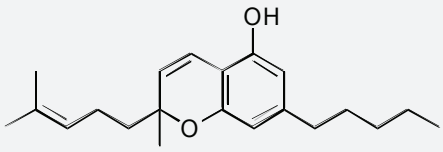
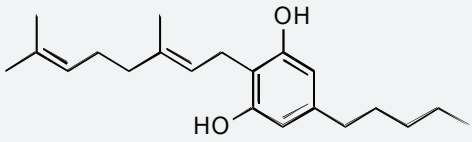
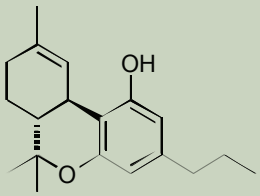
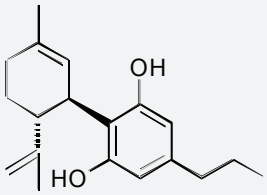
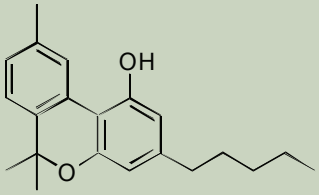
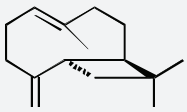
Compound	Putative Medicinal Action
 Δ⁹-Tetrahydrocannabinol (Δ⁹-THC)	**Primary psychotropic cannabinoid** Activates PPAR-y and TRPA1 at nano- and micromolar concentrations, respectively (Pertwee 2008). Analgesic via CB_1 and CB_2 agonism (active at ~20–40 nM) (Rahn and Hohmann 2009). Antiemetic (Haney et al. 2007; Hollister 1971; Machado et al. 2008). Anti-inflammatory, antioxidant (Hampson et al. 1998). Antipruritic, cholestatic jaundice (Neff et al. 2002). Benefits duodenal ulcers (Douthwaite 1947). Bronchodilatory (Williams et al. 1976). Muscle relaxant (Kavia et al. 2010). Reduces Alzheimer's symptoms (Eubanks et al. 2006; Volicer et al. 1997).
 Cannabidiol (CBD)	**Non-psychotropic cannabinoid** Anandamide (AEA) reuptake inhibitor (De Petrocellis et al. 2011). Analgesic (Davis and Hatoum 1983). Anticonvulsant (Jones et al. 2010). Antidepressant in rodents (Deyo and Musty 2003). Anti-emetic (5HT1A agonist; 5 mg/kg ip) (Rock et al. 2010). Antifungal (ElSohly et al. 1982). Anti-inflammatory (Booz 2011). Antagonizes effects of THC in humans (Pertwee 2008). Antioxidant (Hampson et al. 1998). Anxiolytic via 5HT1A agonism (Campos and Guimaraes 2008; Resstel et al. 2009; Russo et al. 2005). Decreases sebum/sebocytes proliferation (Biro et al. 2009). Effective against methicillin-resistant *Staphylococcus aureus* (MRSA) (Appendino et al. 2008). Increases adenosine A2A signaling (Carrier et al. 2006). Pro-apoptotic against breast cancer cell lines (Ligresti et al. 2006). Treatment of addiction (Xi et al. 2010). Treatment of psychosis (Russo et al. 2007).
 Cannabichromene (CBC)	**Non-psychotropic cannabinoid** Analgesic (weak) (Turner et al. 1980b). Anandamide reuptake inhibitor (weak) (De Petrocellis et al. 2008; Ligresti et al. 2006). Anti-inflammatory (Davis and Hatoum 1983). Antimicrobial (Turner and ElSohly 1981). TRPA1 agonist (De Petrocellis et al. 2008; Ligresti et al. 2006).

Table 6 (continued) Structure and activity of primary phytocannabinoids

Compound	Putative Medicinal Action
 Cannabigerol (CBG)	**Non-psychotropic cannabinoid** Analgesic via α-2 adrenergic blockade (Cascio et al. 2010). Anandamide reuptake inhibitor (low micromolar range) agonist (De Petrocellis et al. 2008; Ligresti et al. 2006). Anti-fungal (ElSohly et al. 1982). Anti-inflammatory, anti-hyperalgesic (Bolognini et al. 2010). Effective against MRSA (Appendino et al. 2008). GABA uptake inhibitor (Banerjee et al. 1975). Reduces keratinocytes proliferation in psoriasis (Wilkinson and Williamson 2007). 5HT1A antagonist; counters antiemetic effects of CBD (Rock et al. 2010). TRPM8 antagonist (De Petrocellis et al. 2011). TRPV1, TRPA1, and cannabinoid agonist (De Petrocellis et al. 2008; Ligresti et al. 2006).
 Δ^9-Tetrahydrocannabivarin (THCV)	**Non-psychotropic cannabinoid** Antagonizes Δ^9-THC at low doses (< 3 mg/kg); acts as CB_1 agonist at higher doses (10 mg/kg) in mice (Pertwee et al. 2007, 2008). Anticonvulsant (Hill et al. 2010). Reduced food intake in mice (Cawthorne et al. 2007). Improved glucose tolerance, insulin sensitivity, and insulin signalling in vivo (Wargent et al. 2013).
 Cannabidivarin (CBDV)	**Non-psychotropic cannabinoid** Anticonvulsant in vitro and in vivo (Hill et al. 2010, 2012).
 Cannabinol (CBN)	**Non-psychotropic cannabinoid; minor by-product of Δ^9-THC oxidation** Decreases breast cancer resistant protein (Holland et al. 2008). Effective against MRSA (Appendino et al. 2008). Reduces keratinocytes proliferation in psoriasis (Wilkinson and Williamson 2007). Sedative (Musty et al. 1976). TRPV2 agonist for burns (Qin et al. 2008).
 β-Caryophyllene	**Non-psychotropic sesquiterpene of the essential oil** Common compound of many aromatic plants. Anti-inflammatory, antibiotic, antioxidant, anticarcinogenic, and local anaesthetic activities (Leandro et al. 2012).

Source: Modified from Izzo et al. (2009); Russo (2011).

in cannabis include β-pinene, *cis*-ocimene, *trans*-ocimene, and linalool.

β-Myrcene is recognized to have sedative, muscle-relaxant (do Vale et al. 2002), anti-inflammatory, and analgesic activities (Lorenzetti et al. 1991; Rao et al. 1990). Limonene, a precursor to other monoterpenoids and fairly ubiquitous in nature (Noma and Asakawa 2010), is highly bioavailable and has been suggested to be anxiolytic (do Vale et al. 2002), anticarcinogenic (Elson et al. 1997), and radical-scavenging (Malhotra et al. 2009), while also used to treat gastro-esophageal reflux and gallstones (Sun 2007). α-Pinene is one of the most widely encountered terpenoids in nature, being especially common to coniferous trees (Chalchat et al. 1985; Persson et al. 1996). This terpenoid is reported to have anti-inflammatory (Gil et al. 1989), bronchodilatory (Falk et al. 1990), and antibiotic (anti-MRSA) (Kose et al. 2010) activities, and is an AChE inhibitor that may be of use as a memory aid (Perry et al. 2000). Terpinolene has been reported to be a sedative (Ito and Ito 2013) and antispasmodic (Riyazi et al. 2007) agent. Linalool, common to lavender (*Lavandula* spp.) and coriander (*Coriandrum sativum*), has anxiolytic (Souto-Maior et al. 2011), local anaesthetic, analgesic (Peana et al. 2004), sedative, and anticonvulsant (Karlaganis 2002) properties, and is used as a topical treatment for burns (Gattefosse 1993). Pulegone is a minor terpenoid in cannabis (Turner et al. 1980b), and is also found in rosemary (*Rosmarinus officinalis*), and possesses sedative (Miyazawa et al. 1997) and anti-pyretic (Ortiz de Urbina et al. 1989) properties. Turner et al. (1980b) report *p*-cymene in cannabis; it has anti-microbial properties (Kisko and Roller 2005) and is able to effect AChE inhibition (Perry et al. 1996).

As with cannabis flavonoids, many of these proposed uses are extrapolated from the same compounds in other medicinal plants, with their relevance to cannabis effects equally unknown.

Monoterpenoids are exceptionally volatile and particularly susceptible to loss during drying and storage. As demonstrated by Ross and ElSohly (1996), the relative percentage of monoterpenoids in the essential oil fraction gradually reduced from 92.48 to 62.02% after cannabis was dried and stored at room temperature in closed paper bags for 3 months. Specifically, for example, the content of β-myrcene gradually decreased from 67.11% to 32.88% of the oil, while linalool increased from 2.80% to 5.07%, and α- and β-pinenes and limonene remained seemingly unaffected (no statistical analysis was reported in the study). However, none of the major (> 0.1% of the total) compounds decreased to unquantifiable levels.

Sesquiterpenoids

Sesquiterpenoids comprise 6.84%–47.5% of the essential oil extracted from fresh plant material (Mediavilla and Steinemann 1997; Ross and ElSohly 1996). The primary sesquiterpenoid in cannabis is usually β-caryophyllene. This sesquiterpenoid surpasses β-myrcene as the overall predominate terpenoid in some fiber-type plants (Bertoli et al. 2010; Mediavilla and Steinemann 1997). Caryophyllene oxide, reportedly the volatile compound sensed by drug detection dogs (Stahl and Kunde 1973), is common to all cannabis strains. Other common sesquiterpenoids in cannabis include α-humulene (ie., α-caryophyllene), trans-nerolidol, α-guaine, elemene, and isomers of farnesene and bergamotene (Bertoli et al. 2010; Fischedick et al. 2010; Hillig 2004; Mediavilla and Steinemann 1997; Ross and Elsohly 1996).

β-Caryophyllene is a dominant constituent in black pepper (*Piper nigrum*) and clove (*Syzygium aromaticum*). It reportedly has anti-inflammatory (Basile et al. 1988), gastrocytoprotective (Tambe et al. 1996), analgesic (Ghelardini et al. 2001), and anti-malarial properties (Campbell et al. 1997). This terpenoid was demonstrated to be a selective CB_2 receptor agonist (Gertsch et al. 2008). β-caryophyllene and α-humulene, which along with monoterpenoids myrcene and β-farnesene, predominates in hops (*Humulus lupulus*), imparts its cannabis-like odor.

The relative levels of sesquiterpenoids may increase after drying and in storage, due to loss of the more volatile monoterpenoids. Prolonged storage of the material characterized by Ross and ElSohly (1996) resulted in the sesquiterpenoids content of 35.63%, compared with 6.84% in the oil extracted from the fresh plant. The content of β-caryophyllene increased from 1.33% to 5.45% after 3 months of storage of dried plant material, compared with fresh plant (Ross and ElSohly 1996).

Flavonoids

To date, more than 29 flavonoids have been identified in cannabis (Clark and Bohm 1979; ElSohly and Slade 2005; Ross et al. 2005; Vanhoenacker et al. 2002). cannabis flavonoids belong mainly to 2 classes: flavones (e.g., vitexin, apigenin, isovitexin, luteolin, and orientin and their *O*-glucosides) and 3-hydroxyflavones, or flavonols (e.g., kaempferol and quercetin). Clark (1978) examined 9 cannabis accessions grown in a common garden, and a canonical analysis of flavonoid profiles separated drug-type plants from fiber-type plants (see also Clark and Bohm 1979). Flavones act as phytoestrogens; Sauer et al. (1983) report that a cannabis extract and cannabis smoke condensate showed affinity for estrogen receptors in a heterologous competition assay. The displacement of [^{3}H]estradiol was *not* due to THC, rather apigenin was implicated.

Cannabis also biosynthesizes 3 unique prenylated aglycone flavanones, cannflavins A, B, and C, (Crombie et al. 1980; Radwan et al. 2008a). The cannflavins have only been reported in studies of drug-type plants (Barrett et al. 1985; Crombie et al. 1980; Radwan et al. 2008a;) and appear to be absent in fiber-type plants (Vanhoenacker et al. 2002). Cannflavins are potent inhibitors of cyclooxygenase enzymes and prostaglandin E2 production (Barrett et al. 1985). The cannflavins are structurally related to 8-prenylnaringenin, a potent phytoestrogen from hops. The pharmacology of cannabis flavonoids was reviewed in McPartland and Russo (2001), who propose many potential uses, predominantly

extrapolating from research on numerous other medicinal plants. Whether these uses have clinical relevance to cannabis is unknown.

Other Constituents

To date, 527 compounds have been isolated from cannabis (Appendino et al. 2011; ElSohly and Slade 2005). These other compounds occurring in cannabis include carbohydrates (monosaccharides, disaccharides, polysaccharides, sugar alcohols, cyclitols, and amino sugars), amino acids, amines (e.g., piperidine, hordenine, ammonia), non-cannabinoid phenols (spiro-indan-type, dihydrostilbene-type, cannabidihydrophenanthrene derivatives, simple phenols, simple phenolic glycosides, and phenol methyl esters), simple alcohols, aldehydes, ketones, acids, esters, lactones, steroids (phytosterols and brassinosteroides), vitamins, xanthones, coumarins, and pigments. Two unique spermidine-type C21-alkaloids, (+)-cannabisativine (Turner et al. 1976) and anhydrocannabisativine (ElSohly et al. 1978), have been found in cannabis and are reviewed in Mechoulam (1988).

Among the 527 compunds, some predominate in achenes or roots, and are marginally relevant to flowering tops. These include amides, fatty acids and their esters (oxylipins), quaternary bases (e.g., choline, trigonelline), and proteins.

Pharmacological effects have been established for many of these compounds. Notably, β-sitosterol, a phytosterol ubiquitous in the plant kingdom and found in cannabis (Mole and Turner 1974) and cannabis smoke (Adams and Jones 1975; Foote and Jones 1974), was shown to reduce topical inflammation and chronic edema in skin models (Gomes et al. 2008). A group of unique stilbenoids, canniprene and its spiranized (cannabispirans) and quinoid (denbinobin) derivatives (Turner et al. 1980b), were shown to have anti-inflmmatory, antibacterial, and antifungal activities (Flores-Sanchez and Verpoorte 2008; Pagani et al. 2011). Whether these actions are of clinical relevance remains to be determined.

Analytical

There are a number of analytes of interest in cannabis. Historically and presently, the quantitation of THC has been the focus of greatest interest. In recent decades, other cannabinoids have gained interest (e.g., CBD, THCV) due to their therapeutic benefits, as have terpenoids. Gas chromatography (GC) has been the primary methodological technique used for federal regulatory and toxicology purposes (e.g., ElSohly et al. 2000; Mehmedic et al. 2010, among others). Generally, there are a host of non-standardized, non-validated methods across several analytical platforms being used that give a wide range of total or THC values with unknown reliability. Thus, there is a need for standardized and validated testing methodologies.

THC is present only at very low levels in fresh or dry plant material. This compound is derived by decarboxylation of the naturally occurring non-psychoactive THCA during storage (small amounts) and heating (e.g., more complete decarboxylation when smoked) (Sirikantaramas et al. 2004; Yamauchi et al. 1967). In absence of a specific legislative directive regarding THC quantification, it is most common to quantify "total THC" (THCA + THC), as this best represents the potential activity associated with THC. Total THC content more closely reflects the amount of THC potentially yielded when smoked. Because of this, many legal systems consider total THC content as the primary quantitative value desired.

Decarboxylation from THCA to total THC can be achieved prior to and during analysis. Decarboxylation prior to analysis can be accomplished by placing a plant sample that has been extracted into a solvent into a heating block at 150 °C in an open glass vial. When the extraction solvent has evaporated, decarboxylation can occur within 5 min; however, individual analysts need to validate this process in their own laboratories (UNODC 2009).

During GC analysis, a sample elutes through a column within an oven, which decarboxylates most of the THCA into THC. Therefore, GC typically measures total THC. However, if the goal of the analysis is to quantify both THCA and THC by GC, prior derivatization is required (UNODC 2009). Additionally, varied degrees of decarboxylation can occur during injection in some GC systems, and high injection temperatures in the liner may cause a decomposition of THC. Decarboxylation may be partial, complete, or inconsistent depending on the temperature and geometry of the injector. Therefore, if decarboxylation is not performed prior to analysis, the specific gas chromatograph system and analysis conditions must be validated to ensure that complete decarboxylation of THCA is attained without undue decomposition of THC (Dussy et al. 2005; UNODC 2009).

High Performance Liquid Chromatography (HPLC) is also applicable for the quantification of cannabinoids. HPLC allows for the quantitation of the naturally occurring acid compounds, as well as the neutral forms, as both acids and neutrals are detected, and the peaks for both compounds can be added together for "total THC" or individual cannabinoids can be quantified. HPLC is therefore the optimal testing methodology for quantifying the authentic plant compounds prior to decarboxylation.

Thin-layer chromatography (TLC) is predominantly of value for the identification of cannabis. Currently, there are no validated TLC or high performance TLC (HPTLC) methods for the quantitation of THC that give results equal to those obtained from LC or GC analyses, although, some commercial laboratories are attempting to do so.

Some US states that have legalized the use of cannabis for either medicinal or non-medical use have proposed mandates requiring quantitative analysis. Both growers and dispensers are making claims of varying quantitative values of THC, other cannabinoids, and terpenoids in herbal cannabis and associated products. However, as cannabinoids are closely related in structure and molecular weight,

adequate chromatographic separation of these molecules is requisite to accurately report quantitative values. For. example, Debruyne et al. (1994) compared TLC and HPLC to their gold standard: capillary column GC-MS. Analysis of a single cannabis specimen produced different quantitative peak sizes using these three methods. With GC-MS, THC=CBD>CBN; with HPLC, CBN>THC=CBD; with TLC, CBN>THC=CBD.

With appropriate sample preparation, analytical methods can be applied to a variety of cannabis preparations (foods or topicals), extractions (tinctures or oils), or concentrates; however method extensions must be performed for various matrices. To aid laboratories in the analysis of cannabis, the cannabis committee of the American Herbal Products Association (AHPA) developed a set of draft guidelines outlining recommended practices for labs to follow (AHPA 2013b), and Americans for Safe Access (ASA) has developed a laboratory certification program (ASA PFC 2013).

Lastly, and of significant importance in the analysis of cannabis, is to employ a formal sampling protocol (e.g. [OMC] BMC 2010; WHO 1998 among others) to assure the sample being tested is representative of an entire batch. This is critical, as dosing decisions either for medical or non-medical use can be based on claimed potencies, and there can be significant variation in constituent concentration between plants and even within a single plant itself. For cannabis, the sampling program being applied may differ between products being tested (e.g., raw material versus extracts). For crude cannabis, specific guidance is provided by the Bureau voor Medicinale Cannabis (BMC) monograph of the Netherlands ([OMC] BMC 2010).

Thin-Layer Chromatography Characterization of Cannabis and Its Major Cannabinoids

The following method was developed by the University of Mississippi and provides a characteristic fingerprint that can be used for the identification of cannabis and its primary cannabinoids as well as distinguish between THC-dominant, CBD-dominant, and fiber types. Two different reagents for visualization can be used. Both identify the primary cannabinoids, and either of them can be used for purposes of basic identification of crude cannabis plant material. Additionally, some different bands are visible with the 2 reagents. Therefore, examination using the 2 reagents allows for a more complete visualization of cannabis compounds.

Sample Preparation

Weigh approximately 100 mg of dried powdered cannabis, and extract by maceration with sonication in 10 mL dichloromethane for 1 h. Filter the extract and evaporate the solution under nitrogen. Redissolve the residue in methnol, adjusting the concentration to 10 mg/mL.

Decarboxylation of Cannabinoid Acids (optional)

To decarboxylate cannabinoid acids (e.g., to convert THCA to THC), heat the dried plant extract at 120 °C for 2 h* and adjust the concentration to 10mg/mL as indicated above.

* Alternatively, heating at 210 °C for 15 min can facilitate sufficient decarboxylation.

Table 7 R_f **values for cannabinoid standards**

Phytocannabinoid	R_f
CBC	0.21
Δ^9-THC	0.26
CBN	0.29
CBG	0.33
CBD	0.40
THCV	0.42
Δ^9-THCA	0.61
CBDA	0.77

Note: Due to its relatively high concentration in drug type samples, Δ^9-THC can overlap with CBN. CBN is a degradation compound of Δ^9-THC.

Standards Preparations

Cannabinoid standards are dissolved in methanol at a concentration of 1 mg/mL.

Note: All cannabinoid standards utilized in the development of this method were isolated at the University of Mississippi. There is limited availability of commercially prepared cannabinoid standards.

Standards Solution Stability

CBD, CBG, and CBN are stable in methanol, both at room temperature and with freezing. THC, THCV, and CBC methanolic solutions are stable only when frozen and acid compounds are only stable in a freezer. Due to their instability, acid compounds should be prepared cool and stored and shipped frozen.

Reagent Preparation

Fast Blue reagent: Dissolve 0.5 g Fast Blue B salt (MP Biochemicals, LLS) in 100 mL distilled water.

Vanillin/H2SO4: Dissolve 6 g vanillin in 90 mL ethanol (95%). Add 10 mL of 98% H_2SO_4. This reagent is relatively unstable and is best to use fresh each time.

Chromatographic Conditions

Stationary Phase:

C18 (UV 254) TLC plates 150 µm, 10 cm × 10 cm (Sorbent Technologies).

Figure 17a **TLC chromatogram of cannabis and its primary cannabinoids (Fast Blue reagent; white light)**

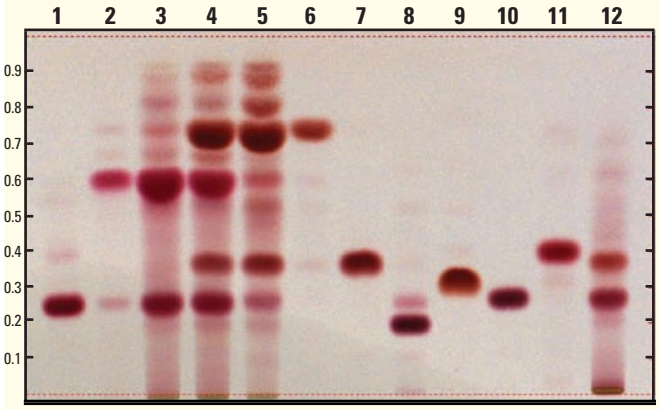


Figure 17b **TLC chromatogram of cannabis and its primary cannabinoids (Vanillin/H2SO4 reagent; white light)**

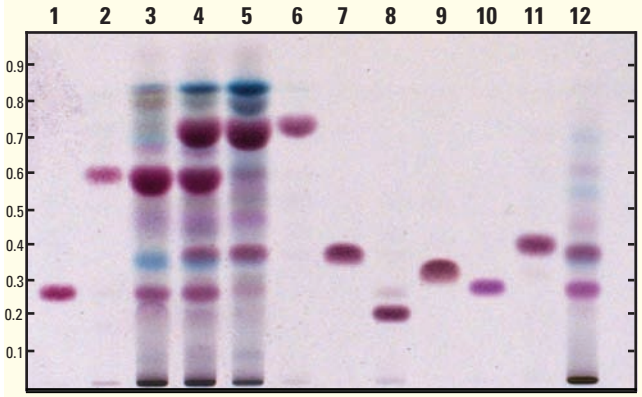
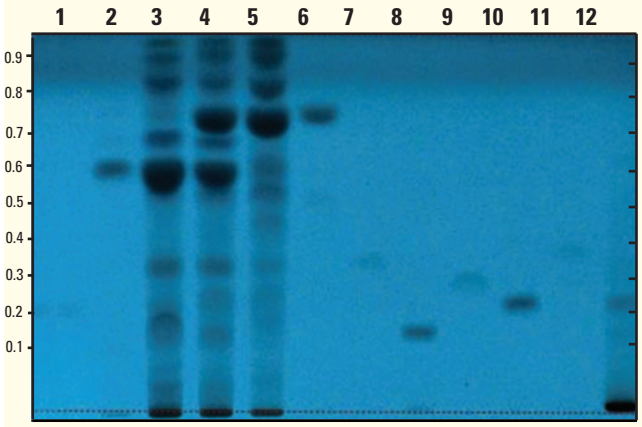


Figure 17c **TLC chromatogram of cannabis and its primary cannabinoids (UV 254 nm)**



Discussion of chromatograms

Observations (Fast Blue reagent; white light)

17a) In the cannabis THC drug type, the most prominent visible bands are those for Δ^9-THC, and THCA with 4 primary bands in the upper Rf region, including CBDA. In the intermediate type, the most prominent visible bands are those for Δ^9-THC, CBD, THCA, and CBDA with additional bands showing for CBC in the lower Rf; unknown bands in the middle R_f; and 3 bands in the upper Rf, including CBDA. In the cannabis fiber type, the pattern of banding is very similar to the intermediate type, but reflects a much lower concentration of THCA and a similar concentration of CBD and CBDA. When subjected to decarboxylation, a degradation of a number of the original cannabinoid acids occurs, leaving characteristic bands for Δ^9-THC and CBD and a faint band for CBC. THCA-dominant types are most often notably lacking in CBD, while fiber types yield very low concentrations of Δ^9-THC and relatively high concentrations of CBDA. Thus, these 3 clearly delineated types can be readily distinguished. However, other materials, which are highly crossed, may not be readily distinguished.

17b) All standards (Lanes 1, 2, 6-11) appear as purple bands with varying intensities. In the cannabis drug type (Lane 3), the most prominent visible bands are those for THCA (R_f 0.61) and Δ^9-THC (R_f 0.26). In the intermediate type (Lane 4), the most prominent visible bands are those for CBDA, THCA, CBD, and Δ^9-THC. In the cannabis fiber type (Lane 5), the strongest bands are seen for CBDA and CBD. In the decarboxylated intermediate cannabis type (Lane 12), the only visible bands are for Δ^9-THC and CBD due to decarboxylation of the cannabinoid acids by heating.

17c) All cannabinoids are of varying intensities. THCA (Lane 2), CBDA (Lane 6), CBC (Lane 8), and CBN (Lane 10) are more intense than the others. In the cannabis drug type (Lane 3), a strong band is seen at the position of THCA. In the intermediate type (Lane 4), the most prominent visible bands are those for THCA and CBDA, while in the cannabis fiber type (Lane 5), the band for CBDA is most prominent. In the decarboxylated intermediate cannabis type (Lane 12), a band corresponding to CBN occurs in the lower third R_f (0.3).

Figure 17a-c lane assignments

Lane 1: Δ^9-THC
Lane 2: THCA
Lane 3: THC-type cannabis
Lane 4: Intermediate-type cannabis
Lane 5: Fiber-type cannabis
Lane 6: CBDA
Lane 7: CBD
Lane 8: CBC
Lane 9: CBG
Lane 10: CBN
Lane 11: THCV
Lane 12: Cannabis intermediate type decarboxylated (UM)

Mobile Phase:

75:25 (v:v) methanol/water with 0.1% glacial acetic acid.

Sample Application

Apply 5 µL of the sample preparations and 2 µL of the standards preparations on the plate as 5 mm bands 2 mm apart from each other. The application position should be 8 mm from the lower edge of the plate and at least 15 mm from the left and right edges of the plate. For visualization using both reagents, separate plates should be prepared.

Development

Line a flat bottom chamber (14 cm x 14 cm x 8 cm) with a filter paper or chromatography paper. Add a sufficient amount (~25 mL) of the Mobile Phase solution to ensure that the filter paper is covered to a a height of at least 5 mm, and let saturate for 15 min. Measure and mark on the plate the developing distance 60 mm from the application position. Introduce the plate into the chamber, and allow the developing solvent to reach the mark. Remove the plate and dry for 2 min at 70 °C in an oven.

Detection

Visualize the plates under UV 254 nm, then spray one set of the plates with the Fast Blue reagent and the other set of plates with the vanillin/H_2SO_4 reagent, followed by visualization under white light. For basic identification of the primary cannabinoids, either reagent can be used.

Results

See Table 7 and refer to the chromatograms provided (Figure 17a–c).

High-Performance Liquid Chromatography (HPLC) for the Determination of Major Phytocannabinoids in Cannabis

This HPLC method was adapted from Swift et al. (2013) and can be used for quantitation of THCA-A, Δ^9-THC, CBDA, CBD, CBGA, CBG, and CBN in cannabis preparations. The method was adapted from an earlier method developed by DeBacker et al. (2009), which also quantified Δ^8-THC. The original method of DeBacker et al. (2009) was validated for cannabis raw material and fully validated using total error approach in accordance with ISO17025 and the guidelines of the French Society of Pharmaceutical Sciences and Techniques (SFSTP). This modified and optimized method of Swift et al. (2013) was subjected to validation for selectivity, linearity, accuracy, precision, and recovery according to the US Food and Drug Administration (FDA) guidance for bioanalytical method validation (FDA 2001).

With appropriate modifications in sample preparations, the same chromatography can be used for the analysis of other cannabis materials (i.e. concentrates, extracts, foods). However, the robustness of this chromatography when applied to various matrices requires further validation (e.g., recovery, spiking experiments).

Sample Preparation

Crude Cannabis

Test samples are dried for 24 h in a 35 °C forced ventilation oven. Dried samples are ground to a fine powder. 200 mg of the sample is weighed in a glass vial and extracted with 10 mL of a mixture of methanol/chloroform (v/v: 9:1) by sonication for 30 min. The extract is filtered into an amber vial and diluted with methanol/chloroform solution (v/v: 9:1) to a concentration of 1:10. A 100-µL aliquot of the dilution is evaporated under a gentle stream of nitrogen and re-dissolved in 100 µL of a mixture of water/acetonitrile (v/v: 5/5).

Note: For analysis, the UNODC (2009) recommends that crude cannabis be dried to a finished moisture content of 8–13%, pulverized, and sieved through a 1 mm sieve. The UNODC provides the following sample preparations for different matrices. This specific method was not validated with these matrices, but these guidelines may be useful to the analyst.

Sample Preparation of Cannabis Resin

Grate into small pieces to a particle size of approximately 1 mm, or if sticky, cool with liquid nitrogen, pulverize, and sieve through a 1 mm sieve (UNODC 2009). Dissolve 50 mg in 10 mL of a mixture of methanol/ chloroform (v/v: 9:1) by sonication for 30 min.

Sample Preparation of Cannabis Oil

For HPLC analysis, cannabis oil requires no prior preparation. Dissolve 50 mg in 10 mL of a mixture of methanol/ chloroform (v/v: 9:1) by sonication for 30 min.

Standards Preparation

The availability of cannabinoid reference materials varies due to federal legal restrictions. A variety of cannabinoids are sold pre-diluted at concentrations of one mg/mL or less. Stock solutions for the standard curves are prepared across a broad range of concentrations to account for variable concentrations of cannabinoids. For accuracy, it is necessary to include at least 4 points in the standard curve. Standards should be run with every sample set and a relative bias not greater than 10% should be achieved. Limits of quantitation (LOQ) should be established using a calibration curve covering a range from 0.5 µg/mL to 100 µg/mL.

Internal Standard

Diazepam (50 mg/L). Diazepam is a schedule IV controlled substance. Use of an alternative internal control, such as methyl or propylparaben (e.g., 30 mg/L), should be validated for acceptable recovery and chromatographic separation.

Standard Stability

CBD, CBG, and CBN are stable in methanol, both at room temperature and with freezing. Δ^9-THC, THCV, and CBC methanolic solutions are stable only when frozen and acid compounds are only stable in a freezer. Due to their instability, acid compounds should be prepared cool and stored and shipped frozen.

Linearity Range

Compound	r_2	LOQ (%)	LOD (%)
THCA	0.9969	0.05	0.025
Δ^9-THC	0.9940	0.05	0.025
CBDA	0.9939	0.05	0.05
CBD	0.9951	0.075	0.075
CBGA	0.9948	0.05	0.05
CBG	0.9959	0.15	0.1
CBN	0.9917	0.05	0.025

r_2=coeficient detrmination: LOQ=Limit of Quantitation: LOD=Limit of Detection

Note: This method was not validated for quantitation of Δ^8-THC.

Storage of Reference Standards

For long-term storage of reference standards, store at -20 C protected from light and air. When properly stored, reference standards are stable for up to 12 months.

Chromatographic Conditions

Apparatus:

Validation was performed on a Shimadzu ADVP module (Kyoto, Japan) equipped with a SIL-10 autoinjector with sample cooler and LC-10 in-line vacuum degassing solvent delivery unit.

Column:

Waters X-Bridge C18 (4.6 mm x 150 mm, 3.5 µm) reverse-phase column (Waters, Australia) coupled with a 1-mm Opti-Guard C18 pre-column (Optimize Technologies, Alpha Resources, Thornleigh, Australia).

Column Temperature:

30 °C.

Injection Volume:

30 µL.

Mobile Phase:

A. 50 mM ammonium formate (adjusted to pH 3.75 with 10% acetonitrile)

B. 90% acetonitrile.

Time (min) B in A (%)

0 70

15 90

30 90

31 70

40 70

Flow Rate:

1 mL/min.

Detection (diode array detector):

Full spectra monitoring from 190-370 nm is recommended. Non-acidic cannabinoids are typically detected at approximately 228 nm and acidic cannabinoids at approximately 270 nm. Note: The validation was performed using a photodiode array detector. For routine use, a standard UV detector is suitable.

Run Time:

30 min.

Post-run Time:

6 min.

Note: CBD and CBG peaks may slightly overlap if present in high concentrations (> 10%).

Quantitation

Inject each standard preparation and generate a standard curve based on the peak area vs. concentration, as a ratio of standard to internal standard.

Cannabinoid contents in the sample are quantified using the linear equation based on least squares regression for each cannabinoid compound: (y = mx + c)

where:

x = concentration of the individual cannabinoid in the sample (µg/mL);

y = peak area of the invidivual cannabinoid;

c = calculated y-intercept of the calibration curve;

m = calculated slope of the calibration curve.

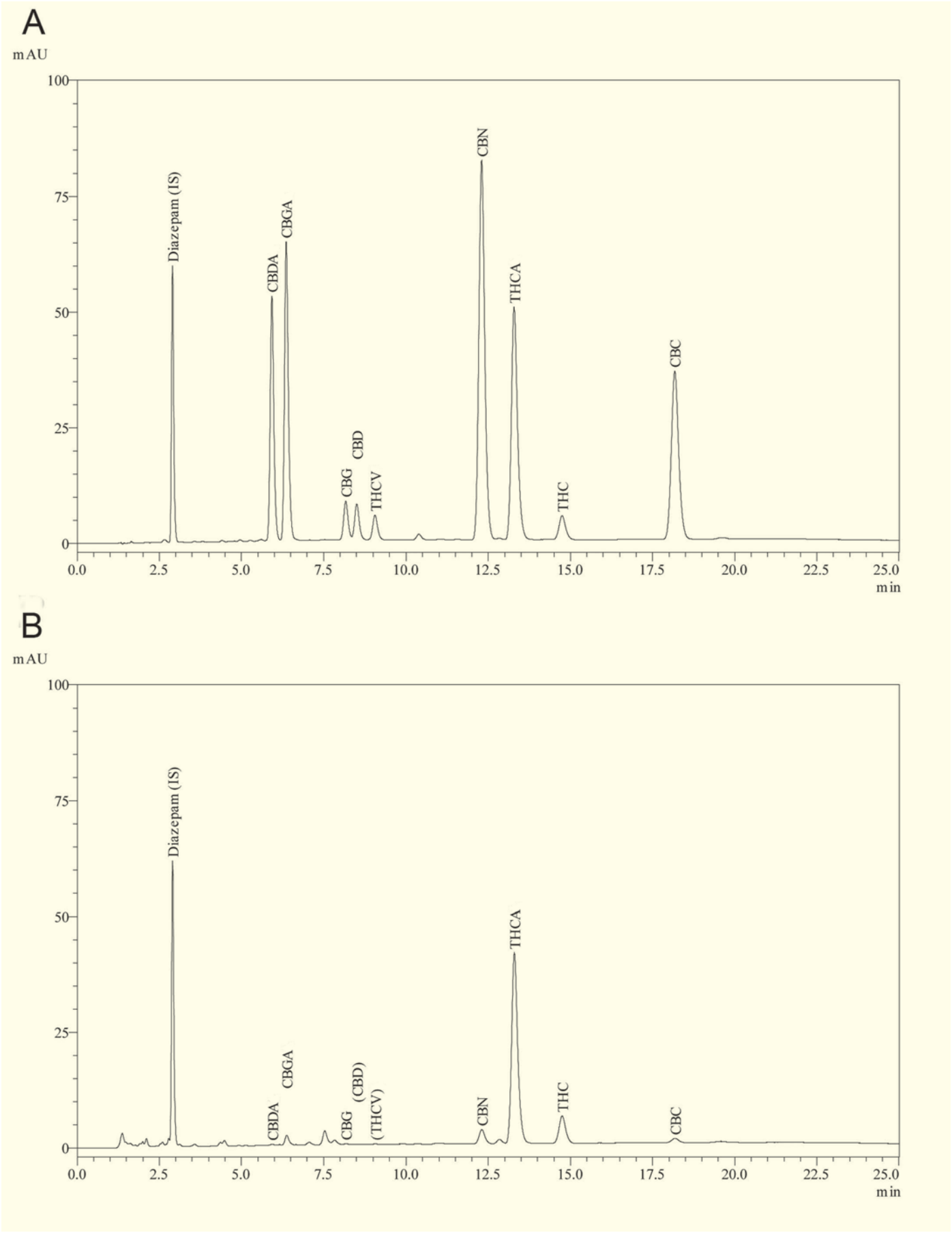
Using the concentration from the equation (y = mx + c), total content (C_{CBXT}) in the sample can be calculated as a sum of the concentrations of the neutral (C_{CBX}) and the acidic (C_{CBXA}) components. A conversion factor of 0.877 is used for adjustment of the molar masses of THCA-A and CBDA; a conversion factor of 0.878 is used for CBGA; both after decarboxylation. These conversion factors may not apply for other cannabinoids:

$$C_{CBXT} = C_{CBX} + C_{CBXA} \times 0.877$$

The individual cannabinoid content in the material is then calculated according to the following equation:

$$W_{CBX(T)} = \frac{C_{CBX(T)} \times V_{sample} \times D}{m_{sample} \times 10^6} \times 100\%$$

Figure 18 **Representative HPLC chromatograms of cannabinoid standards (A at 11 µg/mL) and cannabis raw material (B)**



where:

$W_{CBX(T)}$ = (total) cannabinoid content in the material (% weight);

$C_{CBX(T)}$ = (total) cannabinoid content in the sample (µg/mL);

V_{sample} = sample volume (mL);

D = dilution factor;

m_{sample} = sample mass (g).

Calibration Range

Linear from 2 µg/mL to 100 µg/mL. Extrapolations from this curve should not be made; however, cannabinoid concentrations in samples greater than 100 µg/mL can be appropriately diluted, or the curve can be extended out to 1000 µg/mL (with 7 or more points in the curve) to ensure the reading is within the calibration range.

Gas Chromatography with Flame Ionization Detection (GC-FID) for the Quantitation of Phytocannabinoids

The following GC-FID method is used for the quantitation of the major phytocannabinoids of confiscated cannabis material submitted to the University of Mississippi by the DEA and other United States law enforcement agencies as part of NIDA's Marijuana Potency Monitoring Program (ElSohly et al. 2000; Mehmedic et al. 2010). Due to the high temperature of the GC injector port, in situ decarboxylation of the acidic cannabinoids occurs upon injection. This method, therefore, quantifies total cannabinoids (acidic and neutral) simultaneously. If quantitation of free (neutral) and acidic compounds is required for a specific cannabinoid, a non-destructive method, e.g., HPLC, or derivatization, e.g., silylation or formation of the alkylboronates, should be employed and validated.

Sample Preparation

Crude cannabis and hashish: To 100 mg of dried, powdered cannabis material with seeds and stems removed, add 3 mL of the internal standard solution (see below on the preparation instructions). Macerate for 1 hour at room temperature Sonicate for 5 min. Filter the extract into GC vials, and cap the vials.

Hash oil: To 100 mg of hash oil, add 4 mL of hash oil extraction solution (see below). Macerate for a minimum of 2 h at room temperature. Sonicate for 5 min. Add 20 mL of absolute ethanol, and sonicate again for 5 min. Filter the extract into GC vials, and cap the vials.

Internal Standard Preparation (use for extraction of cannabis and hashish)

Dissolve 100 mg of 4-androstene-3,17-dione in 100 mL of 1:9 v/v chloroform/methanol mixture.

Hash Oil Extraction Solution: Dissolve 50 mg of 4-androstene-3,17-dione in 50 mL of absolute ethanol.

Chromatographic Conditions

Column:

DB-1MS: 15 m x 0.25 mm id x 0.25 µm film (J&W Scientific, Inc, US [Agilent Technologies]).

Mobile Phase:

Helium.

Column\Head Pressure:

14 psi (1.0 mL/min).

Traps:

Moisture and oxygen traps for the purification of the helium.

Injection Volume:

1 µL.

Injection Mode:

Split (can be selected based on the sensitivity needed and analytical goal).

Injector Temperature:

240 °C.

Temperature Program (Column Control):

170 °C (hold 1 min) to 250 °C (hold 3 min) at 10 °C/min, 12 min total run time.

Detection Temperature:

260 °C.

Make-up Gas:

Helium (UHP): 20 psi, 20 mL/min (nitrogen may be used as an alternative make-up gas).

Combustion Gases:

Hydrogen (UHP): 30 psi, 30 mL/min and compressed air (suitably purified) at 30 psi, 400 mL/min.

Split Flow:

50 mL/min.

Split Ratio:

50:1.

Septum Purge:

5 mL/min (will vary on different systems).

Detection (FID):

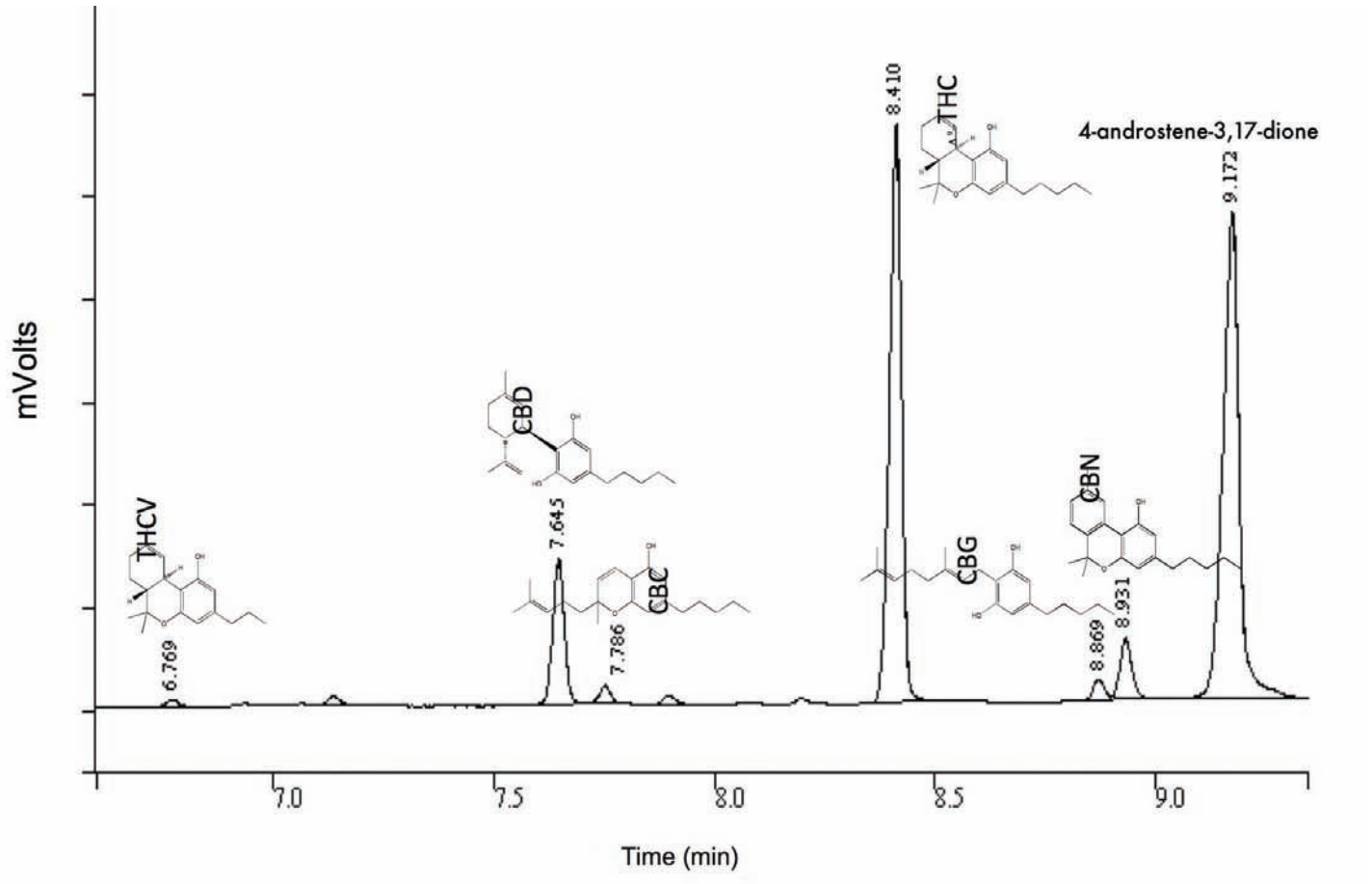
Relative retention times are provided in Table 8.

Calculations:

Cannabinoid potency is calculated as shown in the following equation:

$$W_C = (I_C \times m_{is}) / (I_{is} \times m_{sample}) \times 100\%$$

Figure 19 **Characteristic gas chromatography (GC) chromatogram of cannabis with an internal standard**



where:

W_C = relative cannabinoid content of the material, % weight;

I_C = integrated area of the cannabinoid peak from GC-FID chromatogram;

I_{is} = integrated area of the peak of the internal standard from GC-FID chromatogram;

m_{is} = mass of internal standard

Table 8 **Relative retention times of phytocannabinoids and 4-androstene-3,17-dione, as observed using GC-FID**

Cannabinoid	Time (min)	
	Without internal standard	With internal standard
THCV	6.772	6.769
CBD	7.649	7.645
CBC	7.786	7.786
Δ^9-THC	8.420	8.410
CBG	8.869	8.869
CBN	8.930	8.931
4-androstene-3,17-dione	–	9.172

Limit Tests

Limits that are applicable to cannabis include those that are generally applied to herbal materials, such as tolerance levels of microbial and fungal contamination, content of certain metals, as well as limits of solvent and pesticide residues. With exception to loss on drying and moisture content of dry material, the following limits are based on general recom-

mendations for botanical ingredients established by various national and international bodies. Tests can be performed according to standard pharmacopoeial instructions (e.g., European Pharmacopoeia, United States Pharmacopeia, among others).

Foreign Organic Matter (crude cannabis material): Not more than 5.0% of stems 3 mm or more in diameter; not more than 2.0% of other foreign matter.

Total Ash (crude cannabis material): Not more than 20.0%.

Acid-insoluble Ash (crude cannabis material): Not more than 4.0%.

Loss on Drying (crude cannabis material): Not more than 10.0% of its weight, determined on 1.000 g of the powdered drug by drying in an oven at 105 ºC for 2 h (BMC 2010).

Moisture content of dry material (crude cannabis after packaging): Not more than 15% (BMC 2010).

Microbial and Fungal Limits

The presence of microbes is typical for all natural products. Unless carefully cultivated, illegal supplies may not meet the prescribed specifications. Conversely, reports in which a causal association between microbial exposure through cannabis use and infections has been established (e.g., Carod Artal 2003) appear to be rare considering the prevalence of use and exposure.

Tolerance limits for microbial and fungal contamination in cannabis and its products should be consistent with applicable state, federal, and international regulations, whenever applicable. Recommended tolerance limits for cannabis products are provided in Table 9 and were based on a review of national and international recommendations for botanical products as well as discussion with a variety of stakeholders (e.g., Washington State). Additional guidance for botanical products is provided in national and international compendia based on oral consumption of finished botanical products. Additionally, more restrictive limits may be adopted for medical use of cannabis, most notably when used by immune compromised individuals. Microbes such as *Aspergillus* spp., for example, can be transmitted through inhalation and are of specific concern in those with specific medical conditions (e.g. chronic granulamatous disease and cystic fibrosis) and when employing specific medical treatments (e.g., immunosuppressive therapies). Reducing total microbial risk may require specific microbial reduction treatment to the greatest level possible without compromising the putative medicinal activity. Appropriate methods for testing microbial loads can be found in the *Bacteriological Analytical Manual* (FDA 2013a).

It is important to note that microbial and fungal values do not typically represent pass or fail criteria. Rather they are recommended levels when plants are produced under normal circumstances and growing conditions. Individual herbs, such as mints (*Mentha* spp.), which have a high concentration of trichomes, are prone to higher levels of molds than crops with fewer trichomes. As cannabis also possesses high concentrations of trichomes, this may be a factor and recommended limits may require adjustment over time. Higher levels of molds can also occur in seasons of heavy rain without undue damage to the crop and may justify a material exceeding the proposed limits as long as there is no visible damage to the plant and other qualitative specifications are met. Limits must also be appropriately applied to the various preparations being made. Typical microbial and fungal limits may not be relavant to materials that are to

Table 9 Microbial and fungal limits recommended for orally consumed botanical products in the US (CFU/g)

	Total viable aerobic bacteria	Total yeast and mold	Total coliforms	Bile-tolerant gram-negative bacteria	*E. coli* (pathogenic strains) and *Salmonella* spp.
Unprocessed materials*	10^5	10^4	10^3	10^3	Not detected in 1 g
Processed materials*	10^5	10^4	10^3	10^3	Not detected in 1 g
CO_2 and solvent-based extracts	10^4	10^3	10^2	10^2	Not detected in 1 g

* Unprocessed materials include minimally processed crude cannabis preparations such as inflorescences, accumulated resin glands (kief), and compressed resin gland (hashish). Processed materials include various solid or liquid infused edible preparation, oils, topical preperations, and water-processed resin glands ("bubble hash"). Significant microbial contamination can occur during post-harvesting hadling.

Table 10 **Pesticides commonly used in cannabis cultivation**

Pesticide	Use	Residue Analytical Methods (RAM) Environmental Protection Agency (EPA)[1] or Literature[2]
Abamectin (Avermectins B1a and B1b)	Insecticide/acaricide	LC-FLD[1]; LC-MS/MS[2]
Acequinocyl	Insecticide/acaricide	LC/MS/MS[1]
Bifenazate	Acaricide	LC[1]; LC-MS/MS[2]
Bifenthrin (synthetic pyrethroid)	Insecticide	GC-ECD[1]; GC-MS/MS[2]
Chlormequat chloride	Plant growth regulator (PGR)	IC, LC-MS/MS[2]
Cyfluthrin (synthetic pyrethroid)	Insecticide	LC[2] (WHO 2004); GC-MS/MS[2]
Daminozide (Alar)	Plant growth regulator (PGR)	UV Spectroscopy[1]; LC-MS/MS[2]
Etoxazole	Acaricide	GC-MS(/MS)[1]
Fenoxycarb	Insecticide	LC/UV[1]; LC-MS/MS[2]
Imazalil	Fungicide	GC-ECD[1]; LC-MS/MS[2]
Imidacloprid	Insecticide	LC-MS/MS[2]
Myclobutanil	Fungicide	GC-ECD; GC-NPD[1]; GC-MS/MS)[2]; LC-MS/MS[2]
Paclobutrazol	Plant growth regulator (PGR); fungicide	LC-MS/MS[2]
Pyrethrins*	Insecticide	GC-ECD[1]
Spinosad	Insecticide	LC-MS/MS; immunoassay[1]
Spiromesifen	Insecticide	GC-MS[1]; LC-MS/MS[2]
Spirotetramat	Insecticide	LC/LC-MS/MS[2]
Trifloxystrobin	Fungicide	GC-NPD[1]; GC-MS/MS[2]; LC-MS/MS[2]

ECD = Electron capture detector; FLD = Fluorescence detector; GC = Gas chromatography; LC = Liquid chromatography; IR = Infrared spectroscopy; MS = Mass spectrometry; NMR = Nuclear magnetic resonance; NPD = Nitrogen phosphorous detector.
* Natural pyrethrins are tolerance exempt; synthetic pyrethrins are not.

be subjected to processing, such as infusing, decocting, or extracting with heat, alcohol, or other processes that introduce a microbial reduction step prior to consumption.

Metal Limits

When grown in contaminated soil, cannabis accumulates heavy metals to the extent that it has been proposed as a candidate for bioremediation of toxic waste sites (Shi and Cai 2009). Siegel et al. (1988) measured 440 ng mercury per gram of cannabis in Hawaii, whose volcanic soil contains naturally high levels of mercury. Siegel notes that mercury is absorbed 10 times more efficiently by the lungs than by the gut. He calculated that smoking 100 g of volcanic cannabis per week could lead to mercury poisoning. The American Herbal Products Association (AHPA) provides manufacturers of herbal products with general recommendations for maximum heavy metals levels in herbal products, based on the daily product intake amount (Table 11). The most appropriate method for quantification of metals in medicinal products is an inductively coupled plasma-mass spectrometry (ICP-MS) method of the US Food and Drug Administration (FDA), which analyzes arsenic, cadmium, chromium, lead, and mercury (FDA 2011). The cannabis monograph of the Netherlands BMC (2010) considers the risk of metal contamination of cannabis grown under controlled conditions to be low.

Pesticide Limits

In the US, pesticides are regulated by the Environmental Protection Agency (EPA), which registers or licenses pesticides for use in the United States, and by individual states (usually, by that state's department of agriculture), which may regulate pesticides more stringently than EPA. Pesticide tolerances are approved on an individual or crop group basis, so that the approval of a pesticide for use on one commodity does not confer the approval of its use on another. Where no limits are specifically established for a specific crop or class of crops, the limit is zero (0), generally considered as < 0.01 ppm or 10 ppb according to analytical methods set forth in the *Pesticide Analytical Manual* (PAM; available from the US Food and Drug Administration) (FDA 2013b).

To date, there are no pesticides specifically approved for use on cannabis in North America on the federal level. However, some pesticides with tolerance exempt ingredi-

Table 11 Metal limits recommended for herbal products in the US

Contaminating metal	Limit, µg/daily dose
Inorganic arsenic	10
Cadmium	4.1
Lead	6
Methyl mercury	2.0

Source: AHPA (2008).

ents have broad use sites that could allow for their use on cannabis. Additionally, some states, (e.g., Massachusetts, Washington, and Colorado) are formulating guidelines for pesticide use in cannabis cultivation, whose ingredients are approved in that state for organic production, or are listed by the Organic Materials Review Institute (OMRI). Use of unapproved pesticides in those states that allow for OMRI-listed or exempt pesticides represents a public safety license violation and can result in the cancellation of a cannabis producer's license. State allowance for pesticide use on cannabis may be in conflict with federal pesticide regulations.

Presence and Testing of Pesticides in Cannabis

Specialty agricultural supply stores for the cannabis industry, have proliferated across the US, many of which are categorized as "hydroponic". This aspect of the industry lacks any meaningful regulation or guidance. Products found in such stores have been reported to contain banned substances, and often fail to accurately disclose ingredients or provide adequate information for proper use. For example, the California Department of Food and Agriculture (CDFA) in 2011 issued cease and desist orders against the sale of a number of popular cannabis cultivation products due to their inclusion of a number of banned plant growth regulators including daminozide (Alar) and paclobutrazol (CDFA 2011). A number of these products are labeled as "organic" though they may not be compliant under the National Organics Program of the United States Department of Agriculture (USDA).

The use of such agents on cannabis crops is widespread. Daley et al. (2013) compiled a list of 148 pesticide products used in cannabis cultivation, based on a survey of California growers. Insecticides and miticides are often used on cannabis grown indoors, while fungicides are used on both indoor and outdoor crops. Inappropriate use of insecticides, miticides, and fungicides (such as improper product selection, application rate, concentration, and/or timing) can lead to pests becoming resistant and/or medical users being exposed to inappropriate residue levels.

Appropriate testing methodologies, as recommended by the Environmental Protection Agency (EPA Residue Analytical Methods [RAM]) or those of the Food and Drug Administration (FDA Pesticide Analytical Manual [PAM]), should be employed when appropriate. However, as these tests were developed for commodity food products, the amount of sample needed may be prohibitive to apply to the cannabis industry. Alternatively, The food testing QuEChERS screen uses smaller quantities and may be more applicable to a variety, though not all, of cannabis products (Schoen 2013, personal communication to AHP, unreferenced).

In the cannabis industry today, the most commonly used screening technology for organophosphates, organochlorines, carbamates, and ethylenediaminetetraacetic acid (EDTA) are immunoassays (e.g., enzyme-linked immunosorbent assays [ELISA]) and broad spectrum field tests that may or may not be validated for use on cannabis. Similarly, immunoassays for a broad range of PGRs and fungicides commonly used in cannabis cultivation are not available. Because of their relative inexpense, immunoassays are routinely used by analytical labs specializing in cannabis testing and are at high risk of not detecting pesticide residues and reporting samples to be "pesticide-free" or "non-detected". Before commercial use, any immunoassay should be validated against a standard testing methodology.

Table 10 provides a list of the most common pesticides (including acaricides, insecticides, fungicides, and plant growth regulators) used in cannabis production.

Solvent Residues

Limits on solvents used in the manufacture of botanical products are established by the International Conference on Harmonization (ICH) (ICH 2011), with exceptions made for ethanol and acetic acid in products formulated to contain these substances (e.g., tinctures and vinegars). According to the ICH guideline, solvents are categorized in 3 classes. Class 1 includes known carcinogens, toxic substances, and environmental hazards such as benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene, and 1,1,1-trichloroethane. These are to be avoided in the manufacture of herbal and/or pharmaceutical products. Class 2 and 3 solvents (Table 12) are distinguished based on their relative toxicity level. Limits established for permissible daily exposures (PDE) are determined individually for Class 2 solvents. Limits for Class 3 solvents are set at a general limit of 50 mg/day. In addition, the ICH guideline lists solvents for which no adequate toxicological data was found (Table 13) and requires manufacturers of pharmaceutical products that choose to use these solvents to supply justification for residual levels of these solvents in their final products. Petroleum ether, found in this group, is reportedly used in the production of hash oil (UNODC 2009).

Solvent extracted products made with Class 3 or other solvents, are not to exceed 0.5% residual solvent by weight or 5000 parts per million (PPM) per 10 gram of solvent-based product and are to be quantified according to the United States Pharmacopeia (USP <467>), Residual Solvents, Option 1. Higher concentrations may also be acceptable provided they are realistic in relation to safety, manufacturing, and good manufacturing practices.

Table 12 Permissable and restricted solvents in the manufacture of cannabis preparations

Class 2 solvents		Class 3 solvents Permissible daily exposure: 50 mg/day
Solvent	**Permissible daily exposure, mg/day**	
Acetonitrile	4.1	Acetic acid[†]
Chlorobenzene	3.6	Acetone
Chloroform*	0.6	Anisole
Cyclohexane	38.8	1-Butanol
1,2-Dichlorothene	18.7	2-Butanol
Dichloromethane*	6.0	Butyl acetate
1,2-Dimethoxyethane	1.0	tert-Butylmethylether
N,N-Dimethylacetamide*	10.9	Cumene*
N,N-Dimethylformamide	8.8	Dimethyl sulfoxide
1,4-Dioxane*	3.8	Ethanol*[†]
2-Ethoxyethanol	1.6	Ethyl acetate
Ethyleneglycol	6.2	Ethyl ether
Formamide	2.2	Ethyl formate
Hexane	2.9	Formic acid
Methanol*	30.0	Heptane
2-Methoxyethanol	0.5	Isobutyl acetate
Methylbutyl ketone	0.5	Isopropyl acetate
Methylcyclohexane	11.8	Methyl acetate
N-Methylpyrrolidone*	5.3	3-Methyl-1-butanol
Nitromethane*	0.5	Methylethyl ketone
Pyridine*	2.0	Methylisobutyl ketone
Sulfolane	1.6	2-Methyl-1-propanol
Tetrahydrofuran	7.2	Pentane
Tetralin	1.0	1-Pentanol
Toluene*	8.9	1-Propanol
1,1,2-Trichloroethene	0.8	2-Propanol
Xylene	21.7	Propyl acetate

* Listed as chemicals known to the state of California to cause cancer or reproductive toxicity under Proposition 65 (CAEPA 2013).
Source: AHPA (2008); CAEPA (2013); ICH (2011); United States Pharmacopeia (USP 30-NF 25 2007).

Table 13 Solvents for which no adequate toxicological data was found

1,1-Diethoxypropane	Methylisopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

Source: ICH (2011).

International Status

Definitions and regulations of what constitutes a "controlled substance" and medically useful substance differ greatly between countries. There are also varying levels of tolerance for use of mind-altering substances such as alcohol and cannabis. Internationally and domestically, regulations regarding the medical and recreational use of cannabis are changing rapidly. In the US, individual states have enacted their own rights, regulations, and prohibitions regarding both medical and recreational cannabis use, which conflict with federal law. Similarly, a number of countries (e.g., the US, Canada, Israel, the Netherlands, and others) provide an official source of medicinal-grade cannabis to certain chronically ill patients. Additionally, several countries (e.g., Canada, Denmark, Germany, Spain, New Zealand, United Kingdom) have approved pharmaceutical preparations made from cannabis extracts (e.g., Sativex®) as prescription-only medicines (MHRA 2010).

The regulation of cannabis is a subject of international treaties that include the US as a signatory (United Nations 1973). The US Controlled Substances Act (CSA) was designed to fulfill the country's treaty obligations under the United Nations' Single Convention on Narcotic Drugs (1961). This treaty restricts cannabis to appropriate medical use only, and places strict controls on cannabis cultivation in a manner similar to those imposed on opium poppies. The treaty does not apply to cannabis plants grown exclusively for industrial (fiber and seed) or horticultural purposes. As of 2013, there were 61 signatories to the Convention (United Nations 2013a) and 54 signatories to the Protocol that amended the convention in 1972 (United Nations 2013b). Following is a brief review of the manner in which cannabis is regulated domestically and internationally. Due to the rapidly changing regulatory environment, interested readers must refer to primary regulatory policies in various states and countries as well as expected requirements under international treaties.

United States

The term "marihuana" is defined in the United States Code (USC) as "all parts of the plant Cannabis sativa L., whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture, or preparation of such plant, its seeds or resin. Such "marihuana" term does not include the mature stalks of such plant, fiber produced from such stalks, oil or cake made from the seeds of such plant, any other compound, manufacture, salt, derivative, mixture, or preparation of such mature stalks (except the resin extracted therefrom), fiber, oil, or cake, or the sterilized seed of cannabis, such plant which is incapable of germination" (USC 2010). This language remains essentially unchanged from the Marihuana Tax Act of 1937.

Drug (Federal): "Marihuana," "tetrahydrocannabinols" and CBD are classified by the Drug Enforcement Administration (DEA) as Schedule I controlled substances (DEA 2011a). The findings required to place a substance on Schedule I of the Controlled Substances Act are: (a) the drug or other substance has a high potential for abuse; (b) the drug or other substance has no currently accepted medical use in treatment in the United States; and (c) there is a lack of accepted safety for use of the drug or other substance under medical supervision. Several formal petitions for the rescheduling of cannabis have been denied (DEA 2011b).

Rescheduling to Schedule II by the DEA requires for the following 5-part test to be fulfilled: 1) the drug's chemistry must be known and reproducible; 2) there must be adequate safety studies; 3) there must be adequate and well-controlled studies proving efficacy; 4) the drug must be accepted by qualified experts; and 5) the scientific evidence must be widely available. Alternatively, rescheduling could occur by Executive Order of the President or by Congress. The DEA rescheduled synthetic THC (dronabinol, Marinol®) to Schedule II in 1985, and Schedule III in 1999.

An exception is made for the "Compassionate Use" Investigational New Drug (IND) Program: In 1976, the DC Superior Court found a defendant suffering from glaucoma not guilty of possession of marijuana based on the Common Law Doctrine of Necessity (US v Randall). The defendant successfully argued that inhalation of marijuana smoke had a beneficial effect, normalizing intraocular pressure and lessening visual distortions (DC Superior Court 1976). In 1978, the same glaucoma patient brought a lawsuit against the federal government (Randall v US) for its role in disrupting his legal access to marijuana. An outcome of the lawsuit settlement by the Department of Health and Human Services (HHS), which became the basis for the Food and Drug Administration (FDA) Compassionate IND Program, was that the National Institute on Drug Abuse (NIDA) would begin supplying cannabis to patients whose physicians applied for and received use permits from the FDA. The NIDA provides funding to the University of Mississippi for growing, harvesting and storage of cannabis as well as potency monitoring and other services for the DEA (NIDA 1988). The NIDA is responsible for shipping the marijuana to registered patients. Medical diagnosis of Compassionate IND Program patients have included (ProCon.org 2014)

- AIDS
- Glaucoma
- Multiple Congenital Cartilaginous Exostoses
- Multiple sclerosis
- Nail Patella Syndrome

Drug (State): To date, medical cannabis laws have been enacted in 22 states and the District of Columbia (Stroup 2014). These laws exist in conflict with federal laws leaving discretion to US Attorneys on when to enforce federal law against participants in state-sanctioned programs. To date, however, there has been no attempt by the federal government to overturn such state laws. In August 2013 the Department of Justice issued a memo to US attorneys advising that individuals and companies following state laws should not be priorities for prosecution but ultimately

left the decision of whether or not to prosecute up to US Attorneys (Cole 2013).

Recreational (State): In 2012, Washington and Colorado, both of which allow for the medical use of cannabis, through ballot initiative, approved the controlled recreational use of cannabis, limiting its use to legal-age adults and with specific restrictions.

In 2000–2007, there have been approximately 7.9 million cannabis-related arrests in the US (US Bureau of Justice Statistics) making cannabis-related crimes one of the most frequently enforced crimes in the country. In 2012, there were a total of 749,825 marijuana arrests, of which 91,593 were trafficking/sale arrests and 658,231 were for possession (FBI Uniform Crime Report 2012).

Canada

Canadians currently have access to the widest representation of cannabinoid drugs in the world, including dronabinol (Marinol®), nabilone (Cesamet®), Sativex®, and crude cannabis. Canada also re-legalized industrial hemp cultivation in 1998. Cannabis for medical use is regulated under the Marihuana for Medical Purposes Regulations (MMPR), which came into force on June 7, 2013. Under the MMPR, marihuana for one's own medical purposes or for those of another person for whom they are responsible may be obtained only from a) a licensed producer In accordance with a medical document (signed by a licensed health care practitioner), b) from a health care practitioner in the course of treatment, or c) from a hospital in accordance with Narcotic Control Regulations. An individual may obtain up to 30 times the daily quantity from a licensed producer or from a hospital. Individuals must register to become clients of a licensed producer. Adults who reside in Canada and/or corporations with a head office or branch office in Canada are eligible to apply for a producer's license (Government of Canada 2014).

Indications: Potential therapeutic uses are outlined in Health Canada's information for health care professionals on cannabis and the cannabinoids. The listed uses include the following:

- Alzheimer's disease and dementia
- Arthritides and Musculoskeletal Disorders
- Asthma
- Chemotherapy-induced nausea and vomiting
- Epilepsy
- Gastrointestinal system disorders (irritable bowel syndrome, inflammatory bowel disease, hepatitis, pancreatitis, metabolic syndrome/obesity)
- Glaucoma
- Hypertension
- Inflammation (Inflammatory skin diseases [dermatitis, psoriasis, pruritus])
- Movement disorders (dystonia, Huntington's Disease, Parkinson's Disease, Tourette's syndrome)
- Multiple sclerosis, amyotrophic lateral sclerosis, spinal cord injury
- Pain (acute and chronic)
- Palliative care (relief from pain and other distressing symptoms, and the enhancement of quality of life)
- Psychiatric disorders (alcohol and opioid withdrawal symptoms [drug withdrawal symptoms], anxiety and depression, sleep disorders, schizophrenia and psychosis
- Wasting syndrome (cachexia, e.g., from tissue injury by infection or tumor) and loss of appetite (anorexia) in AIDS and cancer patients, and anorexia nervosa (Health Canada 2013).

European Union (EU)

In the EU, rules regarding the commerce of cannabis are not harmonized. Possession of small amounts for medical or personal use has been decriminalized or liberalized to varying degrees in several countries including Belgium, the Czech Republic, Estonia, Germany, Italy, the Netherlands, Poland, Portugal, and Spain, as well as in some non-EU European countries like Switzerland (Reuter 2010; Rosmarin and Eastwood 2012). The Netherlands and the Czech Republic have enacted programs for access to dried cannabis flowers for medical use. The Netherlands represents the most liberal state in terms of access to cannabis for both medicinal and recreational use; France, in contrast, has prohibited cannabis drugs since 1925, but never outlawed fiber-type plants (France was the only country in Western Europe that grew hemp between 1982 and 1985). Spain, because of its proximity to Morocco, leads the world in hashish seizures, accounting for 26% of global seizures with 356 tons seized in 2011 and 326 tons in 2012 (UNODC 2014).. Concerning the cultivation of industrial hemp, the current upper legal limit is 0.2% THC with a ratio of CBD to THC greater than one in most European countries (UNODC 2009).

In November 2013, a European citizens' initiative proposing the legalization of cannabis and the EU to adopt a common policy on the control and regulation of cannabis production, use and sale, was registered with the European Commission. Citizens' initiatives have one year to collect one million signatures of EU citizens old enough to vote. If the requisite number of signatures is obtained, the Commission has 3 months to examine the initiative, meet with the initiative organizers, hold a public hearing, and prepare a formal response. The Commission is not obliged to propose legislation as a result of an initiative. If the Commission decides to put forward a legislative proposal, the normal legislative procedure kicks off, i.e. the Commission proposal is submitted to the legislator (generally the European Parliament and the Council, or, in some cases, only the Council) and, if adopted, it becomes law (European Commission 2013).

India

India enacted the Narcotics Drugs and Psychotropic Substances Act in 1985, which brought India into compliance with the UN's Single Convention on Narcotic Drugs. Ganja (flowering tops) and charas (hashish) are illegal. Bhang (the dried leaf of cultivated or wild-collected Cannabis sativa,) when used in traditional medicine preparations and products, is regulated as an active ingredient of traditional medicines used in the Indian Systems of Medicine (Ayurveda, Siddha, and Unani). Quality standards monographs are published in the Ayurvedic Pharmacopoeia of India (API), Siddha Pharmacopoeia of India (SPI), and Unani Pharmacopoeia of India (UPI).

Indications: Ayurveda: Agnimandya (digestive impairment), anidra (insomnia), atisara (diarrhea), klaibya (male impotence), grahani roga (malabsorption syndrome) (API 1989); Siddha: Kakkirumal (whooping cough), mikupaci (excessive appetite), narampuvali (neuralgia), orraittalaivali (hemicranias/migraine), perumpatu (menorrhagia), vantipeti (vomiting and diarrhea) (SPI 2008). Unani: Ishal (diarrhea), kasrat-e-tams (polymenorrhagia), bawaseer (piles), sual (bronchitis), waj-ul-kabid (hepatalgia), qulanj (colic) (UPI 2007).

Israel

In July 2011, the Israeli Cabinet approved arrangements and supervision regarding the supply of cannabis for medical and research uses in recognition that the medical use of cannabis is necessary in certain cases. The Health Ministry, in coordination with the Israel Police and the Israel Anti-Drug Authority, is responsible for supplies from imports and local cultivation (State of Israel Prime Minister's Office 2011).

In December 2013, the Israeli Cabinet amended the medical marijuana regulations by increasing the pool of physicians allowed to prescribe cannabis to their patients from 21 to 31. The new rule also changes the way in which marijuana can be grown, packaged and distributed in Israel. As of early 2014, approximately 14,000 patients have been given prescriptions to use medicinal marijuana.

Quality: There are currently 10 different strains of marijuana being grown by 8 authorized growers and distributed to patients with a prescription (Israeli Medical Association 2014).

Indications: Cannabis prescriptions are available for these conditions (and others on a case-by-case basis):

- AIDS wasting syndrome
- Asthma
- Chronic pain due to a proven organic etiology
- HIV patients with significant loss of body weight or a CD4 cell count below 400
- Inflammatory bowel disease (but not Irritable Bowel Syndrome)
- Malignant cancerous tumor in various stages
- Multiple sclerosis
- Orphan diseases (i.e., diseases and conditions that affect only a small percentage of the population and for which few, if any, pharmaceutical drugs are developed)
- Parkinson's Disease
- Vomiting and pain associated with chemotherapy for cancer (Stafford Mader 2013).

Netherlands

The Office for Medicinal Cannabis (OMC) is responsible for the production of cannabis (dried flower tips harvested from female Cannabis sativa plants) for medical and scientific purposes and is the exclusive supplier of medicinal cannabis to pharmacies, and on its import and export.
Quality: Medicinal cannabis provided by the OMC is of pharmaceutical quality, produced under controlled cultivation according to Good Agricultural Practices (GAPs). Three types of medicinal cannabis are available through pharmacies: Bedrocan, Bedrobinol, and Bediol. The recommended modes of administration are by making tea or through inhalation.

Indications: According to OMC, there is sufficient reason to believe that medicinal cannabis can help in cases of:

- Pain and muscle spasms or cramps associated with multiple sclerosis or spinal cord damage;
- Nausea, loss of appetite, weight loss, and debilitation due to cancer or AIDS;
- Nausea and vomiting associated with chemotherapy or radiotherapy used in the treatment of cancer, hepatitis C or HIV infection and AIDS;
- Chronic pain; primarily pain associated with the nervous system, (e.g., damaged nerve, phantom pain, facial neuralgia or chronic pain which remains after the recovery from shingles);
- Gilles de la Tourette syndrome;
- Therapy-resistant glaucoma (OMC 2011).

Switzerland

Medical use: Obtaining marketing authorization from Swissmedic for Complementary and Herbal Medicinal Products (KPAV) that contain preparations made from Cannabis sativa as an active ingredient is possible (Swissmedic 2013a). In November 2013 the first cannabis product received marketing authorization, Sativex® Spray (Cannabis sativae folii cum flore extractum spissum) (Swissmedic 2013b).

Indications: Treatment for symptom improvement in adult patients with moderate to severe spasticity due to multiple sclerosis who have not responded adequately to other antispasticity medication and who demonstrate clinically sig-

nificant improvement in spasticity related symptoms during an initial trial of therapy (Almirall AG 2013).

Recreational use: In 2012, the Federal Assembly amended the federal law on narcotics and psychotropic substances, ostensibly decriminalizing possession of up to 10 grams of cannabis with the implementation of a simplified procedure for imposing a flat fee fine in the amount of 100 Swiss Francs (Federal Assembly of the Swiss Confederation 2012).

Uruguay

In December 2013, Uruguay became the first country to legalize the growing, sale, and smoking of cannabis. The government-sponsored bill that led to this approval provides for regulation of the cultivation, distribution, and consumption of cannabis. The primary stated motivation of the legislation was to fight drug trafficking of cannabis (Uruguay, Law No. 19.172). The law allows for Uruguayan residents over the age of 18 to become a registered user and to purchase up to 40 grams (1.4 ounces) per month from licensed pharmacies. A government database will monitor consumer monthly purchases. Additionally, Uruguayans will be able to grow 6 cannabis plants in their homes a year, or as much as 480 grams (approximately 17 ounces), and form smoking clubs of 15–45 members that can grow up to 99 plants per year. Regional leaders in Latin America consider legalization as a way to help curb the criminal activity and violence associated with the illegal drug trade.

Under the law, a drug control board will be convened that will regulate cultivation standards, fix prices, and monitor consumption of registered users. The use of cannabis is legal in Uruguay, but until this law, cultivation and sale of the drug was not.

Select Countries with Severe Penalties for Cannabis Possession or Trafficking

Indonesia

Penalties for possession, use, or trafficking in illegal drugs in Indonesia are severe, and convicted offenders can expect long jail sentences and heavy fines. A life sentence or the death penalty can be given in cases of drug trafficking (US Department of State 2014).

Iran

Iran executes many people each year on drug-related charges (US Department of State 2014). Under the 2011 Anti-Narcotics Law the term "narcotic" (for certain offenses) refers to bhang (preparation of the leaves and flower tops of Indian hemp), Indian hemp juice, opium, opium juice or residue, or synthetic non-medical psychotropic substances listed by Parliament. The drugs cocaine, heroin, GHB, LSD, and MDMA, among others, fall under a separate "narcotic" definition with different punishments. Offenses that carry the death penalty include fourth conviction for cultivation of cannabis; third conviction for purchase, possession, concealment or transport of 5–20 kg of cannabis; and import, export, production, manufacture, distribution, sale, or supply of more than 5 kg of cannabis. The death penalty is commuted for first-time offenders when distribution or sale was not accomplished and the amount was less than 20 kg (Amnesty International 2011).

Malaysia

Malaysian legislation provides for a mandatory death penalty for convicted drug traffickers. Those arrested with possession of 200 grams (7 ounces) of cannabis will be presumed by law to be trafficking in drugs (US Department of State 2014). The majority of those sentenced to death in Malaysia were convicted of marijuana or hashish offenses with an estimated 77 executions during 2008–2010 (Gallahue 2011).

Saudi Arabia

Those convicted of the import, manufacture, possession, and/or consumption of illegal drugs in Saudi Arabia can expect long jail sentences, heavy fines, public floggings, and/or deportation. The penalty for drug trafficking in Saudi Arabia is death. Saudi officials make no exceptions (US Department of State 2014).

Singapore

Singapore has a mandatory death penalty for many narcotics offenses including trafficking cannabis. Police have the authority to compel both residents and non-residents to submit to random drug analysis (US Department of State 2014). Any person having in his/her possession more than 15 grams of cannabis, 30 grams of cannabis mixture (any mixture of vegetable matter containing THC and CBD in any quantity), or 10 grams of cannabis resin (any substance containing resinous material and in which THC and CBD are found in any quantity) shall be presumed to have had that drug in possession for the purpose of trafficking. The punishment for trafficking in cannabis where the quantity is not less than 330 grams and not more than 500 grams is maximum 30 years or imprisonment for life and 15 strokes of the cane. The minimum punishment is 20 years and 15 strokes of the cane. The penalty for trafficking more than 500 grams is death (AGC Singapore 2008).

United Arab Emirates (UAE)

Legislation enacted in January 1996 imposes the death sentence for convicted drug traffickers. Since January 2006, possession of even trace amounts of illegal drugs, which include cannabis, has resulted in lengthy prison sentences for foreign citizens transiting the UAE. It is possible to be convicted for drug possession based on the result of a drug test even if no other evidence exists, regardless of when or where the consumption originally occurred (US Department of State 2014).

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Female cannabis plant
Source: Elizabeth Blackwell, Herbarium Blackwellianum (1757). Courtesy of the Lloyd Library and Museum, Cincinatti, OH.

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