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Review

Cannabis Inflorescence for Medical Purposes: USP Considerations for Quality Attributes

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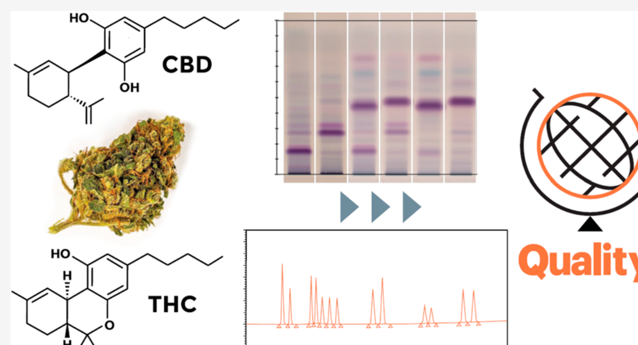
ABSTRACT: There is an active and growing interest in cannabis female inflorescence (*Cannabis sativa*) for medical purposes. Therefore, a definition of its quality attributes can help mitigate public health risks associated with contaminated, substandard, or adulterated products and support sound and reproducible basic and clinical research. As cannabis is a heterogeneous matrix that can contain a complex secondary metabolome with an uneven distribution of constituents, ensuring its quality requires appropriate sampling procedures and a suite of tests, analytical procedures, and acceptance criteria to define the identity, content of constituents (e.g., cannabinoids), and limits on contaminants. As an independent science-based public health organization, United States Pharmacopeia (USP) has formed a Cannabis Expert Panel, which has evaluated specifications necessary to define key cannabis quality attributes. The consensus within the expert panel was that these specifications should differentiate between cannabis chemotypes. Based on the secondary metabolite profiles, the expert panel has suggested adoption of three broad categories of cannabis. These three main chemotypes have been identified as useful for labeling based on the following cannabinoid constituents: (1) tetrahydrocannabinol (THC)-dominant chemotype; (2) intermediate chemotype with both THC and cannabidiol (CBD); and (3) CBD-dominant chemotype. Cannabis plants in each of these chemotypes may be further subcategorized based on the content of other cannabinoids and/or mono- and sesquiterpene profiles. Morphological and chromatographic tests are presented for the identification and quantitative determination of critical constituents. Limits for contaminants including pesticide residues, microbial levels, mycotoxins, and elemental contaminants are presented based on toxicological considerations and aligned with the existing USP procedures for general tests and assays. The principles outlined in this review should be able to be used as the basis of public quality specifications for cannabis inflorescence, which are needed for public health protection and to facilitate scientific research on cannabis safety and therapeutic potential.



■ INTRODUCTION

The use of the female cannabis (*Cannabis sativa* L.; Cannabaceae) inflorescence for medical purposes has increased greatly in the past decade. As of early 2020, a number of countries have implemented or are in the process of implementing legislation and systems for medical access, and millions of people are using cannabis products for medical purposes for a reported array of medical conditions. In 2018, it was estimated that there were more than 2 million people in the United States (U.S.) using cannabis for a variety of illnesses,[1] while in Canada in 2017, 1.6 million people reported using cannabis for medical purposes, an increase of 24% since 2015.[2] Recalls due to contamination and incorrect cannabinoid composition labeling in jurisdictions where cannabis is sold, such as Canada and some U.S. states, highlight the pressing need for quality control standards.[3]

In 2017, the National Academies of Sciences, Engineering, and Medicine reported the current state of evidence and recommendations for research on the health effects of cannabis and cannabinoids.[4] Although the U.S. Food and Drug Administration (FDA) has not approved cannabis inflorescence as a safe and effective botanical drug for any indication,[5] the FDA has publicly highlighted the need to conduct research to obtain data on the safe and effective medical use of cannabis or

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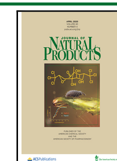

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its active constituents.[6] Resources to help ensure the validity of such studies include the FDA Botanical Drug Development Guidance,[7] which provides appropriate requirements for plant materials as drugs. Furthermore, recognizing the complexity of natural products, the National Institutes of Health (NIH) has a natural products integrity policy to address botanical study materials by outlining special requirements for their characterization.[8] A search of ClinicalTrials.gov, the Web site maintained by the U.S. National Library of Medicine at the NIH, returned 246 open clinical trials for the search term "cannabis" as of November 6, 2019.[9]

Need for Quality Control. In light of the permitted use of cannabis for medical purposes in a majority of U.S. states and in many countries around the world, the known and demonstrated quality issues with cannabis and the active ongoing clinical research in this field, healthcare professionals, the research community, and perhaps most importantly patients and the public will benefit from increased quality control of cannabis. Information on the quality attributes of materials in terms of identity, composition, and purity, and the scientific resources to test for these, can help prevent patient harm resulting from exposure to substandard, contaminated, or adulterated cannabis products. In addition, availability of cannabis, or its constituents, prepared according to consistent manufacturing practices will increase the reproducibility and applicability of preclinical and clinical data.[10,11]

Need for Public Quality Standards. The absence of federal guidance for cannabis testing has led some U.S. states to develop their own, unstandardized approaches.[12] Similarly, the need for harmonized laboratory testing protocols has been highlighted due to concerns related to interlaboratory differences.[13] There is a wide disparity among the guidelines adopted by the various U.S. states and countries around the world. Important considerations for cannabis testing include the development and adoption of validated analytical methods to help address the challenges faced by state laboratory directors and to improve the ability of cannabis producers and consumers to have confidence in the composition and quality of cannabis products. Test methodologies should also be available to confirm the absence of synthetic cannabinoids, which are an emerging issue. They have already been identified in cannabis products and shown to cause serious harm.[14]

Standards that set forth specifications for quality attributes are fundamental to meet the above challenges and to conduct the tests for quality attributes. In the case of complex substances such as cannabis, which can contain a diverse and heterogeneous metabolome and uneven distribution of constituents, the standards should include (1) laboratory verification of identity as cannabis, including any distinctions from hemp depending on the jurisdiction; (2) quantitative composition of cannabinoids; and (3) tests to help ensure minimal exposure to contaminants such as pathogenic microorganisms, toxic elemental contaminants, mycotoxins, and pesticide residues.

USP Standards-Setting Efforts. The United States Pharmacopeia (USP) is an independent, scientific, nonprofit public health organization devoted to improving health through the development of public standards for medicines, food ingredients, and dietary supplements and related programs. The organization publishes the *United States Pharmacopeia* and *National Formulary* (*USP-NF*), two official compendia of the United States recognized in the Federal Food, Drug, and Cosmetic Act. To conduct its work, USP has evolved its expertise in the development of standards for articles of botanical origin, including analytical procedures and acceptance criteria to help ensure the identity and content of constituents of botanical articles.

In 2016, USP published a *Stimuli* article analyzing the advisability and feasibility of developing public quality standards for cannabis for medical purposes and USP's potential role in developing such standards.[15] On the basis of public feedback, USP concluded that the development of quality standards for dried cannabis female inflorescence was feasible and necessary, but that inclusion of such standards in a legally recognized official compendium was not advisible given the current legal status of cannabis at the U.S. federal level.

The following sections of this review article describe the scientific quality attributes and related standards developed by the Cannabis Expert Panel convened by USP in 2016 at the direction of the USP Botanical Dietary Supplements and Herbal Medicines Expert Committee. The methods and specifications described below provide (1) fit-for-purpose analytical methods for the identification of cannabis inflorescence (specifically, the pistillate or female inflorescence, often referred to as the "bud" in the vernacular; hereinafter referred to simply as the inflorescence unless the staminate or male inflorescence is specified) using macroscopic, microscopic, and chromatographic procedures, (2) methods to determine the composition of cannabis inflorescence using quantitative tests such as high-performance liquid chromatography (HPLC) and gas chromatography (GC), and (3) quality specifications to limit the content of common contaminants of cannabis. Additional recommendations highlight the importance of naming, definitions, use of reference materials, and packaging/storage conditions.[16] Multiple tests are included to complement each other and thereby provide an appropriate quality characterization.

We present this review as a scientific contribution to further our mission to improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods.

■ CHEMICAL CONSTITUENTS AND PHARMACOLOGY

The constituents of *C. sativa* most widely recognized as responsible for its pharmacological effects are known as cannabinoids. Mono- and sesquiterpenes are the other major components of cannabis. Cannabinoids are terpenophenolic compounds, and the most abundant and well-known phytocannabinoids are Δ^9-tetrahydrocannabinol (THC; CAS 1972-08-3) and cannabidiol (CBD; CAS 13956-29-1). These are produced by cannabis in their carboxylic acid forms, Δ^9-tetrahydrocannabinolic acid (THCA; CAS 23978-85-0) and cannabidiolic acid (CBDA; CAS 1244-58-2), respectively, which are decarboxylated by heating (e.g., smoking or baking), by light, or by natural degradation. Δ^9-THC, the decarboxylated form of THCA, is the cannabinoid predominantly responsible for the psychoactive properties of cannabis. Both THCA and CBDA share the precursor molecule cannabigerolic acid (CBGA; CAS 25555-57-1). This precursor is formed by condensation of olivetolic acid, originating from the polyketide biosynthetic pathway, and geranyl pyrophosphate, originating from the deoxyxylulose phosphate pathway,[17] also known as the methylerythritol phosphate pathway (Figure S1, Supporting Information). Several reviews of cannabinoid biosynthesis and chemical diversity have been published.[18−21]

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The discovery of G protein-coupled receptors has advanced the understanding of the neurobiological basis for the interactions of cannabis constituents with endocrine functions. For example, the activation of cannabinoid receptor 1 (CB_1) can induce psychoactive effects, and cannabinoid receptor 2 (CB_2) is associated with non-impairing effects of cannabis. Broadly speaking, the CB_1 receptors are expressed in many tissues but are most abundant in the central nervous system and play important roles in many functions, including the modulation of mood, appetite, pain, and memory, among others;[22] CB_2 receptors are important for the modulation of immune function.[23] THC has high affinity for both CB_1 and CB_2 receptors and similar partial agonist behavior for both of these sites. CBD, on the other hand, shows several hundred-fold lower binding affinity at CB_1 compared to CB_2 receptors and serves as a negative allosteric modulator at the CB_1 receptor, along with an ability to bind to many other targets.[22−24] CBD is believed to mitigate some of the effects of THC including psychoactivity, sedation, and tachycardia, while contributing to the analgesic and antiemetic properties of THC, among others.[25] Cannabinoids can also target several other receptor systems including other G protein-coupled receptors and serotonin receptors. Other compounds such as cannabigerol (CBG; CAS 25654-31-3) and cannabichromene (CBC; CAS 20675-51-8) do not bind to CB_1 or CB_2 receptors with high affinity, but impact the cannabinoid system by inhibiting the uptake of anandamide, an endogenous ligand for these sites, and through transient effects on receptor potential channels and other receptors.[23]

NAMING AND DEFINITIONS

A standardized nomenclature is the first step to adequately address the quality of products. For example, USP has developed a guideline to assign titles to monographs (documentary standards) for the quality of botanical articles of commerce used to make herbal medicines, with an accompanying glossary.[26] For cannabis, currently the most common article of commerce is the "bud" (i.e., the female inflorescence). Necessary further details defined in the standard include the Latin binomial with its taxonomic authority abbreviations and family (e.g., *Cannabis sativa* L., Cannabaceae), relevant subspecies or varieties, cultivars (cultivated varieties selectively bred) and chemotypes (chemical reflection of the plant phenotype), and the part(s) of the plant present. Since the medicinal effects depend on chemical composition, the concentration range of characteristic phytochemicals in the botanical article may be standardized in order to achieve consistent quality.

The USP approach to documentary standards of quality is hierarchical. First, the standards for the identity, purity, and strength (i.e., the content of specific phytochemicals of relevance to the intended use) of the botanical raw material are established. Then, quality attributes are defined for articles of commerce prepared from the USP-grade botanical raw material, such as a minimally processed powdered plant material. A cannabis inflorescence can be further processed to obtain other products derived from the raw material, meeting the quality attributes in this review article to make a cannabis extract with concentrated cannabinoids, a semipurified material containing just a mixture of cannabinoids or a fully purified (i.e., isolated) single cannabinoid, or a formulated cannabis product containing other ingredients, such as an edible oil or capsule. Documentary standards of quality and associated physical Reference Standards should be established as the need arises for any of these types of articles of commerce derived from cannabis. Therefore, this publication represents just the first step of defining quality attributes for the cannabis inflorescence, recognizing that cannabis-derived products such as extracts, tinctures, oils, concentrates, and isolated cannabinoids, among others, are being investigated as more readily standardized derivatives for medicinal purposes.

In order to achieve the level of standardization required for reliable medical use and for clinical trials, cannabis materials should be derived from clonally propagated homogeneous cultivars grown under conditions that produce consistent chemical profiles or chemotypes.[27] A cultivar is an assemblage of plants that (1) has been selected for a particular characteristic or combination of characteristics and (2) remains distinct, uniform, and stable in these characteristics when propagated by appropriate means; it is named according to the provisions of the current edition of the International Code of Nomenclature for Cultivated Plants.[28] Many of the kinds of cannabis in commerce for medical purposes have been selected according to phytochemical criteria and are not yet officially registered crops or cultivars.

A term commonly used in both the scientific literature and in commerce is a "strain" of cannabis, but the thousands of so-called strains are not consistent in either morphological or chemical profiles and thus cannot be relied upon for consistent categorization of different kinds of cannabis.

The term "chemotype" is used in this review article to describe phytochemical profiles to which individual plants or populations of cannabis may conform. The phenotypic expression of the secondary metabolome is controlled by its genome and the environment (e.g., temperature, light exposure, altitude, water, soil fertilizers, and the insects and microflora in the soil and on the plant). While in cannabis chemotypes are conventionally defined by THC/CBD ratios, the phytochemical classes that are used here to characterize samples of cannabis include these cannabinoids but also consider other cannabinoids and the mono- and sesquiterpenes, for reasons described in detail below. Thus, essential variables affecting chemical expression of the genome should be included in the definitions (e.g., age of the plant, preferred cultivation climate, harvest seasons). Post-harvest processing requirements that affect the chemical composition of the article should also be defined (e.g., drying conditions). Minimum content and ranges of marker constituents are typically included in the compendial definitions.

Botanical ingredients should be designated with standardized and recognizable names. The convention adopted by USP to designate herbal drugs consists of the Latin binomial of the species followed by the part of the plant.[26] In this particular case, the assignment of Latin binomials to *Cannabis* species is a matter of taxonomic debate.

Most authorities recognize cannabis as a single highly variable species, designated as *Cannabis sativa* L., with two subspecies [i.e., *C. sativa* subsp. *sativa* and *C. sativa* subsp. *indica* (Lam.) E.Small & Cronquist].[29] For the purpose of this review article, USP defines cannabis as the dried pistillate (female) inflorescence of the plant *Cannabis sativa* L. (family Cannabaceae) including its subspecies, varieties, and chemotypes. Accordingly, the designation chosen to name the article is "Cannabis Inflorescence" as representative of the genus and the part of the plant used, respectively.

Cannabis Chemotypes. Analysis of large data sets has shown that the prevalent chemotypes of cannabis are genetically evolved to produce predominantly one or more of the

cannabinoids.[30] The following paragraphs include examples of attempts to classify cannabis into groups.

One authoritative publication by Small[31] noted various traits of cannabis selected for domestication (e.g., differing morphology of the mature plant, cannabinoid profiles, terpene profiles, concentration and distribution of the secretory glands that produce the cannabinoids, inflorescence color) and recognized the following six categories:

(1) Non-narcotic plants domesticated for stem fiber (and to a minor extent for oilseed) in western Asia and Europe; cannabinoid levels are typically low, with relatively high CBD levels compared to THC;
(2) Non-narcotic plants domesticated for stem fiber (and to a minor extent for oilseed) in East Asia, especially the People's Republic of China; cannabinoid levels are typically low, with relatively high CBD levels compared to THC;
(3) Narcotic plants domesticated in a wide area of south-central Asia for very high THC content; cannabinoids are mostly or almost completely THC;
(4) Narcotic plants domesticated in southern Asia, particularly in Afghanistan and neighboring countries, for substantial amounts of both THC and CBD;
(5) Hybrid class of cultivated plants that has been generated between the two [non-narcotic] fiber groups (1 and 2 above);
(6) Hybrid class of cultivated plants that has been generated between the two narcotic groups (3 and 4 above)

The first two groups in the above categorization scheme would be what most would recognize as "industrial hemp". According to Small (2015),[31] "it should be understood that the hybrid cultivars or strains are not simply first generation hybrids, but represent various degrees of stabilized intermediacy, essentially representing all degrees of variation between the parental groups, so that there is continuous variation among fiber races, and similarly continuous variation among narcotic races."

The term "narcotic" used by Small (2015) in his categorization of cannabis groups has the root meaning "to make numb", and by extension, sleep-inducing. Cannabis may or may not manifest this property depending on the chemotype (e.g., one high in CBD with no THC), and since the term has come to be associated with opioid drugs, it may not always be the most relevant to use in the context of cannabis. The alternative terminology of "drug type" and "non-drug type" is not accurate either since CBD and other constituents of plants in categories (1) and (2) have potential drug uses. Therefore, it may be better to refer to these groups as Small's categories (1) to (6), where, for example, category (1) comprises plants domesticated for stem fiber (and to a minor extent for oilseed) in western Asia and Europe with generally low cannabinoid levels and CBD levels higher than THC.

The United Nations Office on Drugs and Crime[32] categorization of cannabis into drug and fiber types is based on the GC-FID. Hillig and Mahlberg[33] used THC/CBD ratios and a statistical approach to define chemotaxonomic trends in cannabis and noticed that most samples did not fall within the arbitrary values set by the United Nations Office on Drugs and Crime. Instead, most samples clustered into three chemotypes based on relative content of these cannabinoids. Other classifications exist from the U.S. National Institute on Drug Abuse (NIDA), which classifies and makes available cannabis plant material blends for research in various ratios and concentrations of THC to CBD.[34] Yet another classification example is found in The Netherlands from their Ministry of Health, Welfare and Sports' Office of Medicinal Cannabis, where five varieties of GMP-certified, gamma-irradiated "cannabis flos"—the female flowers (i.e., pistillate) of *Cannabis sativa* L.—are characterized as follows:[35]

- Bedrocan: THC ca. 22% and CBD <1%, from the cultivar "Afina", the longest on the market and most widely prescribed;
- Bedrobinol: THC ca. 13.5% and CBD <1%, from the cultivar "Ludina", considered medium strength;
- Bediol: THC ca. 6.3% and CBD ca. 8%, from the cultivar "Elida", containing a balanced ratio of THC to CBD;
- Bedica: THC ca. 14% and CBD <1%, from the cultivar "Talea", selected for its high content of myrcene, a monoterpene reputedly associated with a calming effect;
- Bedrolite: THC <1% and CBD ca. 9%, from the cultivar "Rensina", intended for patients with treatment-resistant epilepsy

Each of the above approaches to categorizing groups of cannabis materials has its strengths and weaknesses, and generally they fit well in or align with the three grouping criteria we propose in this review article. As research progresses on the pharmacology of the minor cannabinoids, mono- and sesquiterpenes, flavonoids such as the cannflavins, and other potentially bioactive constituents of cannabis, the current categories with their emphasis on THC and CBD are likely to evolve in order to capture all promising pharmacological leads and their interactions with other drugs.[36,37]

The differential activities of the cannabinoids in humans provide a rationale for the classification of the different chemotypes of cannabis. On the basis of the clinical experience with the cannabinoid constituents documented in drug approvals (i.e., THC and CBD), the three main chemotype groups recognized by Small and Beckstead[38,39] have been initially identified in this publication to be relevant for labeling expectations of cannabis: THC-dominant, CBD-dominant, and THC/CBD-intermediate. For the purposes of this review article, unless otherwise indicated, when THC and CBD are referenced, it is taking into account the ability of the carboxylated forms (THCA and CBDA) to convert to their noncarboxylated counterparts. It is explicitly recognized that there are additional chemotypes in which other cannabinoids are abundant (for example, CBG-dominant or enriched in cannabinoids with a C_3 versus a C_5 side-chain and recognized by the suffix "varin"). Furthermore, byproducts such as CBN may be found in old or improperly stored cannabis since it is a nonenzymatic oxidative degradation product of THC. CBN has a 2-fold lower affinity for CB_1 receptors and a 3-fold higher affinity for CB_2 receptors compared to THC, thus affecting cells of the immune system to a greater extent than those of the central nervous system.[40] Other cannabinoids such as tetrahydrocannabivarin (THCV; CAS 31262-37-0) can also display a variety of biological actions and may be found in specially bred chemotypes of cannabis in the form of tetrahydrocannabivarinic acid (THCVA; CAS 39986-26-0). Since the content of these cannabinoids is typically low in the majority of cannabis that is readily available to consumers (less than 10 mg/g, or 1% w/w), and there is still insufficient clinical evidence pertaining to the use and effects of these less commonly seen molecules, the setting of specifications

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for plant material based on the content of these constituents may be premature.[41−43]

Isoprenoids are the largest category of plant secondary metabolites (with the other large groups being phenolics and alkaloids) and are characterized by their synthesis from isoprene building blocks. This class of chemicals spans relatively small molecules such as monoterpenes (containing two isoprene units) to larger secondary metabolites (containing 30 or more carbons). In cannabis, it is the mono- and sesquiterpenes that form the majority of the isoprenoids found in the plant, and these volatile constituents are largely responsible for the flavor and odor of cannabis. Cannabis monoterpenes (C_{10}) and sesquiterpenes (C_{15}) can contribute roughly 2−5% of the cannabis inflorescence dry weight and are referred to with the general term "terpenes" for the purpose of this article. The most dominant terpenes in cannabis include β-myrcene [CAS 123-35-3], D-limonene [CAS 5989-27-5], γ-terpinolene [CAS 586-62-9], α-pinene [CAS 7785-26-4], and β-caryophyllene [CAS 87-44-5]. The synergistic and modulating clinical effects between cannabinoids and terpenes are active fields of research.[44] Due to the contribution of terpenes to the organoleptic characteristics, and limited or anecdotal evidence that these can alter the perceived effects of cannabis, terpenes may form the basis for further subcategorization of cannabis chemotypes in order to establish the impact of these substances on the pharmacological effects of cannabis products when used in clinical practice.

Naming in Laws and Regulations. The federal Controlled Substances Act (CSA) classifies cannabis containing more than 0.3% Δ^9-THC on a dry weight basis as a Schedule I drug (termed as "Marihuana" or "Marijuana" under the CSA),[45] which means that its use, sale, cultivation, and distribution in the United States are federally illegal except for research purposes. The term "cannabis" is used in this article to refer to the plant used for medical purposes, regardless of the legal definition as a controlled substance.

The Agriculture Improvement Act of 2018 defined the term "hemp" to mean "the plant *Cannabis sativa* L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a Δ^9-tetrahydrocannabinol concentration of not more than 0.3% on a dry weight basis". The Act also amended the CSA to exclude hemp from the definition of marihuana and to remove it from the Schedule I category.[46] It updated the definition of the term "marihuana" to mean "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. The term "marihuana" does not include (i) hemp, as defined in section 297A of the Agricultural Marketing Act of 1946 or (ii) 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 such plant which is incapable of germination." Accordingly, hemp is now categorized as an agricultural commodity regulated by the U.S. Department of Agriculture (USDA) and is no longer a controlled substance under federal law. The recent USDA Interim Final Rule (IFR) included provisions for maintaining information on the land where hemp is produced, testing the levels of Δ^9-tetrahydrocannabinol, disposing of plants not meeting necessary requirements, licensing requirements, and measures to ensure compliance with these new requirements.[47] The IFR also clarified that samples must be tested using postdecarboxylation or tested using analytical methods where the total THC concentration level reported accounts for the conversion of Δ^9-THCA into Δ^9-THC.

Hemp is defined differently in the Canadian *Industrial Hemp Regulations*,[48] which provide a more restricted regulatory definition of hemp, stating that "industrial hemp means a cannabis plant—or any part of that plant—in which the concentration of THC is 0.3% w/w or less in the flowering heads and leaves". Only certain products derived from hemp are allowed under this regulatory regime (e.g., fiber, or hemp seeds and their derivatives such as protein and food oils), and any hemp seed or its derivatives must contain less than 10 ppm THC; otherwise the products are regulated as cannabis. Hemp flowers and their extracts, including CBD, fall under the Canadian *Cannabis Regulations* regardless of THC concentration. Health Canada has further clarified that in hemp seed products (i.e., hemp seed, hemp protein, and hemp oils) other cannabinoids such as CBD should be present only in trace amounts from adhering resin as a contaminant of the seed.[49] The Canadian hemp regulations also prescribe that the THC concentration must take into account the potential to convert THCA into THC.

As is the case with cannabis that is not considered hemp, the cannabinoid and terpene content of hemp may vary depending on the nature of the chemotype, the part of the plant, and other factors such as the growth, harvest, and storage conditions. Fiber and seed-type hemp and hemp food products such as hemp oils, hemp proteins, and hemp seeds devoid of CBD or other cannabinoids are used as foods rather than intended for medical use and fall outside the scope of this review article.

IDENTIFICATION TESTS

Establishing the identity of a complex botanical specimen often requires use of multiple analytical procedures with attributes of specificity to identify the correct plant material (e.g., cannabis pistillate inflorescence) and differentiate the material from closely related species that could be used as adulterants or substitutes for the article. USP general chapters describe the general procedures that can aid in the identification of botanicals, e.g., *USP* general chapter ⟨563⟩ *Identification of Articles of Botanical Origin*,[50] *USP* general chapter ⟨203⟩ *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*,[51] and *USP* general chapter ⟨1064⟩ *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure*.[52]

Macroscopic and Microscopic Methods. The typical macroscopic and microscopic characteristics of the cannabis pistillate (female) inflorescence may be recognizable in their native (not powdered) form and may aid in the determination of identity. The identifying features of the cannabis pistillate inflorescence and the associated illustrations are included in Appendix 1, Supporting Information.

HPTLC Chromatographic Profile. The limitation of a macroscopic or microscopic examination in identifying different chemotypes of cannabis can be addressed by an HPTLC fingerprint, which can detect the presence of and discriminate the relative abundance of the major cannabinoids in both acidic and noncarboxylated forms and the conversion of the cannabinoids in the form of carboxylic acids to their decarboxylated counterparts by heat.

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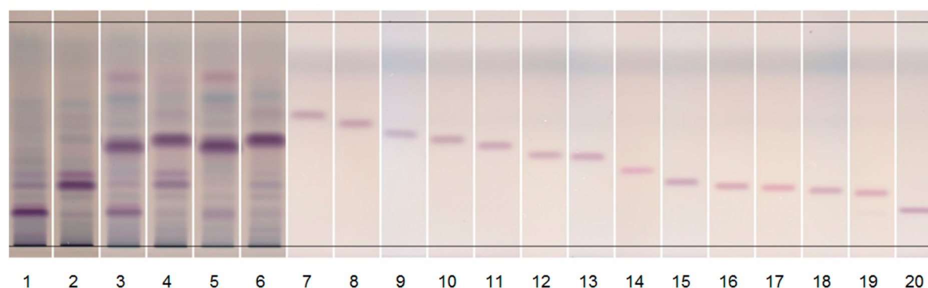

Figure 1. HPTLC profile of 11 cannabinoids and the fingerprints of three cannabis chemotypes before and after decarboxylation. Track assignments: 1: THC-dominant cannabis; 2: decarboxylated THC-dominant cannabis; 3: THC/CBD-intermediate cannabis; 4: decarboxylated THC/CBD-intermediate cannabis; 5: CBD-dominant cannabis; 6: decarboxylated CBD-dominant cannabis; 7: CBDV; 8: CBDVA; 9: CBG; 10: CBD; 11: CBDA; 12: THCV; 13: CBGA; 14: CBN; 15: Δ^9-THC; 16: Δ^8-THC; 17: CBL; 18: THCVA; 19: CBC; 20: Δ^9-THCA.

The chromatographic conditions for this method are described in Appendix 2, Supporting Information. The system suitability requires resolution of cannabinoids without overlap using a mobile phase composed of methanol, water, and glacial acetic acid (80:10:10). Fast blue B salt has been used traditionally as the reagent of choice to visualize cannabinoids. However, it is being banned from analytical laboratories due to toxicity issues, and hence vanillin sulfuric acid as a less toxic reagent is used in the proposed method. Although CBD and CBDA may not be fully resolved in samples with a high content of CBDA under the proposed conditions, this system is still useful to identify the cannabis chemotypes based on THC and CBD content (e.g., THC-dominant, CBD-dominant, or THC/CBD-intermediate). Another limitation of the method is that CBGA may co-migrate with THCVA. Typically, CBGA levels are higher than THCVA levels, unless a cultivar is bred to produce a high content of THCVA. These co-migrations are eliminated after decarboxylation, which converts CBDA into CBD, CBGA into CBG, and THCVA into THCV, and hence would not impact the appropriate identification of the chemotypes if decarboxylated test materials are used.

THC-dominant cannabis inflorescence that has not been decarboxylated shows the most intense band corresponding to THCA, a band corresponding to THC, and very weak or absent bands corresponding to CBD and CBDA. Bands corresponding to other cannabinoids such as CBGA and cannabidivarinic acid (CBDVA; CAS 31932-13-5) may also be observed. CBD-dominant cannabis inflorescence shows the most intense bands corresponding to CBD and CBDA, with weak bands due to THCA and THC. THC/CBD-intermediate cannabis inflorescence shows bands corresponding to THC/THCA and CBD/CBDA of similar intensity. Decarboxylated samples of all three chemotypes in Figure 1 show complete disappearance or weakening of the bands corresponding to carboxylated cannabinoids (e.g., THCA, CBDA, CBDVA) and increased abundance of their decarboxylated forms. A band corresponding to CBN may be observed in decarboxylated samples containing a high concentration of THC as its oxidation product.

HPLC and GC Chromatographic Profiles. Chromatographic methods according to the procedure described in Appendix 3, Supporting Information, may be used to verify the identity of the chemotype stated in the labeling based on the presence and relative abundance of the THC and CBD. For the purposes of classification, "total THC" may be defined as the amount of THC that takes into account the potential of THCA to convert quantitatively to THC with no further degradation, and "total CBD" may be defined as the amount of CBD that takes into account the potential of CBDA to convert quantitatively to CBD with no further degradation using the following formulas:

$$\text{Total THC} = \text{THC} + 0.877 \times \text{THCA}$$

$$\text{Total CBD} = \text{CBD} + 0.877 \times \text{CBDA}$$

These formulas account for the loss of mass due to decarboxylation of THCA or CBDA. The THC and THCA refer specifically to the Δ^9-isomer and do not include Δ^8-THC (CAS 5957-75-5). Criteria for the proposed chemotype classification system are presented below:

- The chromatographic pattern for a THC-dominant chemotype exhibits the principal peak for THCA corresponding in retention time to the peak for the compound in the *Standard solution*. The ratio of total THC content to total CBD content is not less than (NLT) 5:1, and the chemotype contains not more than (NMT) 10 mg/g total CBD and NLT 10 mg/g total THC.
- The chromatographic pattern for a CBD-dominant chemotype exhibits the principal peak for CBDA corresponding in retention time to the peak for the compound in the *Standard solution*. The ratio of total THC content to total CBD content is NMT 1:5, and the chemotype contains NMT 10 mg/g total THC and NLT 10 mg/g total CBD.
- The chromatographic pattern for a THC/CBD intermediate chemotype shows two principal peaks for THCA and CBDA corresponding in retention times to the peaks for the compounds in the *Standard solution*. The ratio of total THC content to total CBD content is NLT 0.2:1 and NMT 5:1, with NLT 10 mg/g total CBD and NLT 10 mg/g total THC.

During the development of the above-proposed classification, several data sets for dried cannabis inflorescence were analyzed to test its appropriateness. With regard to the THC-dominant class, the vast majority of samples assessed had ratios with values higher than 20:1, with a significant proportion of these exceeding a 100:1 ratio. For the CBD-dominant class, the vast majority of the samples had ratios lower than 0.04:1.

TESTS FOR CANNABINOID AND TERPENE CONTENT

Quantitation of the Cannabinoids. The USP Cannabis Expert Panel recommended that the standard should include quantitative determination of the major cannabinoids, coupled with acceptance criteria to specify the appropriate cannabis

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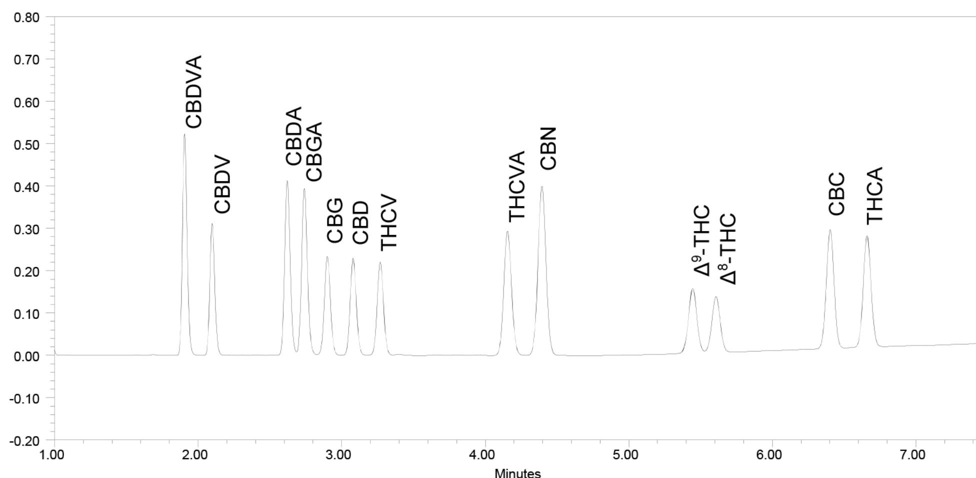

Figure 2. HPLC profile of cannabinoids.

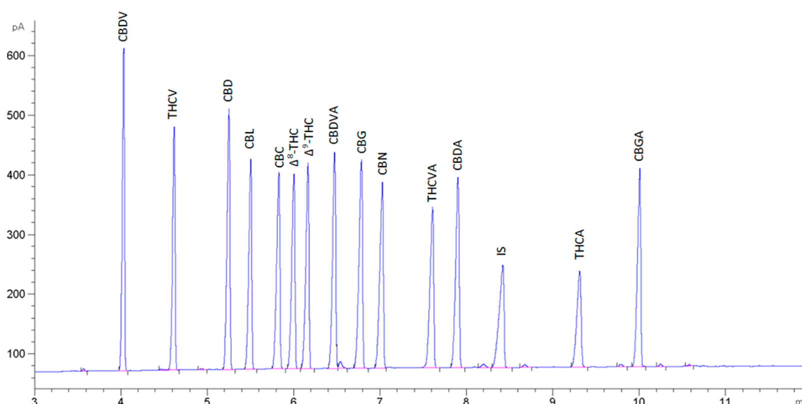

Figure 3. GC profile of cannabinoids.

chemotype based on the ratios of total THC to total CBD. Specifications include acceptance criteria of ±20% the labeled level of total THC and total CBD and the ratio of these constituents to define the particular chemotypes: THC-dominant, CBD-dominant, and THC/CBD-intermediate. A maximum level of CBN is established as an indicator of aging/degradation. Other minor cannabinoids such as the cannabivarin derivatives THCV and cannabidivarin (CBDV; CAS 24274-48-4) and their respective carboxylated forms THCVA and CBDVA, as well as the THC isomer Δ^8-THC, have distinct pharmacological activities. Accordingly, these are not combined in calculating the total THC or CBD content and should be reported separately, especially for cultivars bred to produce a relatively high content of these constituents. Both HPLC- and GC-based procedures are provided in Appendix 3, Supporting Information, and the Labeling section is utilized to indicate the analytical procedure used. While chemotypes producing higher content of minor cannabinoids such as THCVA, CBDVA, CBGA, or CBCA may be available, there is still insufficient information to set specifications for cannabis inflorescence based on these constituents beyond the requirement to label any cannabinoid exceeding 10 mg/g (1% w/w), although the same approach to establish a tolerance of ±20% of the target labeled level could be used.

Several investigators have published HPLC- and GC-based procedures for quantitation of the cannabinoids.[53−56] Validated HPLC and GC procedures are included in Appendix 3, Supporting Information, and illustrated in Figures 2 and 3. *USP* general chapter <1225> *Validation of Compendial Procedures*[57] provides the principles for analytical procedure validation. System suitability is determined based on the chromatographic similarity with the standard solution, the relative standard deviation of NMT 2.0% for THC or CBD peaks in repeated injections, resolution between CBD and CBG, between CBDA and THCA, and between Δ^9-THC and Δ^8-THC, and the tailing factor of NMT 2.0 for the Δ^9-THC peak in the standard solution. The Panel initially selected the method by Mudge et al, which AOAC Int. adopted as First Action in the Official Methods of Analysis as AOAC Official Method 2018.10.[53] However, the pH must be controlled, as variations in the pH of the mobile phase affect the relative retention of the carboxylated cannabinoids in the chromatogram and the reproducibility of retention times. The use of a fixed amount of formic acid to fully protonate the carboxylated cannabinoids provides a consistent chromatography system because it eliminates the need to adjust the pH of the mobile phase to achieve the desired separation.

An HPLC procedure using a C_{18} column packed with solid core superficially porous shell particles of 2.7 μm has been chosen by the expert panel for the analysis of cannabis inflorescence (see Procedure 1 in Appendix 3, Supporting Information). Similar separation can be achieved in ultra-high-performance liquid chromatography in the procedure published by the Olemiss group[56] using a C_{18} column packed with solid core with superficially porous shell particles of 1.7 μm.

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Under these chromatographic conditions, CBNA and Δ^8-THC may coelute. The content of Δ^8-THC and CBNA is typically low to not-detected in cannabis inflorescence samples, present above detection limits only in highly oxidized samples, where conversion of THCA to CBNA may occur.

In the event that the Δ^8-THC/CBNA peak is detected, it may be necessary to have an additional test to determine if the peak is due to Δ^8-THC, CBNA, or a mixture of both. In these cases, a peak analysis by UV spectrum can help to determine the identity of the peak. If their individual quantitative determination is desired, the First Action AOAC Official Method 2018.10 by Mudge et al.[53] can be used, or the aqueous component of the mobile phase may be modified by the addition of a fixed amount of ammonium formate at a concentration between 2.5 and 5 mM. This addition shifts the locus of the peak for CBNA to an earlier retention time before the locus of Δ^9-THC, leaving unchanged the position of Δ^8-THC and effecting the separation. THCA and THCVA also shift their loci to earlier retention time in the chromatogram with the addition of ammonium formate, although in a lesser extent and without change in the elution order. Alternatively, detection by mass spectrometry can also resolve these peaks, as their mass is different.

Calibration for each individual cannabinoid reference standard will provide optimal results. The addition of an internal standard improves the reproducibility and accuracy of results to compensate inaccurate dilutions and losses during extraction; butylparaben (CAS# 94-26-8) or ibuprofen (CAS # 15687-27-1) can be used successfully with this chromatographic system for such a purpose. Some laboratories may choose to determine each cannabinoid against a single reference standard using relative response factors (analyte response factor/reference compound response factor) or conversion factors (reference compound response factor/analyte response factor). As variability in relative response can occur between HPLC instruments, laboratories should verify the system suitability with regard to accuracy at the wavelength of detection and establish response factors for each instrument. Appendix 3, Supporting Information, lists response factors that can be used as a guide. Conversion factors in this appendix are derived by dividing the response factor of CBD (the reference peak) by the response factor of analyte.

Reference standard mixtures for carboxylated and non-carboxylated cannabinoids used in combination with the labeled peaks in the reference chromatograms provided with the lots of the USP Reference Standards could be used to determine peak loci and system suitability. In order to avoid decarboxylation of the carboxylated cannabinoids in the GC procedure at the high temperatures of injection ports, the sample preparation uses a derivatization procedure to convert the carboxylated cannabinoids into their trimethylsilyl (TMS) derivatives without decarboxylation.

The typical chromatographic profiles of cannabinoid standards obtained from Cerilliant are shown in Figures 2 and 3.

The Cannabis Expert Panel recommended the following acceptance criteria to classify the cannabis inflorescence into chemotypes based on the total THC and total CBD content, as determined by the quantitative chromatographic analysis described in Appendix 3, Supporting Information. The contents of total THC and total CBD take into account the potential of carboxylated forms to convert quantitatively to decarboxylated forms with no further degradation using the following formulas:

$$\text{Total THC} = \text{THC} + 0.877 \times \text{THCA}$$

$$\text{Total CBD} = \text{CBD} + 0.877 \times \text{CBDA}$$

THC-dominant chemotype:

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

CBD-dominant chemotype:

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

THC/CBD intermediate chemotype:

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

Considering the high inherent variability of the cannabinoid content between cannabis flowers from plants collected from the same facility, the Cannabis Expert Panel suggested limits of NLT 80% and NMT 120% of the labeled amount of the cannabinoids. The conventional range of NLT 90% to NMT 110% for pharmaceutical products was considered to be overly burdensome for the inherently variable cannabis inflorescence. The permitted wider variability is not intended to be applied as a way to address the variable content of different cannabis chemotypes, or different growing conditions or processing techniques, but rather for within-batch variability for cannabis (e.g., container-to-container variability of a cannabis batch). In the context of the quantitative assessment of cannabinoids, the Cannabis Expert Panel had also suggested that the content of cannabinoids should be calculated on a fixed water activity basis. This panel suggested that the cannabis inflorescence be tested as received to reflect the cannabinoid content of the material as received by the consumer. The panel recommended that the storage conditions of dried cannabis maintain the water activity (a_W) at 0.60 ± 0.05 (see below: Other Quality Attributes: Water Activity) to align with the ASTM specification to maintain water

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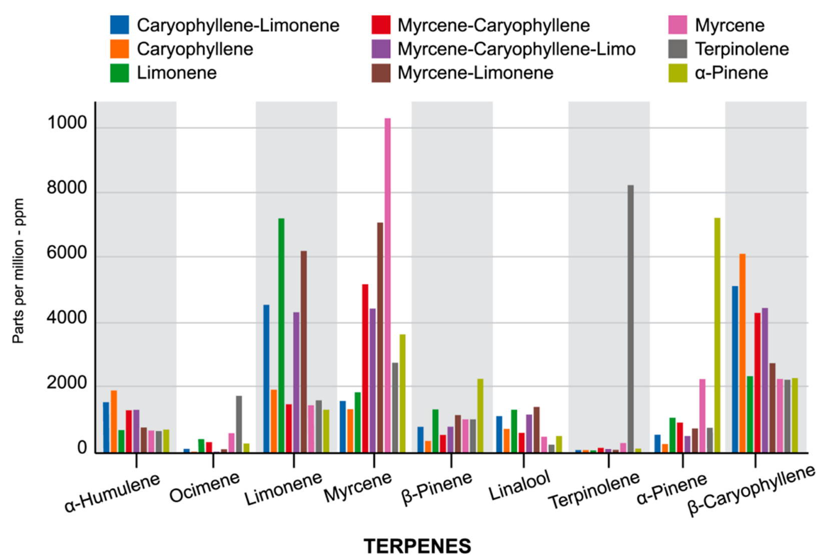

Figure 4. Commonly observed dominant and co-dominant terpenes in commercial cannabis inflorescence. Samples are grouped by dominant terpene, indicated by the color legend above the figure. The *y*-axis represents mean values of terpenes in ppm. The figure shows the dominance and co-dominance of terpenes and that humulene, ocimene, linalool, and *β*-pinene are not dominant terpenes.

activity, and laboratories must ensure they maintain the water activity of cannabis samples prior to testing so that cannabinoid levels are accurately measured.[58] The recommended water activity level is intended to prevent the material from degradation due to excessive drying (water activity below 0.55) or microbial growth (water activity above 0.65). This approach departs from analytical determination on the dried basis, such as that used in the definition of "hemp" in the Agriculture Improvement Act of 2018. The rationale for the expert panel recommendation for cannabis is based on the fact that cannabis contains a substantial amount of volatile constituents (such as terpenes) and a substantial amount of substances sensitive to decarboxylation. Recognizing the potential for loss of volatile components at elevated temperatures, a Dutch monograph has suggested heating at 40 °C above phosphorus pentoxide under vacuum for over 24 h to determine the loss on drying.[59,60]

The Cannabis Expert Panel recommended adoption of best practices for sampling for analysis and recommended that the quantitative tests should be performed on samples representative of the entire inflorescence batch. Representative samples are critical to ensure reproducibility of the results for the appropriate labeling of the product composition and that containers in a lot or batch will be in compliance with the required 80−120% of the labeled amount for cannabinoid content. Improper sampling methods could lead to overestimation of cannabinoid content (for example, by sampling from only the top two inches of the plant when a batch contains flowers that are also found in the middle or bottom of the plant). It is important to use well-defined collection methodology to ensure representative sampling of the entire product batch. In order to achieve representative sampling, sampling should occur from different loci within containers of that batch.[61] Considering that cannabis glandular hair trichomes (technically called types of "trichomes", as are the nonglandular types of hairs also seen in the cannabis inflorescence) contain the highest levels of cannabinoids, sampling errors due to decapitation of these glandular hairs and adhesion of resin to surfaces should be avoided. Sampling procedures should take this into account and should include a sample homogenization process to increase the representativeness of the portion being used for a test. Before and after a homogenization step, stratification of detached trichomes could also lead to varying results due to sample inhomogeneity (i.e., stratification of fines) and should be accounted for in order to avoid bias. Proper equipment must be used for sampling, with documentation that follows an approved standard operating procedure. *USP* general chapter <561> *Articles of Botanical Origin* describes the sampling procedures applicable to vegetable drugs, including procedures for gross sampling from multiple batches and the test sampling methods, and involves thorough mixing and adequate sample sizes for the necessary tests.[62] According to *USP* general chapter <561>, for articles in containers holding less than 1 kg, the contents should be mixed, and a sufficient quantity should be withdrawn for the required tests. For articles in containers holding between 1 and 5 kg, equal portions should be withdrawn from the upper, middle, and lower parts of the container, with each of the samples being sufficient to carry out the tests. For containers holding more than 5 kg, *USP* general chapter <561> requires three samples from the upper, middle, and lower parts of the container, and these samples must be a minimum of 250 g. As cannabis is a high-value material, the USP Cannabis Expert Panel recognizes that these quantities may be too large to be practical to use, and smaller quantities may be justifiable. The ASTM International's cannabis committee is drafting guidance[63] for the sampling of cannabis products for subsequent laboratory analyses of process lots including extracts and concentrates.

Content of Terpenes. The differences between some properties of different cannabis varieties have been attributed to the potential interplay between cannabinoids and terpenes[64,65] and that the relative ratios of terpenes can differ between cannabis chemotypes. The Cannabis Expert Panel recommended determining five of the most commonly abundant terpenes found in cannabis by GC: a sesquiterpene *β*-caryophyllene and four monoterpenes: D-limonene, *β*-myrcene, *α*-pinene, and *γ*-terpinolene. Appendix 4, Supporting Information, presents a GC-FID method for analysis of terpenes[66,67] and the acceptance criteria based on the relative dominance of the terpenes. Analysis of data from a large set of samples has shown that each one of these terpenes could occur as the dominant terpene or as co-

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dominant (i.e., with ratios <2:1). Co-dominance is typically observed in the pairs β-myrcene/D-limonene, D-limonene/β-caryophyllene, and β-myrcene/β-caryophyllene, or as the triad β-myrcene/D-limonene/β-caryophyllene (Figure 4).

More clinical research is necessary to define the influence of terpene profiles on the pharmacology of a cannabis product for specific conditions. Cannabis should be labeled with the total content of terpenes and the profile in terms of dominant or co-dominant terpenes so correlations between the terpene chemotypes and any clinical relevance or pharmacological effects can be adequately researched and established.

Design of the *USP* Reference Standards. The use of reference standards (RSs) is necessary for analytical procedures to accurately identify and measure the content of constituents in a material. For the purpose of establishing the identity of a botanical ingredient, RSs may be used for qualitative applications such as identification tests, system suitability tests, or chromatographic peak markers. Additional information regarding USP RSs is available from the *USP* general chapter <11> *USP Reference Standards*.[68] The Cannabis Expert Panel recommended that USP develop appropriate RSs for quantitative measurement of the following cannabinoids: Δ^9-THC, Δ^8-THC, THCA, CBD, CBDA, CBG, CBGA, CBC, CBDV, CBDVA, THCV, and THCVA, as well as CBN as a marker for degradation. As cannabis research continues to develop, other cannabinoids for RS development may become commercially available and may be added to the RS as appropriate, such as when novel chemotypes of cannabis are developed that may contain significant amounts of these cannabinoids.

The stability of carboxylated cannabinoids is discussed in the section below and is addressed through the design of two sets of RS mixtures that contain either carboxylated or noncarboxylated forms. Suitability of the RSs was evaluated for the intended purposes referenced in this review article. In order to provide RSs for use in the test for identification and quantitation, the following standards for pure compounds in solution or solid form and for cannabinoid mixtures of defined compositions are presented:

- USP Cannabinoid Acids Mixture RS 1 mL (in acetonitrile and triethylamine with stabilizer) [Catalog #1089172]:
  - 0.25 mg of tetrahydrocannabinolic acid (THCA) [CAS 23978-85-0]
  - 0.25 mg of cannabidiolic acid (CBDA) [CAS 1244-58-2]
  - 0.050 mg of tetrahydrocannabivarinic acid (THCVA) [CAS 39986-26-0]
  - 0.025 mg of cannabidivarinic acid (CBDVA) [CAS 31932-13-5]
  - 0.025 mg of cannabigerolic acid (CBGA) [CAS 25555-57-1]
- USP Cannabinoids Mixture RS 1 mL (in methanol) [Catalog #1089183]:
  - 0.075 mg of Δ^9-tetrahydrocannabinol (Δ^9-THC) [CAS 1972-08-3]
  - 0.025 mg of Δ^8-tetrahydrocannabinol (Δ^8-THC) [CAS 5957-75-5]
  - 0.050 mg of cannabidiol (CBD) [CAS 13956-29-1]
  - 0.025 mg of cannabinol (CBN) [CAS 521-35-7]
  - 0.025 mg of cannabichromene (CBC) [CAS 20675-51-8]
  - 0.025 mg of cannabigerol (CBG) [CAS 25654-31-3]
  - 0.025 mg of tetrahydrocannabivarin (THCV) [CAS 31262-37-0]
  - 0.025 mg of cannabidivarin (CBDV) [CAS 24274-48-4]
- USP Δ^9-Tetrahydrocannabinol RS 1 mL (1 mg/mL) [Catalog #6151621]
- USP Cannabidiol Solution RS 1 mL (1 mg/mL) [Catalog #1089161]
- USP Cannabidiol RS 25 mg [Catalog #1089149]

Stability of the USP Reference Standards. Stability of cannabinoids can be impacted due to storage and working conditions, particularly for carboxylated cannabinoids. The preliminary stability testing with THCA in acetonitrile was conducted at three temperatures, −20, 4, and 25 °C, in the presence of different stabilizers, compared with control samples stored at −70 °C. A THCA solution with a combination of stabilizers showed decreased impurity levels observed after 3 and 10 days. These outcomes indicate the need for stabilizers if stored at temperatures warmer than −70 °C. Based on the stability studies, an aprotic solvent composed of 38% acetonitrile and 62% triethylamine containing 0.15 mg/mL of ascorbic acid was found to be an optimal stabilizer. Further stability testing for the mixture of carboxylated cannabinoids in the presence of a suitable combination of stabilizers prevented the generation of impurities.

LIMITS FOR CONTAMINANTS

Pesticide Residues. Cannabis plants are susceptible to pest infestation, whether grown outdoors or indoors. Integrated pest management practices should be applied to the control of cannabis pests and may include measures such as sanitation programs, physical or temporal barriers, appropriate use of approved pest control products, intercropping, and biological controls, among others.[69] Recent cases in the U.S. and Canada of consumers being exposed to residues of pesticides unauthorized for use or used off-label on cannabis have resulted in recalls and increased public and regulatory concern.[70] In the U.S., crop-specific pesticide residue limits are established by the U.S. Environmental Protection Agency (EPA) for foods, but no approved pesticides or pesticide limits exist for cannabis. Furthermore, levels of pesticides deemed appropriate to protect public health and safety in foodstuffs may not necessarily apply to cannabis inflorescence, as dried cannabis is typically smoked or vaped, introducing pyrolysis products and a different route of exposure (inhalation). In contrast, other jurisdictions where cannabis cultivation and use are legal have established permissible pesticides and limits. For example, Canada has published guidance on pest control product use on cannabis and has authorized[71] certain pesticides for this use. Furthermore, it has established mandatory third party testing requirements for pesticides that consist of a list of pest control product active ingredients and quantitation limits that must be met by validated methods in order to detect and deter the unauthorized use of pesticides.[72] To date, the requirements established in Canada are the most stringent among the countries and U.S. states that regulate the legal use of cannabis.[12]

Pesticide residue levels for oral botanical drugs are controlled through the limits presented in *USP* general chapter <561>. The limits in this chapter were established based on acceptable daily exposure, body weight, amount consumed, and a safety margin,

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but these assume an oral route of exposure. The list found in *USP* general chapter <561> is not exhaustive and largely accounts for toxic, environmentally persistent, or widely used pesticides. Pesticides that may be used on specific crops must also be considered if there is reason to believe they may be present in a botanical product. In cannabis, many of the pesticides used to control pests such as powdery mildew, botrytis, or spider mites are not listed in *USP* general chapter <561>; therefore, several states and Canada have established guidelines specific to the cannabis industry. In U.S. states, this is either a permissible or a negative list of pesticides that may be used or avoided in cannabis cultivation. For example, California has a two-tier system of pesticides comprising those that should not be present (Category 1) and those with specified limits on products for oral or inhalation use (Category 2), while in Canada the list has been developed to control for the unauthorized use of pesticides on cannabis and is based on the lowest limits of quantitation reasonably achievable given current laboratory technologies available to the cannabis testing industry. The method used by Health Canada to achieve detection and quantitation of pesticides to the levels required in Canada is available in the publication by Moulins et al.[73] There are also ongoing efforts by standards-development organizations, such as those of the AOAC, which has drafted a standard method performance requirements (SMPRs) document by compiling the lowest action levels of the pesticide residue limits from various state regulations and setting the minimum limit of quantitation (LoQ) for an analytical method at half the lowest action levels.[74] Validated multiresidue methods for analysis of cannabis samples are published and have been adopted widely by cannabis quality control testing laboratories.[73]

Although U.S. state requirements may provide some guidance to control pesticide contaminants,[12] additional residues that are not expressly permitted by these states may also be detected on cannabis due to environmental drift or persistence or through incidental contamination. Other major considerations with regard to the toxicity of these contaminants are that cannabis may be consumed by inhalation by at-risk populations (e.g., those with a pre-existing lung disease, infectious diseases, or immunocompromised function), which may further increase the risk of harm. A high level of uncertainty exists for smoked and vaporized cannabis containing pesticide residues since pyrolysis of such substances is not well studied, nor is it included in pesticide registration safety data (e.g., for products such as tobacco) since their use is typically registered for foods, and an inhaled route of exposure bypasses first-pass metabolism.[75] Given the limited information, the expert panel proposed adopting the limits based on acceptable daily intake (ADI) as published by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO),[76] with consideration of the body weight of the consumer, the amount consumed, and a safety factor to account for the inhalation exposure pathway. Considering the high level of uncertainty that pyrolysis and inhalation introduce and the absence of appropriate toxicological data, a 1000-fold safety factor was suggested. Accordingly, the pesticide residues on cannabis may be limited using the following approach:

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

$$V(\text{mg/kg}) = AM/1000B$$

where A is the ADI, as published by FAO-WHO,[77] in mg/kg of body weight; M is body weight, in kg (60 kg); B is the daily dose of the article, in kg; and V, in mg/kg, is the calculated value of the pesticide residue. The calculated value is used to determine limits based on the requirements that the general maximum residue limit is 0.1 ppm if the calculated value is more than 0.1 ppm, and 0.01 ppm if the calculated value is less than 0.1 ppm.

This risk-based and precautionary approach helps limit the risk from exposures to pesticide residues by assigning conservative limits. Specifically, for high-toxicity pesticides for which the calculated value is lower than 0.1 ppm, the requirement caps the limit at NMT 0.01 ppm (10 ppb), and for pesticides with values above 0.1 ppm, the limit of NMT 0.1 ppm is imposed. These limits are intended to address pesticide residues resulting from incidental contamination (e.g., through environmental drift) and are not intended to permit the use of pesticides that are not authorized by the applicable regulatory body.

For the quantitative analysis of pesticide residues, *USP* general chapter <561>[62] requires the use of validated analytical procedures, such as those in accordance with the latest version of the European Union guideline on analytical quality control and validation procedures for pesticide residue analysis (current version Document No. SANTE/12682/2019)[78] or the EPA method validation principles (OPPTS 860.1340).[79]

Elemental Contaminants. Cannabis has been identified as a hyper-accumulator for heavy metals.[80] These elemental impurities may be introduced from soils, water, and other inputs, and exposure of consumers to cannabis products containing such contaminants is an important quality and safety consideration.[81−83] Toxicologically based limits for elemental contaminants are described in the *USP* general chapter <232> *Elemental Impurities—Limits*, while analytical methodologies are discussed in the *USP* general chapter <233> *Elemental Impurities—Procedures*. Considering the potential inhalation use of cannabis inflorescence, the panel had suggested adoption of acceptance criteria from the *USP* general chapter <232> for inhalation products:

- arsenic: NMT 0.2 μg/g
- cadmium: NMT 0.2 μg/g
- lead: NMT 0.5 μg/g
- mercury: NMT 0.1 μg/g

When contamination with other elemental impurities may be possible (e.g., due to past or nearby industrial activities), in addition to the above specifications, *USP* general chapter <232> also requires that "when additional elemental impurities are known to be present, have been added, or have the potential for introduction, assurance with the specified levels is required".

Microbial Contaminants. Contamination of cannabis inflorescence with pathogenic bacteria, yeast, and mold during cultivation, harvesting, drying, storage, and/or distribution is a serious risk, especially considering that cannabis may be consumed by at-risk patient populations such as those with compromised immune function.[84] Moreover, cannabis products should be held to microbial specifications that help ensure that practices used in cannabis production are indeed effective and to verify that cannabis for medical purposes is held to a high quality standard. Such specifications reduce patients' exposure to risks posed by microbial contamination.

USP general chapter <61> *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* includes

methods for enumerating total aerobic bacterial count and the total yeasts and molds count. *USP* general chapter <62> *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* includes methods and specifications for the absence of *Salmonella* species and *Escherichia coli* and the enumeration of total bile-tolerant Gram-negative bacteria.

The Cannabis Expert Panel recommended the following specifications for the microbial quality of cannabis inflorescence:

- *USP* general chapter <61>:
  - The total aerobic bacterial count: NMT 10^5 cfu/g
  - The total combined molds and yeast count: NMT 10^4 cfu/g
- *USP* general chapter <62>:
  - The total bile-tolerant Gram-negative bacteria: NMT 10^3 cfu/g
  - Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

While the available information indicated that about 25% of the market samples may fail the above-mentioned USP specifications, the Cannabis Expert Panel was of the opinion that improved sanitation practices such as the ASTM cleaning guidelines, *Standard Guide for Analytical Laboratory Operations Supporting the Cannabis Industry*,[85] good postharvest practices, and good production practices required for Canadian cannabis production should help in achieving acceptable microbial loads.

Testing methodologies and specifications found in the *European Pharmacopeia* (*Ph.Eur.*) 5.1.8 *Microbiological quality of herbal medicinal products for oral use and extracts used in their preparation* generally align with the above requirements and methods in *USP* general chapters <61> and <62>. In Canada, it is the specifications in *Ph.Eur.* 5.1.8 that have been most widely adopted among cannabis producers. The sample amount required for *Salmonella* testing according to *Ph.Eur.* 5.1.8 is larger (25 g) than that of the analogous test according to *USP* general chapter <62> (10 g). The specification found in *Ph.Eur.* 5.1.8 for bile-tolerant Gram-negative bacteria is also 10^4 rather than 10^3, and the maximum allowable total aerobic microbial counts and total yeasts and molds counts are 5-fold the limits, rather than 2-fold as specified here (see below). Stakeholders are invited to provide feedback regarding these differences between the *Ph.Eur.* and *USP* in relation to cannabis quality control or the burdens involved in meeting the proposed requirements.

For the detection and quantitation of microorganisms not amendable to a plating method, such as the common cannabis pathogen powdery mildew, molecular methods such as quantitative polymerase chain reaction (qPCR) may be used. Powdery mildew is an obligate parasite and cannot grow using standard plating techniques. *USP* general chapter <1223> *Validation of Alternative Microbial Methods* provides guidelines for microbial recovery and identification and the applicability of method validation characteristics such as accuracy, precision, specificity, detection limit, quantification limit, linearity, range, ruggedness, and robustness for such molecular tools in the context of microbiological method validation.

At-risk populations such as patients with immunocompromised function who inhale cannabis inflorescence may be at greater risk of microbial infection compared to healthy individuals. Therefore, some healthcare practitioners and patients may be interested in identifying products with more stringent microbial specifications. The limits for inhalation products in the *USP* general chapter <1111> *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* are more stringent than those proposed above:

- The total aerobic bacterial count: NMT 10^2 cfu/g
- The total combined molds and yeast count: NMT 10^1 cfu/g
- Meets the requirements of the tests for the absence of bile-tolerant Gram-negative bacteria, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*

According to *USP* general chapter <61> and *USP* general chapter <1111>, when an acceptance criterion for microbiological quality is prescribed, it is interpreted as follows:

10^1 cfu: maximum acceptable count = 20;
10^2 cfu: maximum acceptable count = 200;
10^3 cfu: maximum acceptable count = 2000; and so forth

Products that meet the more stringent acceptance criterion for inhaled limits in *USP* general chapter <1111> may be identified and labeled to indicate to healthcare practitioners and patients that such products (particularly for inhalation products) have a reduced microbial load.

Reduced microbial loads may be accomplished through good cultivation, harvesting, and postharvesting practices[69] or some form of microbial load reduction method such as ozonation or irradiation. Irradiation of botanical materials to reduce microbial load is specifically prohibited in the United States, except on certain species that have been granted an exception. Hazekamp noted that treatment with gamma irradiation did not cause changes in the content of THCA and CBDA, but significantly reduced the concentration of some terpenes such as terpinolene by 38% in the tested Bedrolite variety.[86] The quality of treated products may also depend upon the intensity of irradiation, the length of exposure time, the temperature at which products are irradiated, and whether steps have been taken to reduce the presence of oxygen during irradiation. In cases where irradiation or other treatments to reduce the microbial load of the finished product is permitted, testing for microbial contaminants should be done before the treatment to prevent the sale of spoiled cannabis where the fungi or other microbes are simply not viable when tested by plating methods. Treatment methods such as irradiation should not be used as a means to remediate cannabis contaminated above the allowed limits, but rather to increase the shelf life of the cannabis or further reduce loads of cannabis that passes the microbial requirements in order to achieve even more stringent limits.

In order to control the water available to microbes in the cannabis inflorescence for microbial growth, the Cannabis Expert Panel suggested adoption of the ASTM guidelines for water activity, which limit the content to values between 0.55 and 0.65 (see below: Other Quality Attributes: Water Activity). Reduced water activity will greatly assist in the prevention of microbial proliferation and spoilage. Additional ASTM documents that may be used for controlling the microbial load of cannabis include guidelines for cleaning and disinfection, packaging, labeling, and disposal.[58]

***Aspergillus* Species.** Inhalation of cannabis contaminated with *Aspergillus* spp. may have serious effects, especially on immunocompromised users.[87,88] Many states with legalized cannabis markets now require that all cannabis goods intended for consumption by inhalation be tested for the four pathogenic *Aspergillus* species *A. niger*, *A. flavus*, *A. fumigatus*, and *A. terreus*. When inhaled, all four of these species are known to cause a variety of immune lung disorders, ranging from asthma, allergic bronchopulmonary aspergillosis, and hypersensitivity pneumo-

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nitis to invasive and life-threatening systemic fungal infections in immunocompromised hosts.[89−91]

Proper testing for *Aspergillus* spp. in cannabis and cannabis products has been a challenge to testing laboratories, as there are not yet officially validated methods available for *Aspergillus* detection. Molecular methods such as qPCR to detect specific *Aspergillus* species in cannabis can lead to false positives due to cross-reactivity with nonspecified *Aspergillus* species. However, these methods are by far the most sensitive and can be made more specific (depending on primer/probe selection) compared to culture-based methods. It is important to note that there are a variety of commercially available kits on the market intended for pathogenic *Aspergillus* species detection; however, their use should be thoroughly evaluated in-house to ensure proper sensitivity and specificity according to regulatory guidelines.[92] In addition, enrichment of cannabis matrices in fungus-specific media for at least 48 h is recommended so *Aspergillus* fungi grow to detectable levels. Due to the tendency of these contaminating organisms to form clumps and nonuniform dispersal in an enrichment media, an increased sample volume for downstream DNA extraction is strongly recommended to increase the sensitivity of molecular-based qPCR methods.

Detection of pathogenic *Aspergillus* species using culture-based methods is very difficult, requiring a highly trained and experienced mycologist to correctly identify these pathogens by colony appearance and morphology, as there are many nonpathogenic species of *Aspergillus* that may be indistinguishable from those that are pathogenic.[93] While a compendial method for determination of *Aspergillus* species is not available, *USP* general chapter <1223> *Validation of Alternative Microbial Methods* should be considered during method validation efforts. AOAC has recently developed a standard method performance requirements document to invite testing methods for the four species of pathogenic *Aspergillus* in cannabis.[94]

Mycotoxins. Mycotoxins are harmful metabolites produced by fungi. The primary mycotoxins of concern with cannabis are aflatoxins, ochratoxins, and vomitoxin (also known as deoxynivalenol, or DON). Aflatoxins may suppress the immune system, mutate DNA, and cause liver cancer (hepatocarcinoma). Ochratoxin A can cause nephrotoxicity and is a suspected carcinogen. Even if a cannabis product were to be treated for microbial reduction or passes a total yeast and mold enumeration test, it could still contain mycotoxins.

Aflatoxins B1, B2, G1, and G2 are a group of structurally related toxic compounds produced by certain strains of fungi. Under favorable growth conditions, *Aspergillus flavus* and *A. parasiticus* were shown capable of producing aflatoxins on cannabis.[95] Aflatoxicosis is the acute or chronic poisoning that results from ingestion of aflatoxins, and aflatoxins are recognized as highly carcinogenic substances. Next-generation sequencing of the ITS2 nuclear ribosomal region of the fungal communities found in dispensary-acquired cannabis flowers yielded several toxigenic *Penicillium* and *Aspergillus* species, including *P. citrinum* and *P. paxilli*, that were not detected by culture-based methods.[96]

State-to-state requirements may differ, but they provide some guidance to control mycotoxin contaminants. The USP Cannabis Expert Panel recommended that, in addition to conforming with the relevant state or regulatory body requirements, aflatoxin testing should be done according to Method II or Method III in the *USP* general chapter <561> *Articles of Botanical Origin: Test for Aflatoxins*. The toxicologically based acceptance criteria for aflatoxin limits are NMT 20 ppb for the total of aflatoxins B1, B2, G1, and G2 combined and NMT 5 ppb of aflatoxin B1.

The USP Cannabis Expert Panel anticipates making further appropriate recommendations regarding controls for other mycotoxins as the understanding of cannabis safety and quality control evolves.

OTHER QUALITY ATTRIBUTES

Water Activity. The Cannabis Expert Panel determined that a water activity test was more appropriate in order to control the water available to microbes in the cannabis inflorescence for microbial growth. The expert panel suggested that the water activity should be controlled at 0.60 ± 0.05. *USP* general chapter <1112> *Application of Water Activity Determination to Nonsterile Pharmaceutical Products* provides information regarding control of water activity for reducing the susceptibility of formulations to microbial contamination. The proposed *USP* general chapter <922> *Water Activity* outlines the recommended methods to qualify, calibrate, and use water activity meters to accurately measure the water activity of raw materials and products.[97]

Foreign Organic Matter. Any other plant parts except for cannabis inflorescence or vegetable matter other than the intended article, such as seeds or stalk, represent foreign organic matter that should be controlled. Similarly, the article should be free of visibly contaminated material such as infestation with powdery mildew or other molds. When good agricultural and collection practices are followed, cannabis inflorescence should contain NMT 5% of stems 3 mm or more in diameter and NMT 2% of other foreign matter (e.g., seeds). These limits could be determined by physically removing all parts of a sample that should not be present, weighing them, and expressing them as a percent total of the sample assessed. *USP* general chapter <561> *Articles of Botanical Origin* describes the method for analysis of foreign organic matter.[62]

Total Ash and Acid-Insoluble Ash. Inorganic compounds such as minerals are absorbed by plants. Their content varies depending on factors such as the nature of the soil, cultivation conditions, and age of the plant. Ash representing the inorganic portion of a plant provides a measure of the amount of residue that remains after the incineration of the botanical. Ash value is of importance because it tends to indicate the amount of care taken in preparation of the crude botanical. The test for acid-insoluble ash measures the residue remaining after boiling the total ash with 3 N hydrochloric acid. This residue consists mainly of sand and other silicates and is an indication of the amount of dirt, soil, clay, and related material that is present in the sample. For cannabis inflorescence, the total ash should be NMT 20.0%, and acid-insoluble ash should be NMT 4.0%. *USP* general chapter <561> describes the method of analysis of ash value and acid-insoluble ash.[62]

Packaging and Storage. Cannabis inflorescence should be stored in a cool and dry place in well-closed containers and protected from light and moisture. Water activity during storage should be maintained at 0.60 ± 0.05. *USP* general chapter <659> *Packaging and Storage Requirements* defines cool conditions at any temperature between 8 and 15 °C (46 °F and 59 °F), and a dry place to be a place that does not exceed 40% average relative humidity at 20 °C (68 °F) or the equivalent water vapor pressure at other temperatures.

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LABELING

Appropriate labeling information helps patients and healthcare practitioners assess whether a product is suitable for particular needs. In addition to ensuring compliance with the applicable state or country requirements for labeling, standardized definitions for the ingredients in cannabis products help describe an article appropriately. USP nomenclature guidelines may be useful in this regard.[26]

Considering the wide variety of cannabis chemotypes, product labeling should specify the nature of the article and whether the plant chemotype is THC-dominant (commonly referred to as Type I), THC/CBD-intermediate (commonly referred to as Type II), or CBD-dominant (commonly referred to as Type III). The label should state the name of the article as *Cannabis* Inflorescence and the scientific Latin binomial. The label should state in mg/g the amount of the "total THC", taking into account the potential of THCA to convert to THC, the amount of the "total CBD", taking into account the potential for CBDA to convert to CBD, and any other cannabinoids above 10 mg/g. The determination of the "total THC" and "total CBD" is described above in the section Quantitation of the Cannabinoids. Since there is a need to investigate the pharmacological interplay between cannabinoids and terpenes, as well as the effects of some of these terpenes on certain clinical conditions, the label should also indicate the dominant or co-dominant terpene(s) as determined by appropriate testing methodologies. In cases where the product conforms to limits for inhaled use found in *USP* general chapter <1111>, and in order to aid at-risk populations choose lower-risk products, the label should identify the product as having a reduced microbial load. When the material is subjected to a microbial reduction process such as irradiation, the method used must be indicated.

Additional requirements for proper packaging and labeling are also necessary to protect the article and to communicate to potential patients, consumers, and healthcare practitioners certain characteristics of the product. These characteristics may include specific ratios between CBD and THC, information about other cannabinoids and terpenes, and any stability/storage information.

ADULTERATION WITH SYNTHETIC CANNABINOIDS

Many psychoactive synthetic cannabinoid analogues of naturally occurring cannabinoids are available on the consumer market and are sold under misleading names, like "spice" or "incense". While adulteration of cannabis inflorescence with synthetic cannabinoids may not be likely, such compounds can be used readily to adulterate products labeled as derived from cannabis, such as vaping products or extracts. Studies have reported serious health effects associated with the use of synthetic cannabinoids.[98] The need to develop and validate screening procedures to detect these synthetic compounds has been emphasized by researchers, regulators, and law enforcement agencies.[99] Addition of synthetic cannabinoids to cannabis products could expose consumers to the risk of adverse effects, overdoses, and death. The life-threatening outcomes of the consumption of synthetic cannabinoids, as detailed at a recent congress of clinical toxicologists from the U.K., Lithuania, Hungary, Slovenia, and The Netherlands, has confirmed this threat to public health as being broadly international.[100] Resources such as the *USP* general chapter <2251> *Screening for Undeclared Drugs and Drug Analogues* could be used to complement the specifications proposed in this paper in cases where cannabis inflorescence is suspected to be adulterated with synthetic cannabinoids. Additional complementary information could be useful to address this issue, e.g., a relational database for cataloging known and newly discovered synthetic cannabinoids and their chemotaxonomic characteristics.

CONCLUSIONS

Several countries and 33 states and the District of Columbia in the U.S. currently allow the use of cannabis for specific medical conditions. As a result, many people have access to cannabis with variable product quality, largely due to differing regulatory requirements in their jurisdictions and a general lack of standards for cannabis specifications, testing, and production.

Standards for cannabis facilitate the use of well-characterized or standardized investigational substances for use in scientifically validated tests by ensuring the identity, content of constituents, and limits of contaminants. Exposure to toxic substances, pathogenic microorganisms, and adulterants such as synthetic compounds that are designed to mimic the effects of phytocannabinoids can result in patient harm or confound research efforts. Gaps in quality control have resulted in recalls where cannabis for medical purposes is sold, such as in Canada and some U.S. states, highlighting the need for robust quality control practices to consistently achieve appropriate product specifications.

Considering the gap in uniform national quality standards for cannabis, several organizations such as ASTM International, AOAC International, and the American Herbal Pharmacopoeia (AHP) have initiated programs to engage stakeholders and develop voluntary guidelines and consensus standards for the industry.

ASTM International formed Committee D37 to develop standards for cannabis, its products, and quality control processes. The activities are focused on the development of test methods as well as practices and guides for cultivation, quality assurance, packaging, and security, among others. As of March 2020, the ASTM committee has published several guidelines including ones for water activity measurement, cleaning and disinfection, packaging, labeling, disposal, corrective action and preventive action (CAPA), hazard analysis critical control points (HACCP), and a standard practice for laboratory test method validation and method development.[58]

AOAC International recently initiated a Cannabis Analytical Science Program for the development of analytical methods, to establish a proficiency testing program, and to provide analytical and laboratory management training, which includes International Organization for Standardization (ISO) accreditation training.[101] As of March 2020 AOAC has published the minimum recommended standard method performance requirements for the following methods: quantitation of cannabinoids in cannabis inflorescence, chocolate, and concentrates; determination of pesticide residues in cannabis dried materials; quantitation of cannabinoids in plant materials of hemp; quantitation of selected residual solvents in dried cannabis materials; and detection of *Aspergillus* spp. in cannabis.[101] In December 2018, two AOAC Official Methods of Analysis were approved as voluntary consensus methods for the quantitation of cannabinoids in cannabis dried plant materials, concentrates, and oils. Additional SMPRs currently under development include those for determination of moisture content in cannabis and hemp, detection of *Salmonella* and Shiga toxin-producing *E. coli* (STEC) in cannabis and cannabis

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products, and the detection of heavy metals in cannabis materials.

In 2013, the American Herbal Pharmacopoeia published a monograph for cannabis inflorescence describing the nomenclature, identification, commercial sources, handling, analytical methods, and international status of cannabis.[102] Industry associations such as the American Herbal Products Association also provide recommendations regarding cultivation and processing, manufacturing, packaging, labeling, holding, laboratory operations, and dispensing operations for cannabis.[69]

The specifications in this review complement the efforts by ASTM, AOAC, and AHP, and this article has provided the context around the development and appropriate use of public standards as well as proposes appropriate specifications (i.e., tests, analytical procedures, and acceptance criteria) to assess the quality of cannabis. The unique value of a standard from USP is that it includes comprehensive interrelated tests with scientifically valid analytical procedures and acceptance criteria for the identity, content of constituents, and limits of contaminants, and additional requirements for nomenclature and definitions, labeling, and storage, supported by reference standards that are verified for fitness for purpose.

THC and CBD are the most studied cannabinoids since they have been reviewed and approved as drugs for specific indications by regulatory agencies. Based on clinical studies and drug approvals for THC and CBD, the Cannabis Expert Panel has recommended labeling requirements for cannabis inflorescence that is either a THC-dominant chemotype, a CBD-dominant chemotype, or an intermediate chemotype containing both THC and CBD. Cannabis inflorescence could be further subcategorized based on the content of minor cannabinoids and their mono- and sesquiterpene profiles. In addition, the principles outlined in this article could be used as the basis of public quality specifications for cannabis inflorescence, which will be helpful for research on potential therapeutic applications and public health protection.

Some of the gaps identified in the current cannabis research are the following areas:

1. Besides CBD and THC, the cannabis plant contains several other phytoconstituents that may be relevant for cannabis bioactivity.[21,64] The current understanding of the molecular targets and the bioactivity of these other constituents remains very limited. Analytical methods to characterize varieties of cannabis that produce elevated content of minor cannabinoids such as THCVA, CBCA, and CBDVA, among others, may be useful to study their various biological activities. The understanding of how additional cannabis constituents modulate the activity of major cannabinoids on endogenous receptors continues to be a developing area of science.[36,37]
2. The validation and adoption of officially recognized analytical methods for detecting contamination with pathogenic *Aspergillus* spp. is still in process. The development of a such methods requires further studies to demonstrate acceptable recovery/sensitivity and specificity, successful cross-validation with traditional culture-based methods, and application to a wide variety of cannabis product types.

Overall, research based on well-established principles of botanical and natural products chemistry and robust quality control can help in understanding the safety and potential uses of cannabis for various medical purposes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01200.

Additional information as described in the text (PDF)

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Notes

The authors declare the following competing financial interest(s): Certain authors are employees of USP, which is a non-for-profit organization that sells documentary and physical reference standards to sustain its activities. The views presented in this article do not necessarily reflect those of the organizations for which the authors work. No official support or endorsement by these organizations is intended or should be inferred.
The proposed standards of identity, content of the constituents, and limits on contaminants that appear in this article were developed by the USP Cannabis Expert Panel and are intended

to provide scientifically valid methods for the analysis of cannabis inflorescence. The standards and specifications presented in this article do not reflect official text of the *USP-NF* or any other compendium published by USP. USP and the Cannabis Expert Panel make no recommendations or representations with respect to the use or utilization of the standards or methods herein for legal or compliance purposes in the U.S. or elsewhere. At the time of publication of this article, cannabis is a Schedule 1 controlled substance under U.S. federal law. This article is not intended to support, encourage, or promote the cultivation, use, or marketing of cannabis in contravention of applicable laws or regulations in the U.S. or elsewhere.

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This paper was published ASAP on April 13, 2020, with errors in the Supporting Information. The corrected version was reposted on April 24, 2020.

Supporting Information

Cannabis Inflorescence for Medical Purposes – USP Considerations for Quality Attributes

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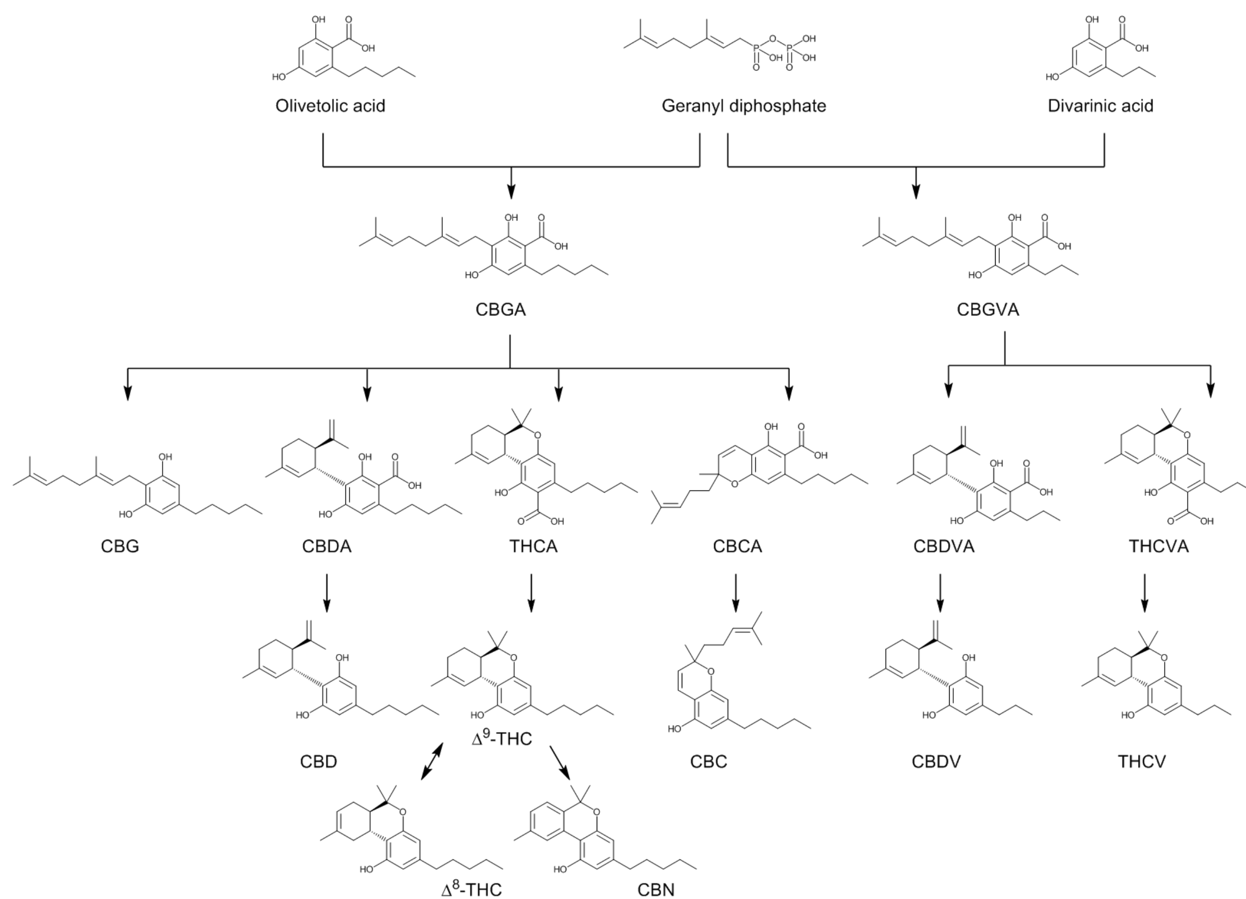

Figure S1. Cannabinoid biosynthetic pathway

APPENDIX 1. BOTANICAL CHARACTERISTICS

The primary literature source for the following description of macroscopic and microscopic botanical characteristics of cannabis is the American Herbal Pharmacopoeia's monograph on *Cannabis* inflorescence,[1] quoted extensively with the permission of the Editor-in-Chief. Additional details from other sources have been added, as indicated by their citations.

The vernacular word "cannabis" has evolved as a generic abstraction from the genus name *Cannabis*, conventionally italicized. Non-italicized, cannabis is employed as a noun and adjective, and frequently (often loosely) used both for cannabis plants and/or any or all of the intoxicant preparations made from them.[2] In the description that follows, the term "cannabis" refers strictly to the cannabis plant.

Macroscopic Features:

For macroscopic examination of material that is stuck together, soak the material in strong alcohol (70%) to dissolve the resin. Then pour off the alcohol and 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.[1]

Cannabis raw material has various characteristic morphological features. Those described here are generally visible with a hand lens of 10× or 20× power. In a few cases, such as some of the features of the very diagnostic trichomes, additional fine details are described, which might be difficult to see clearly without a 40× microscope. The visual detection of every fine detail described here is not required. The intent of providing this additional information is to facilitate the interpretation and understanding of what can be seen with a hand lens, such as how the readily visible resin chamber is formed and what makes the reddish tip of a multicellular trichome that can be seen when its glandular head has fallen off.

Cannabis is most often supplied as variously sized (1.5–15 cm or longer) branches and branchlets, which are sometimes broken up from the dried inflorescences of pistillate plants. Cannabis is generally dioecious, meaning that the pistillate (female) and staminate (male) flowers occur on separate plants, although monoecious (male and female flowers on the same plant) "races" have been created by hemp breeders, and monoecious individuals sometimes also occur in normally dioecious "races."[1,3,4]

The pistillate inflorescence segments, colloquially known as "buds," are often closely trimmed by hand or machine, sometimes leaving portions of the leaf bases and stiff petioles (Figure A1-1).

Figure A1-1. Cannabis dried pistillate inflorescences (“buds”): top and middle are trimmed, bottom are untrimmed and show subtending leaves (photo courtesy of Roy Upton, American Herbal Pharmacopoeia).

The segments are generally light to dark green, various shades of purple to dark purple, or from green-brown to brown. The segments may include whole or fragments of reduced upper leaves, stems, bracts, bracteoles, rudimentary calyx, immature ovules, styles, and glandular and non-glandular trichomes. In response to demand for very high levels of THC, there has been human selection for congested female inflorescences with the production of numerous, well-formed “buds” (Figure A1-2).

Figure A1-2. Cannabis “buds” showing brownish color variation, congested morphology and fragments of stems (photo courtesy of Ronan Yu, British Columbia Institute of Technology).

Thus, cannabis plants vary with regard to 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 in the size and prominence of the various parts exists between cannabis groups and are influenced by human selection as well as environmental factors including light, water, nutrients, and methods of cultivation.

Color: Color is influenced by human selection and the methods of cultivation, handling, harvest, and curing. Pistillate inflorescence parts vary in color from bright, light green to deeper, dark green through dark purple (due to accumulation of anthocyanin pigments), to light yellow-gold to brown. Sometimes flowers have 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 (Figure A1-3). 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.[1]

Figure A1-3. Examples of color variation among chemotypes of cannabis dried pistillate inflorescences: (a) "SFV OG", (b) "Magna", (c) "Laaaav", (d) "Glamour" (photos courtesy of Josh Wurzer, SC Labs).

Stems: The stems may be light brown, pale green, variously mottled, or entirely purple in color. Stems within inflorescences are 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. The stem texture is fibrous and the surface is longitudinally furrowed with short, stiff hairs. The cortex and xylem are thin, and the pith is 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.[1]

Upper Leaves: The upper leaves are 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; they also appear 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.[1]

Pistillate (Female) Inflorescence: Pistillate inflorescences are small, obscure, congested, axillary spicate cymes of approximately 1–5 cm in length and in width, with slightly protruding bracts. Development of the flowers proceeds from the base upward to the top of the inflorescence[5] (Figure A1-4). The tightly compacted inflorescences are the so-called "buds" of cannabis.

Figure A1-4. Pistillate inflorescence with senesced reddish-brown styles and stigmas, an indicator of inflorescence maturity (photo courtesy of Roy Upton, American Herbal Pharmacopoeia).

Bracts: Bracts are light to dark green or brownish-green and scabrous due to the presence of trichomes. Bracts are also numerous, alternating with overlapping edges and narrow stipules at the base. Some bracts are simple and others tripartite, but in both cases, the segments are lanceolate with an acuminate apex and a serrate or entire margin. Bracts subtending the spikes are often divided into five linear leaflets. Those subtending the individual flowers usually have three-minute leaflets. The bracts enclose the female flower except the exserted stigmas. Bracts and stipules both show a marked tendency to shrivel upon drying; in some cases, only the veins of the bracts remain intact. Bract proximal upper surfaces are densely covered by capitate stalked glandular trichomes that are readily seen with magnification (10×). Trichomes are absent from the distal region. Numerous non-glandular trichomes are also visible[1] (Figure A1-5).

Figure A1-5. Outer surface of a bract showing numerous glandular trichomes with yellow contents and clear non-glandular trichomes (photo courtesy of ElSohly group, University of Mississippi).

Perigonal Bracts: Perigonal bracts are also called bracteoles or floral bracts. They are light to dark green or brownish-green, sometimes purple to red and/or mottled or streaked. Perigonal bracts are 4–8 mm long and form in pairs in the axil of a bract.

Perigonal bracts are ovate with an acute apex, entirely incurved margin, and are fused at the base to form a conical cup-like sheath that completely envelops the ovary and loosely encloses the mature fruit. Perigonal bracts are densely hispid or pilose. With 10× magnification, numerous resinous glandular trichomes and non-glandular trichomes are observed on the abaxial (outer or lower) surface.[1] In sinsemilla production, where seeds do not develop due to prevention of pollination, the perigonal bracts remain quite small and are very densely covered with glandular trichomes.

Pistillate Flowers: The very short pedicel bears a bract that subtends the perigonal bract. A single flower approximately 5–10 mm long is formed in the axil of each perigonal bract, thus appearing essentially sessile. Because the perigonal bracts are in pairs, the pistillate flowers also appear in pairs. The true perianth develops from the base of the ovary, initially divergent from the developing ovary but soon adhering closely to it and covering about two-thirds of the ovary at maturity. The hyaline (thin, translucent or transparent) membrane appears simple, smooth, or slightly fringed along the margin, and is often marbled by patches of pigmented cells.[6] The ovary is superior, composed of two carpels (bicarpellate) united to form a single chamber (uniloculate), may be 1–2 mm long, and whitish, and contains a single campylotropous (so curved that both ends of the embryo are close to each other) ovule. Each flower has a short apical style with two caducous (easily detached and shed), long filiform stigmatic branches (styles are often three-branched in sinsemilla material)[7] spreading at the apex and projecting well above the bracteole, and densely covered with long club-shaped papillae. The style plus stigma is up to 1 cm in length and of a dark reddish-brown to orange color[1] (Figure A1-6).

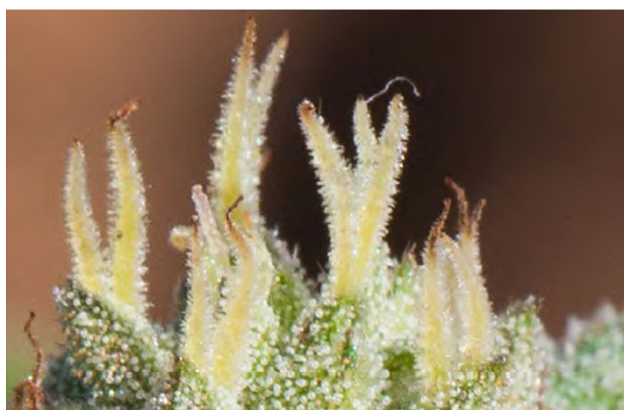

Figure A1-6. Close-up showing spreading stigmatic branches of pistillate flowers, starting to senesce by shriveling and turning from yellow to brown; surrounding bracts are covered in white glandular trichomes (photo courtesy of Roy Upton, American Herbal Pharmacopoeia).

Commercial cannabis samples made from pistillate inflorescences should be lacking staminate flowers. The staminate flower is on a pedicel, 2–4 mm, nodding; calyx of five tepals: yellowish-green to whitish, imbricate, ovate to lanceolate, 2.5–4 mm, membranous, with sessile trichomes petals absent, stamens five, opposite the tepals and prominent on filaments 0.5–1 mm and straight in bud but flaccid at maturity, anthers

oblong, with sparse, sessile glandular trichomes in the furrow, pore appearing near the apex and spreading into longitudinal dehiscence at maturity; rudimentary pistil small.[1,7] Adhering cannabis pollen may be present occasionally. Pollen grains (about 25–30 µm in diameter)[4] are triporate and have a smooth exine.[8]

Fruit: The fruit is an achene (Figure A1-7) and, together with the single enclosed seed, is commonly referred to as the “seed.” When cannabis is grown under the sinsemilla cultivation technique, exposure to pollen is prevented; therefore, the pistillate flowers remain unfertilized throughout maturity and do not develop achenes. Unless specifically desired, achenes should be lacking from properly harvested material. Achenes separate easily from dry samples. The achene is 3–4 mm in diameter (larger in cultivated plants versus ruderal plants[6]) and about 4–6 mm long, solitary, ovoid to oblong in outline, and somewhat compressed (lenticular) in cross-section. The achene may appear glossy, off-white, gray, green, brown-green, or yellowish-green, often mottled in purple—the color is typically darker in chemotypes bred for medicinal use versus fiber or seed crops.[5] In ruderal plants, the base of the achene is elongated.[4] The achene is enclosed within the enlarged, persistent perigonal bract. The perianth at this stage constitutes merely an obscure papery sheath or fragments initially covering 60–90% of the mature achene, the perianth is often missing or present only at the base of the achene after drying. There is generally less adherence of the perianth to the achene in domesticated plants.[6] The thin wall of the ovary (pericarp) tightly covers the coat of the seed. The pericarp is dry, brittle, and finely reticulate. The endosperm is fleshy and oily, and the cotyledons are also fleshy. The embryo is strongly curved.

Figure A1-7. Achenes of cannabis (photo courtesy of ElSohly group, University of Mississippi).

Trichomes: Two primary categories of trichomes are present: glandular, cannabinoid-producing trichomes and non-glandular, non-cannabinoid-producing trichomes. Both can be observed with 10×–20× magnification.

Glandular trichomes are present mainly in three forms: capitate sessile with a multicellular head but no visible stalk, bulbous with a short stalk, and capitate stalked with a multicellular head and multicellular stalk. Staminate flowers have a fourth type of glandular trichome: sessile antherial glandular trichomes.[9,10] Secretory cells at the head

of the stalk form a secretory cavity (resin chamber) by separating an outer zone of their peripheral walls from the cuticle and the subsequent reinforcement of the subcuticular wall and thickening of the cuticle. This cavity fills with membrane-bound secretory vesicles containing cannabinoids and terpenes in an intervesicular matrix.[11] The glandular trichome head has shown increased size with selection for high-THC chemotypes.[12]

Capitate sessile glandular trichomes are the most abundant type, occurring on all aerial epidermal surfaces of mature plants. They are especially abundant on the underside surface of leaves, bracts, and abaxial surface of perigonal bracts. Despite their name, they are not actually sessile. The stalk is one cell high and 2–4 cells thick, but it is hidden beneath the radially-arranged eight cells of the glandular head.[9,10] The glandular head is typically 30–70 µm in diameter and approximately 15–20 µm high.[10,13,7] Two sizes can be distinguished: larger ones are found on the pistillate flowers while smaller ones are found on pistillate flowers, leaves, and stems. The glandular head is comprised of a disc of eight or more secretory cells at the base, above which the resin chamber forms. The glandular head's resin and terpenoid-rich essential oil contents are clear during early stages of development but become opaque-white with maturity and eventually age to become brown.[10] Detached capitate sessile glands can be seen in commercial samples.

Bulbous glandular trichomes, approximately 10–20 µm in diameter and 15–30 µm high,[10,13,7] are also widespread on all epidermal surfaces of the aerial parts, with the highest density on stems and the lowest on the bracts. Most have a two-celled head and a stalk that appears to be one or two cells long and one or two cells thick, but the structure is variable. The stalk may appear to be one-celled but actually has two cells, the upper much larger than the lower; larger ones have a stalk two cells thick. The glandular head may be simple and spherical or complex and multi-compartmented, varying in size from one to four secretory cells. The contents of the resin chamber may be clear or brown in some chemotypes.[10]

Capitate multicellular stalked glandular trichomes are generally abundant, forming a pubescence on both upper and lower epidermal surfaces of the petioles, bracts, and mainly on the abaxial surface of the perigonal bracts; they are rare on staminate plants. During development, stalked glandular trichomes arise first along the veins of the bracts and later spread over the entire surface; they are initiated later than bulbous or capitate sessile glandular trichomes. The stalks, produced by elongation of hypodermal cells, can attain a length of 100–200 µm.[7] The secretory cell disk at the base of the glandular head is about 30 µm in diameter and 15 µm in height and forms a dome-shaped to eventually spherical resin chamber, which is typically 50–100 µm[10,13,7] but may be as large as 129 µm in diameter in some THC-dominant chemotypes.[12] This is compared to industrial hemp cultivars that are 80 µm in diameter[12] with resin and essential oil contents that are clear during early stages of development but become opaque-white with maturity and eventually age to become orange-brown. As the trichomes age it is common for the resin head to become detached from the stalk at an abscission region, leaving the trichome with a tip of supportive neck cells that are reddish brown in color[14] due to the presence of flavonoids.[10] Fragments of multicellular glandular trichomes including portions of the stalk are also seen in commercial samples.

Non-glandular trichomes are all unicellular. The major types are distinguished by differences in size and location.

Stigmas have pollen-trapping, club-shaped trichomes (papillae) approximately 90–180 µm long with rounded ends,[1,5] which often become detached and are found scattered in the powder of commercial samples.[14]

The surfaces of stems, leaves and bracts have abundant covering trichomes of various types; they are all conical, unicellular, and highly silicified, but some contain cystoliths and others do not. A cystolith is a well-defined concretion (sometimes described as grape-like in shape) of calcium carbonate in cannabis (calcium oxalate in some other plants). As described further in the Microscopic Identification section below, calcium oxalate crystals are present in cannabis, but they are found scattered through the tissues, not in the trichomes. The presence of silica on the outer wall surface and cystoliths of calcium carbonate inside helps non-glandular trichomes to persist and to be useful for identifying cannabis material even when it has been burned to ash. The trichomes located on or near the major veins have a verrucose surface (i.e., with warts of cellulose and cutin), whereas those occurring between the veins have a slightly warty or smooth surface.[14]

The cystolithic trichomes are either elongated, not exceptionally enlarged at the base and having a distinctly warted wall, or they are short and much enlarged at the base.[14] The elongated cystolithic trichomes are approximately 150–220 µm long, sharply pointed, and with little enlargement of the base. They often have a distinctly thickened, verrucose wall and are found mainly on the adaxial surface of the leaf, always pointing to the distal part, giving the surface a rough texture.[10]

Very short (approximately 50–125 µm) cystolithic trichomes with a highly enlarged base and smooth or verrucose surfaces are found on the adaxial surface of the bract.[9,7] Some are also found on the abaxial surface of the perigonal bracts.[4] At the base of each cystolithic trichome is the cystolith. Detached fragments of cystolithic trichomes with a warty surface are seen in commercial samples.

Simple, slender unicellular non-cystolithic trichomes approximately 250–370 µm long (some as long as 500 µm) are abundant on the stems and the abaxial surface of leaves, bracts, and to a lesser extent, on the perigonal bracts forming a pubescence much less abundant on the upper surface. They are lying almost flat and oriented toward the distal end.[9,10] Those on the adaxial (inner or upper) surface of the perigonal bract have smooth surfaces and are completely flattened; some have a more cylindrical shape. On the abaxial surface, some covering trichomes, such as those on the lower epidermis of the perigonal bract, are fairly short, rigid, verrucose, slightly enlarged at the base, and abruptly tapered to the apex. Meanwhile, other covering trichomes, such as those on the veins and edges of the perigonal bracts, are larger and more elongated, sometimes having an enlargement at the base that gradually tapers to the apex. Some covering trichomes are bent and some have branches that join (anastomose) with neighboring trichomes.[15] Detached fragments of covering trichomes are seen in commercial samples.

Figure A1-8 shows the heavy coverage of trichomes of various types on the pistillate inflorescence's leaves and bracts. To aid in interpretation of what can be seen with a

hand lens, Figure A1-9 provides labeled photographs, taken with a light microscope and a scanning electron microscope (SEM), of the various trichome types.

Figure A1-8. Cannabis “bud” showing leaf upper surfaces bearing capitate sessile glandular trichomes, bulbous glandular trichomes and cystolithic trichomes; leaf lower surfaces bearing non-glandular non-cystolithic trichomes and capitate sessile glandular trichomes; and bracts bearing capitate stalked glandular trichomes, capitate sessile glandular trichomes and non-glandular non-cystolithic trichomes (photo courtesy of Josh Wurzer, SC Labs).

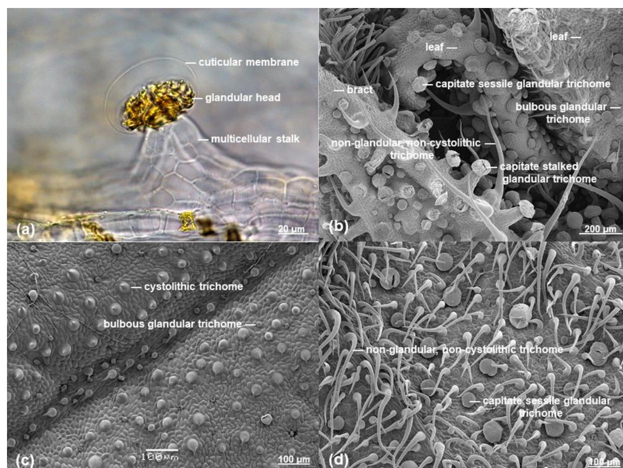

Figure A1-9. (a) Light microscope image of a capitate stalked glandular trichome; (b) SEM image of leaves and bracts of a pistillate inflorescence showing various types of trichomes; (c) SEM image of an adaxial (upper) leaf surface with its characteristic trichomes; (d) SEM image of an abaxial (lower) leaf surface with its characteristic trichomes (photos courtesy of ElSohly group, University of Mississippi).

Powder: Powder may be dull light to dark green, brownish or sometimes purplish. When viewing coarsely ground material under 20× magnification, small fragments of stems, upper leaves, bracts and bracteoles are present, with both attached and detached glandular and non-glandular trichomes, and orange to reddish-brown fragments of stigmas.[14] Fragments of the lower epidermis of leaves contain wavy vertical walls and oval stomata, while upper epidermis pieces have straight vertical walls

and no stomata. Most characteristics require higher magnification, for example, when viewing finely ground powder.[1]

Summary of Raw Material Diagnostic Macroscopic Characteristics: Characteristics of cannabis include the simultaneous presence of the following: rigid, curved cystolithic non-glandular trichomes on the upper leaf and bract surfaces; fine, slender non-cystolithic non-glandular trichomes on both lower and (to a lesser extent) upper surfaces; and capitate sessile glandular trichomes mainly on the lower surface of the leaflets, bracts and perigonal bracts.

Microscopic Features:

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.[1]

Bracts and Leaves: Microscopically, transverse sections of the leaflets and bracts show a dorsiventral structure. The palisade typically consists of a single layer (rarely two layers) of cylindrical cells and the spongy tissue of 2–4 layers of rounded parenchyma. Cluster crystals of calcium oxalate are present in all parts of the mesophyll. The upper epidermis cells bear unicellular, sharply pointed, curved and 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), more slender, and do not have 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 three types: a long multicellular stalk and a multicellular head with approximately eight radiating club-shaped cells; a short unicellular stalk and a bicellular (rarely four-cell) head[14] but they may merely represent stages in the development of the normal stalked glandular trichomes.[16] There are also sessile (without stalk) glandular trichomes with a multicellular head.[16] Both upper and lower epidermises in the midrib region are followed by a few layers of collenchyma. The vascular bundle is composed of phloem, which is made up of small cells and xylem vessels arranged in radial rows. The lower epidermis displays numerous trichomes of three types: non-glandular, non-glandular cystolithic, and glandular. Clusters of calcium oxalate crystals, about 25–30 µm in diameter, are scattered in the cortical parenchyma tissues of the mesophyll and the palisade layer.[7] 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.

Bracteoles: Bracteoles have an undifferentiated mesophyll of about four cell layers, the lower hypodermal layer having a cluster crystal of calcium oxalate in almost every cell. The abaxial surface bears numerous bulbous, sessile, and stalked glandular trichomes as well as unicellular conical trichomes. These trichomes are most numerous where the bracteole curves in to enclose the flower or fruit.[1]

Flowers: Fragments of the stigmas are fairly abundant in cannabis powder. They are orange to reddish-brown. In the stigmatic epidermis, nearly every cell has an extended papilla about 90–180 µm long, thin-walled, cylindrical, with a rounded apex.[1] Many papillae become detached and are found scattered in the powder. The sclerenchymatous layer of the pericarp, when viewed from above, shows cells that are very thick-walled and markedly sinuous, with striations and numerous pits. When viewed from below there is no apparent lumen and the surface is covered with minute, circular pits; a fairly large lumen becomes visible on focusing slightly downwards. These fragments are brown and not very numerous. Occasional fragments of the perianth can be seen. In surface view, they are composed of small, very thin-walled parenchymatous cells. The shape of the cells varies; in some fragments the cells are straight-walled and polygonal; in others they are irregularly elongated and the walls may be markedly sinuous.[14]

Stem: Fragments of the stem's epidermis have long, warty, cystolithic covering trichomes and glandular trichomes. Large, unbranched laticiferous tubes can be seen, coming from the stem phloem; they are elongated, unbranched, thin-walled tubes containing dark orange-brown granular secretions. Well-developed bundles of pericyclic fibers are present, coming from the interior of the phloem. The stem vessels are fairly large and occur in small groups; the walls are lignified and show annular or reticulate thickening. These tissues are found associated with pith and cortex parenchyma fragments containing calcium oxalate cluster crystals, about 25–30 µm in diameter.[1,14]

Images of the microscopic characteristics of cannabis inflorescence powder are provided in Figure A1-10.

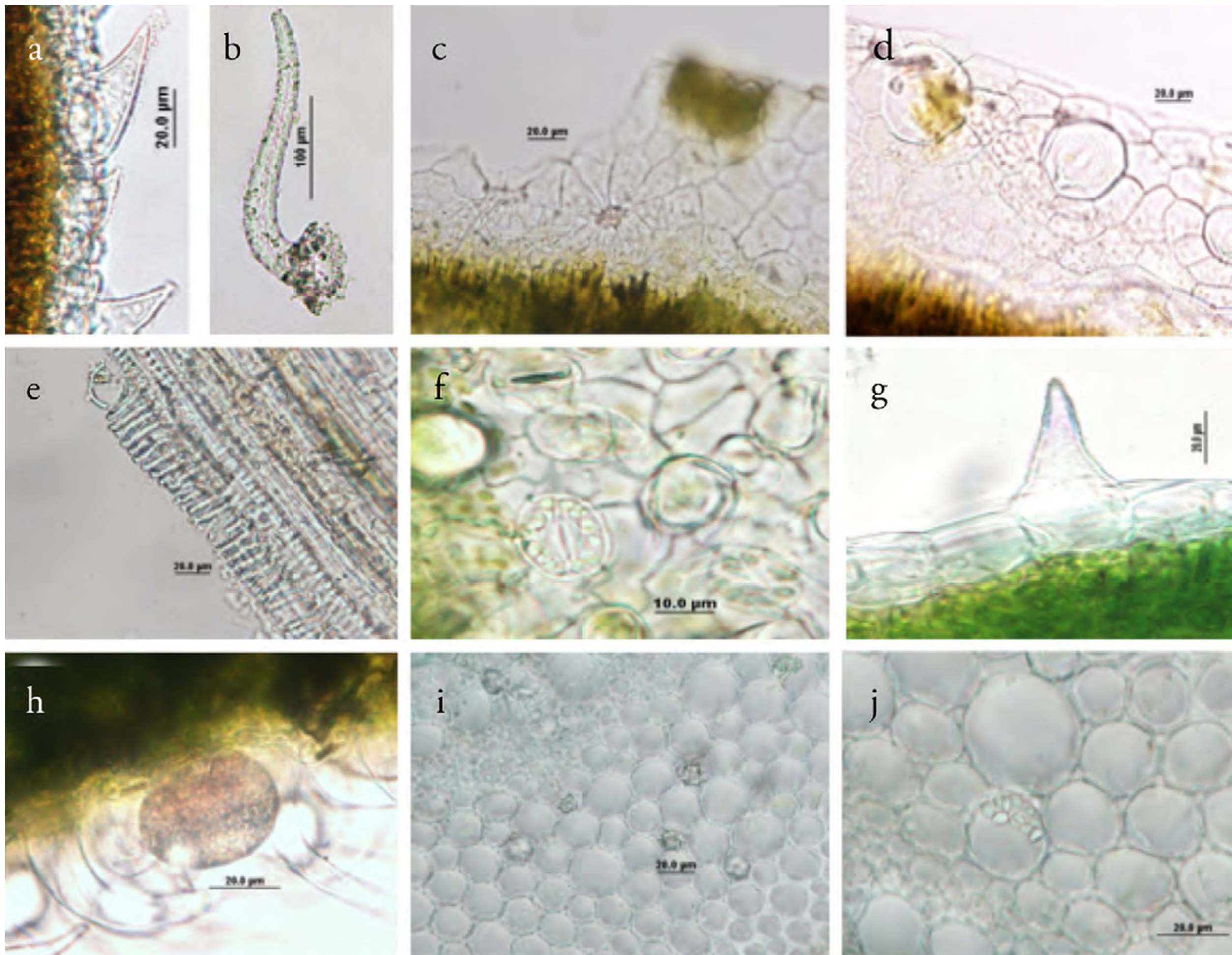

Figure A1-10. Microscopic characteristics of cannabis inflorescence powder: (a) non-glandular conical trichomes on the upper epidermis of leaflet; (b) cystolithic trichome with warty cuticle; (c) head of glandular trichome showing cells radiating from basal cells; (d) surface view of epidermis showing trichomes and cystoliths; (e) fragments of vessel elements showing spiral wall thickenings; (f) lower epidermis showing anomocytic stomata; (g) non-glandular conical trichome with cystolith; (h) head of a glandular trichome covered with cuticle; (i) cortical parenchyma showing crystals of calcium oxalate; (j) cortical parenchyma showing simple starch grains (photos courtesy of ElSohly group, University of Mississippi).

Figure A1-11 provides labeled photographs, taken with a light microscope and an SEM, of key characteristics of the microscopic anatomy of cannabis.

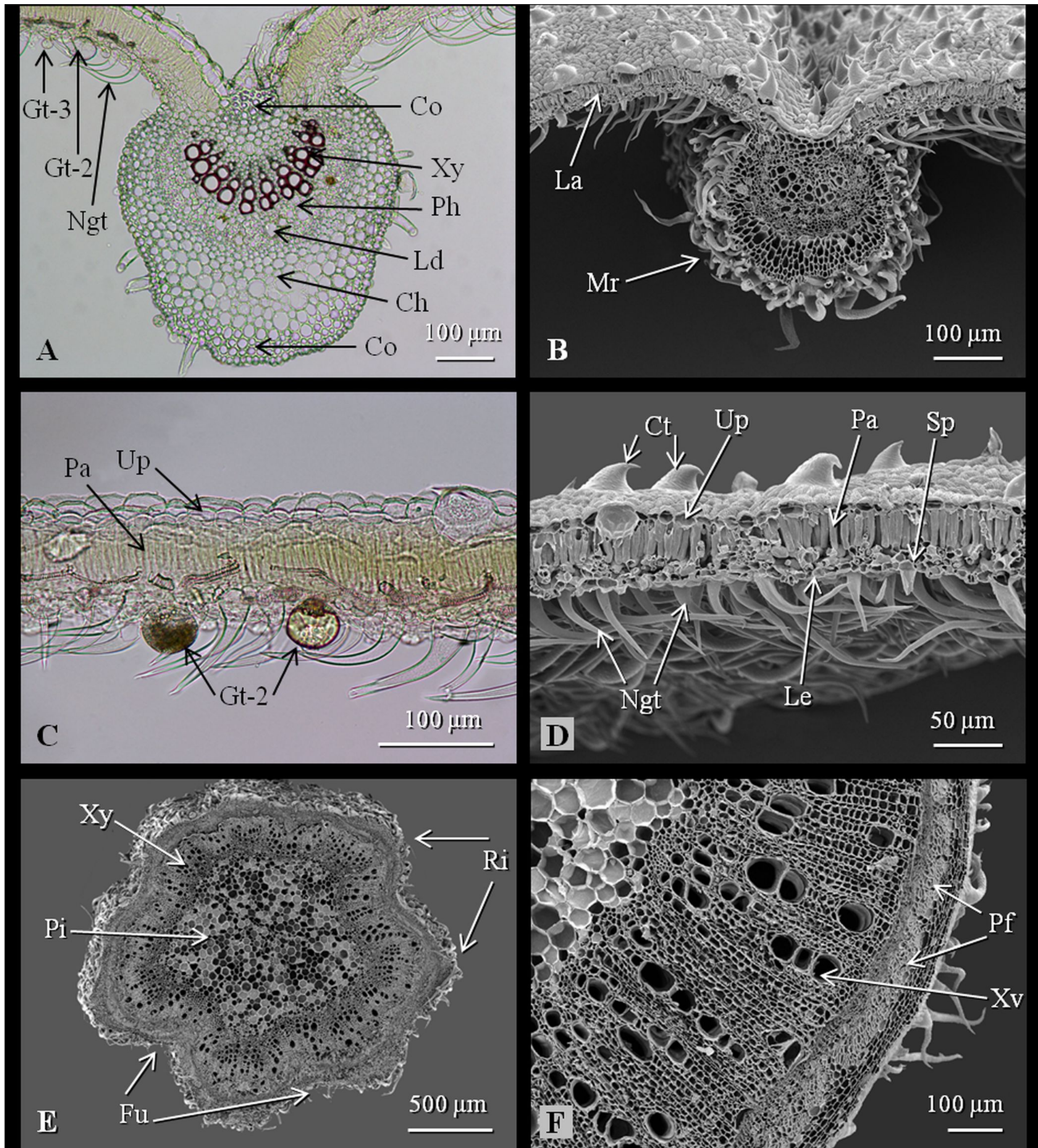

Figure A1-11. Anatomy of cannabis: (A) light microscope and (B) SEM images of a transection (TS) of a leaf through the midrib; (C) light microscope and (D) SEM images of a TS of a leaf through the lamina (blade); (E) SEM image of the TS of a stem, with a portion enlarged (F). Abbreviations: Ch – chlorenchyma, Co – collenchyma, Ct – cystolith trichome, Fu – furrows, Gt-2 – capitate sessile glandular trichome, Gt-3 – bulbous glandular trichome, La – lamina, Ld – laticifer duct, Le — lower epidermis, Mr – midrib, Ngt – non-glandular trichome, Pa – palisade, Pf – pericyclic fibers, Ph – phloem,

Pi – pith, Ri – ridges, Sp – spongy tissue, Up – upper epidermis, Xy – xylem (photos courtesy of ElSohly group, University of Mississippi).

Appendix 1 Photo Credits: The authors gratefully acknowledge the contribution of photographs by Roy Upton of the American Herbal Pharmacopoeia, Scotts Valley, CA; Ronan Yu of the British Columbia Institute of Technology, Burnaby, BC; Joshua H. Wurzer of SC Laboratories LLC., Santa Cruz, CA; and Mahmoud A. ElSohly and colleagues of the National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS.

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APPENDIX 2. HPTLC CHROMATOGRAPHIC PROFILE

Neutralized USP Cannabinoid Acids Mixture RS: Mix USP Cannabinoid Acids Mixture RS with formic acid (4:1).

Standard solution 1: Neutralized USP Cannabinoid Acids Mixture RS, and USP Cannabinoids Mixture RS.

Standard solution 2: USP Delta-9-Tetrahydrocannabinol RS, and USP Cannabidiol Solution RS (or USP Cannabidiol RS prepared as 1 mg/mL solution in methanol) diluted to 0.25 mg/mL in acetonitrile.

Sample solution: Transfer 500 mg of dried cannabis inflorescence, finely powdered and homogenized, into a centrifuge tube and add 5 mL of the mixture of methanol and hexane (9:1, v/v). Vortex the solution for 10 second and sonicate for 15 min in an ultrasonic bath at the room temperature (22±5°) interrupted by additional agitation for 10 seconds on a vortex every 5 minutes. Centrifuge and cool down to the room temperature. Transfer 1.0 mL of supernatant into dark vials.

Decarboxylated sample: The solution in one vial is evaporated to dryness under a stream of nitrogen at the room temperature. The vial is tightly closed and heated at 200° in an oven for 15 min. Cool down to the room temperature and reconstitute with a mixture of methanol and hexane (9:1, v/v) using vortex.

Chromatographic system

See *USP* General Chapter <203>, *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*[1]

Stationary Phase: Reverse phase C_{18} plates with an average particle size of 5 µm (Merck RP-18 HPTLC plates or similar). The plates were pre-washed by developing in methanol then dried at 120^0 for 30 minutes before use.

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

Relative Humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

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

Developing distance: 6 cm

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

Analysis

Samples: *Standard solutions* and *Sample solution*

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

System Suitability: The *Standard solutions* shows the cannabinoid bands with the order of increasing R_F: tetrahydrocannabinolic acid (THCA), cannabichromene (CBC), tetrahydrocannabivarinic acid (THCVA), delta-8-tetrahydrocannabinol (Δ^8-THC), delta-9-tetrahydrocannabinol (Δ^9-THC), cannabinol (CBN), cannabigerolic acid (CBGA), tetrahydrocannabidivarin (THCV), cannabidiolic acid (CBDA), cannabidiol (CBD), cannabigerol (CBG), cannabidivarinic acid (CBDVA), and cannabidivarin (CBDV).

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

REFERENCE FOR APPENDIX 2

1. USP. General Chapter <203> High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin. 2019; https://hmc.usp.org/about/general-chapters/ Accessed March 12, 2020.

ACKNOWLEDGEMENT: The authors acknowledge HPTLC data from University of Mississippi and CAMAG.

APPENDIX 3. CONTENT OF CANNABINOIDS

[Note: Perform either Procedure 1 or Procedure 2. The requirements may be met by following any one of the specified procedures; the procedure used being stated in the labeling only if *Procedure 1* is not used.]

A. Procedure 1

Solution A: 0.1% Formic acid in water

Solution B: 0.1% Formic acid in acetonitrile

Mobile phase: See *Table 1*.

Table 1

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

Standard solution A: USP Delta-9-Tetrahydrocannabinol RS, 1 mg/mL solution, diluted 1 in 10 with methanol to prepare 0.1 mg/mL solution.

Standard solution B: USP Cannabidiol Solution RS 1 mg/mL (or USP Cannabidiol RS prepared as 1 mg/mL solution in methanol) diluted 1:10 with methanol.

Neutralized USP Cannabinoid Acids Mixture RS: Mix USP Cannabinoid Acids Mixture RS with formic acid (4:1).

Standard solution C: Mix 1:1 USP Cannabinoids Mixture RS with Neutralized USP Cannabinoid Acids Mixture RS.

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

If desired, a 1 mg/mL solution of butyl-4- hydroxybenzoate in methanol can be used as internal standard instead of methanol to dissolve the reference standards and as extraction solvent for the inflorescence to compensate dilution and solution

transference errors. Peak response ratio of the internal standard (the reference peak) by the response factor of analyte would have to be included in the calculation in such case. [Response factor = peak area / concentration].

Chromatographic system

See *USP* General Chapter <621> *Chromatography*, *System Suitability*[1]

Mode: LC

Detector: UV with Diode Array Detection, 222 nm

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

Column temperature: 40º

Flow rate: 1.5 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution A* and *Standard solution C*

Suitability requirements

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

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

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

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

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

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Standard solution C* and *Sample solution*.

Using the chromatogram of *Standard solution A, Standards solution B* and *Standard solution C*, identify the retention times of the peaks corresponding to each cannabinoids in the *Sample solution* chromatogram. The relative retention times and Conversion Factors against cannabidiol are provided in Table 2.

Table 2

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

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

Calculate the amount (mg/g) of each cannabinoid in the portion of Cannabis Inflorescence taken:

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

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

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

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

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

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

F = Conversion Factor for the analyte (Table 2)

Calculate total THC (mg/g)

$$\text{Result} = (\text{THCA} \times 0.877) + \Delta^9\text{-THC}$$

Calculate total CBD (mg/g)

$$\text{Result} = (\text{CBDA} \times 0.877) + \text{CBD}$$

Calculate the percentage of the labeled amount of cannabinoids

$$\text{Result} = (P/L) \times 100$$

P = amount of total THC or total CBD as determined above

L = labeled amount of cannabinoids

Acceptance criteria:

The following are the acceptance criteria for the cannabis chemotypes:

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

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

B. Procedure 2

Extraction solution: acetonitrile and methanol (8:2)

Internal standard solution: 10 µg/mL of 4-androstene-3,17-dione in *Extraction solution*.

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

Standard solution B: Dilute USP Cannabidiol Solution RS (or USP Cannabidiol RS prepared as 1 mg/mL solution in methanol) to 20 µg/mL with methanol.

Standard solution C: Combine 400 µL of USP Cannabinoid Acids Mixture RS and 400 µL of USP Cannabinoids Mixture RS in a 4-dram vial. Transfer 8 µL to a derivatization vial, add 100 µL of 2% dimethylaminopyridine (DMAP) in *Extraction solution* and 500 µL of internal standard solution. Evaporate to dryness under stream of nitrogen at 50°C. Derivatize the residue with 100 µL of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) for 30 min at 70°C. Cool the vial to room temperature before injection.

Sample solution: Transfer 300 mg of Cannabis Inflorescence to a centrifuge tube and add 2.5 mL of *Extraction solution*. Sonicate for 20 min and centrifuge for 5 min at 4000 rpm. The extraction is repeated four times and all supernatants are combined

into a 10 mL volumetric flask. Make up to the volume with *Extraction solution*. Transfer 10 µL of this solution to a derivatization vial, and add 50 µL of *Internal standard solution* and 10 µL of 2% DMAP in *Extraction solution*. Vortex and evaporate to dryness under a stream of nitrogen at 50º. Derivatize the residue with 100 µL of BSTFA for 30 min at 70º. Cool the vial to room temperature before injection.

Chromatographic system

See *USP* General Chapter <621> *Chromatography*, *System Suitability*[1]

Mode: GC

Detector: FID

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

Temperature

Injector: 275º

Detector: 300º

Column: See *Table 3*.

Table 3

Initial Temperature (º)	Temperature Ramp (º/min)	Final Temperature (º)	Hold Time at Final Temperature (min)
190	-	190	1
190	30	230	2
230	5	250	1
250	20	300	2.75
190	-	190	10.75

Carrier gas: Helium

Flow rate: 0.8 mL/min

Injection size: 3 µL

Split mode: Split 20:1

System suitability

Sample: *Standard solution A* and *Standard solution C*

Suitability requirements

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

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

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

Analysis

Samples: *Standard solution A*, *Standard solution B, Standard solution C* and *Sample solution*.

Using the chromatogram of *Standard solution A, Standard solution B* and *Standard solution C*, identify the retention times of the cannabinoid peaks corresponding to each cannabinoid in the *Sample solution* chromatogram. The relative retention times against internal standard and relative response factors against CBD are provided in Table 4.

Table 4

Analyte	Relative retention time
CBDV	0.43
THCV	0.48
CBD	0.63
CBC	0.69
Δ^8-THC	0.71
Δ^9-THC	0.74
CBDVA	0.77
CBG	0.81
CBN	0.83
THCVA	0.90
CBDA	0.94
Internal standard	1.00

THCA	1.12
CBGA	1.19

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

Calculate the amount (mg/g) of each cannabinoid in the portion of Cannabis Inflorescence taken:

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

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

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

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

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

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

F = Conversion Factor for the analyte

Calculate total THC (mg/g)

$$\text{Result} = (\text{THCA} \times 0.877) + \Delta^9\text{-THC}$$

Calculate total CBD (mg/g)

$$\text{Result} = (\text{CBDA} \times 0.877) + \text{CBD}$$

Calculate the percentage of the labeled amount of cannabinoid

$$\text{Result} = (P/L) \times 100$$

P = amount of total THC or total CBD as determined above

L = labeled amount of cannabinoid

Acceptance criteria:

The following are the acceptance criteria for the cannabis chemotypes:

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

APPENDIX 4. CONTENT OF TERPENES

Internal Standard Stock Solution: 1000 µg/mL *n*-Tridecane in ethyl acetate

Internal standard solution: 100 µg/mL *n*-Tridecane in ethyl acetate

Standard stock solution: Mix β-caryophyllene, D-limonene, β-myrcene, α-pinene, and α-terpinolene to make 1.0 mg/mL each in Internal Standard Stock Solution

Standard solutions: Dilute the Standard Stock Solution to the concentration of 100 µg/mL using ethyl acetate.

Sample solution: Transfer 1 g of powdered Cannabis Inflorescence into a 15 mL centrifuge tube and extracted with 10 mL of *Internal standard solution*. Sonicate for 15 min. Centrifuge for 5 min and use the aliquot.

Chromatographic system

See *USP* General Chapter <621> *Chromatography*, *System Suitability*.[1]

Detector: FID

Column: 0.25-mm × 30-m fused silica capillary; 0.25-µm film of G27 phase coating (Agilent DB-5ms or similar)

Temperature

Injector: 250º

Detector: 300º

Column: See *Table 5*.

Table 5

Initial Temperature (º)	Temperature Ramp (º/min)	Final Temperature (º)	Hold Time at Final Temperature (min)
70	-	70	2
70	2	85	-
85	3	165	-

Carrier gas: Helium

Flow rate: 1.2 mL/min

Injection size: 2 µL

Split mode: Split 15:1

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between any two peaks

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Using the chromatogram of *Standard solution*, identify the retention times of the terpene peaks corresponding to each terpene in the *Sample solution* chromatogram. The relative retention times are provided in Table 6.

Table 6

Analyte (groups)	Relative Retention Time
α-Pinene	0.72
β-Myrcene	0.86
D-Limonene	1.00
α-Terpinolene	1.18
β-Caryophyllene	2.26

Calculate the percentage of terpenes in the portion of Cannabis Inflorescence taken:

$$\text{Result} = (R_U/R_S) \times C_S \times (V/W) \times 100$$

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

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

C_S = concentration of the terpene in the *Standard solution* (mg/mL)

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

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

Calculate the relative content of each terpene with respect of total detected area between 0.1 and 2.5 RRT with respect to limonene:

$$\text{Result} = (Ai/TDA) \times 100$$

Ai = peak area of each terpene as determined above

TDA = total detected area between 0.1 and 2.5 RRT with respect to limonene

Acceptance criteria:

- For cannabis labeled as α-Pinene dominant: The relative content of α-Pinene is more than 2 times the content of any other peak
- For cannabis labeled Myrcene dominant: The relative content of Myrcene is more than 2 times the content of any other peak
- For cannabis labeled Limonene dominant: The relative content of Limonene is more than 2 times the content of any other peak
- For cannabis labeled Terpinolene dominant: The relative content of Terpinolene is more than 2 times the content of any other peak
- For cannabis labeled ß-Caryophyllene dominant: The relative content of ß-Caryophyllene is more than 2 times the content of any other peak
- For cannabis labeled Myrcene- β-Caryophyllene codominant: Myrcene and β-Caryophyllene are the mayor peaks in the chromatogram and the ratio of relative content of Myrcene/β-Caryophyllene is between 0.5 and 2
- For cannabis labeled Myrcene-Limonene codominant: Myrcene and Limonene are the mayor peaks in the chromatogram and the ratio of relative content of Myrcene/Limonene is between 0.5 and 2
- For cannabis labeled Limonene- β-Caryophyllene codominant: Limonene and β-Caryophyllene are the mayor peaks in the chromatogram and the ratio of relative content of Limonene/β-Caryophyllene is between 0.5 and 2
- For cannabis labeled Myrcene-Limonene- β-Caryophyllene codominant: Myrcene, Limonene and β-Caryophyllene are the mayor peaks in the chromatogram and the ratio of relative content of any pair of the three terpenes is between 0.5 and 2

REFERENCES FOR APPENDICES 3 and 4:

1. USP. General Chapter <621> Chromatography. 2019; https://hmc.usp.org/about/general-chapters/ Accessed March 12, 2020.