American Herbal Pharmacopoeia®

Cannabis Inflorescence
Cannabis spp.

STANDARDS OF IDENTITY, ANALYSIS, AND QUALITY CONTROL

Editors and Technical Advisors

Roy Upton RH DAyu
American Herbal Pharmacopoeia®
Scotts Valley, CA

Lyle Craker PhD
University of Massachusetts
Amherst, MA

Mahmoud ElSohly PhD
University of Mississippi
University, MS

Aviva Romm MD CPM
American Herbal Pharmacopoeia®
Lennox, MA

Ethan Russo MD
GW Pharmaceuticals
Salisbury, UK

Michelle Sexton ND BS
Americans for Safe Access
Washington, DC
The Center for the Study of Cannabis
and Social Policy
Seattle, WA

Research Associates

Jahan Marcu PhD
Green Standard Diagnostics
Henderson, NV

Diana Swisher MA
American Herbal Pharmacopoeia®
Scotts Valley, CA

AHP

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Legal Notification

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

In the United States, cannabis is a Schedule I controlled substance under federal law; therefore, any use or possession of cannabis and its preparations is illegal except pursuant to the compassionate use Investigational New Drug exemption. These standards are not intended to support, encourage or promote the illegal cultivation, use, trade, or commerce of cannabis. Individuals, entities and institutions intending to possess or utilize cannabis and its preparations should consult with legal counsel prior to engaging in any such activity.

The citing of any commercial names or products does not and should not be construed as constituting an endorsement by the American Herbal Pharmacopoeia. Additionally, the reliability, and therefore ability to comply with state or federal regulations, of any conclusions drawn from the analysis of a sample is dependent upon the test sample accurately representing the entire batch. Therefore, when performing all analytical tests, a formal sampling program must be employed.

Authors

Botanical Identification

Mahmoud ElSohly PhD
Suman Chandra PhD
Hemant Lata PhD
University of Mississippi
University, MS

Macroscopic Identification

Suman Chandra PhD
Hemant Lata PhD
Mahmoud ElSohly PhD
University of Mississippi
University, MS

Microscopic Identification

Suman Chandra PhD
Hemant Lata PhD
Mahmoud ElSohly PhD
University of Mississippi
University, MS

Elizabeth Williamson PhD
University of Reading
Reading, UK

Commercial Sources and Handling

Suman Chandra PhD
Hemant Lata PhD
University of Mississippi
University, MS

Roy Upton RH DAyu
American Herbal Pharmacopoeia
Scotts Valley, CA

Constituents

Mahmoud ElSohly PhD
Desmond Slade PhD
University of Mississippi
School of Pharmacy
University, MS

Analytical

Thin-Layer Chromatography (TLC)
Gas Chromatography (GC)
Mahmoud ElSohly PhD
Desmond Slade PhD
Mohammed M Radwan PhD
University of Mississippi
University, MS

High Performance Liquid
Chromatography (HPLC)
Kong M Li PhD
University of Sydney
Sydney, NSW

Reviewers

Wendy Appplequist PhD
Missouri Botanical Gardens
St. Louis, MO

Paula Brown PhD
British Columbia Institute of
Technology (BCIT)
British Columbia, Canada

Rudolf Brenneisen
University of Bern
Bern, Switzerland

Mike Corral
Wo/Men's Alliance for Medical
Marijuana
Santa Cruz, CA

Staci Eisner
Cannabis Committee
American Herbal Products
Association
Silver Spring, MD

Daniel Harder PhD
Museum of Natural History
Santa Cruz, CA

Erik W Johansen
Special Pesticide Registration
Program Coordinator
Washington State Department of
Agriculture
Olympia, WA

James Kababick
Flora Research Laboratories
Grants Pass, OR

ao Prof Dr Liselotte Kren
University of Vienna
Vienna, Austria

Prof Dr Reinhard Länger
AGES Pharm Med
Vienna, Austria

Etienne de Meijer
GW Pharmaceuticals
Salisbury, UK

David Potter PhD
GW Pharmaceuticals
Salisbury, UK

Eike Reich PhD
CAMAG
Muttenz, Switzerland

Jeanette Roberts PhD MPH
University of Wisconsin
Madison, WI

Steph Sherer
Americans for Safe Access (ASA)
Washington DC

Bill Schoenbart Lac OMD
Five Branches University
Santa Cruz, CA

Aman Soumyanath PhD
Oregon Health and Science
University
Portland, OR

Elan Sudberg
Costa Mesa, CA

Elizabeth Williamson PhD
University of Reading
Reading, UK

Hugh Watson
Marijuana Agricultural Chemical
Specialist
Washington State Liquor Control
Board
Olympia, WA

Final Reviewers

Giovanni Appendino Laurea
Department of Pharmaceutical
Sciences
University of the Eastern Piedmont
Novara, Italy

Vincenzo Di Marzo PhD
Endocannabinoid Research Group
(ERG)
Institute of Biomolecular Chemistry
(ICB)
Consiglio Nazionale delle Ricerche
(CNR)
Pozzuoli (NA), Italy

Raphael Mechoulam PhD
Hebrew University of Jerusalem
Jerusalem, Israel

Jonathan Page PhD
National Research Council
Saskatoon, Canada

Ethan Russo MD
GW Pharmaceuticals
Salisbury, United Kingdom

Maged Sharaf PhD
American Herbal Products
Association
Silver Spring, MD

Michael Steenhout
Washington State Liquor Control
Board
Olympia, WA

Medical Disclaimer

The information contained in this monograph represents a synthesis of the authoritative scientific and traditional data. All efforts have been made to ensure the accuracy of the information and findings presented. Those seeking to utilize botanicals as part of a health care program should do so under the guidance of a qualified health care professional.

Statement of Nonendorsement

Reporting on the use of proprietary products reflects studies conducted with them and is not meant to be a product endorsement.

Design & Layout

Michael Parisi
Aptos, CA

Cover Photograph

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

American Herbal Pharmacopoeia®

PO Box 66809
Scott's Valley, CA 95067
831-461-6318
www.herbal-ahp.org
ahp@herbal-ahp.org

Figure 2 (continued) **Botanical characteristics of cannabis inflorescences**

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

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

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

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

hemp, narrow-leaf drug, etc. to account for the plasticity represented in the genus.

Cannabis is a member of the *Cannabaceae* family, together with another well-known member of the family, hops (*Humulus*). The family has recently been expanded to contain 9 other genera (Stevens 2001). The following describes the published range of morphological diversity within plants recognized as *Cannabis* spp.

Morphological Characterization of *Cannabis* L.

Herbaceous annual, taprooted (taproot not developed on vegetatively propagated/cloned plants). Plants dioecious (male and female flowers occur on separate plants) and rarely monoecious (male and female flowers occur on the same plant). Monoecious plants are often referred to as "hermaphrodites." True hermaphrodites bear bisexual flowers and are less common, whereas monoecious plants bear unisexual male and female flowers at different locations on the plant. Staminate (male) plants tend to be taller but less robust than pistillate (female) plants. Height and degree of branching depends on both genetic and environmental factors (UNODC 2009). **Stem:** Erect, furrowed, often hollow, 0.2–6 m (usually 1–3 m) tall, simple to well branched; branchlets densely pubescent; staminate (male) plants usually taller and less robust, compared with pistillate (female)

6a.

6b.

6c.

6d.

6e.

6f.

Figure 6 Macroscopic characteristics of cannabis inflorescence

6a. Dried, untrimmed pistillate inflorescences of morphological type "sativa."

6b. Dried pistillate inflorescences of morphological type "sativa" (bottom – untrimmed; top – trimmed).

6c. Storage effects on color of cannabis material (left – 1-year-old; right – new harvest).

6d. Dried pistillate inflorescences of morphological type "indica" (bottom – untrimmed; middle and top – trimmed).

6e. Close-up of a dried pistillate inflorescence (note the visible glandular trichomes).

6f. Powdered dry cannabis material (leaves and pistillate inflorescences).

Photographs courtesy of: (6a–e) WAMM, Santa Cruz, CA; (6f) University of Mississippi, University, MS.

3a.

3b.
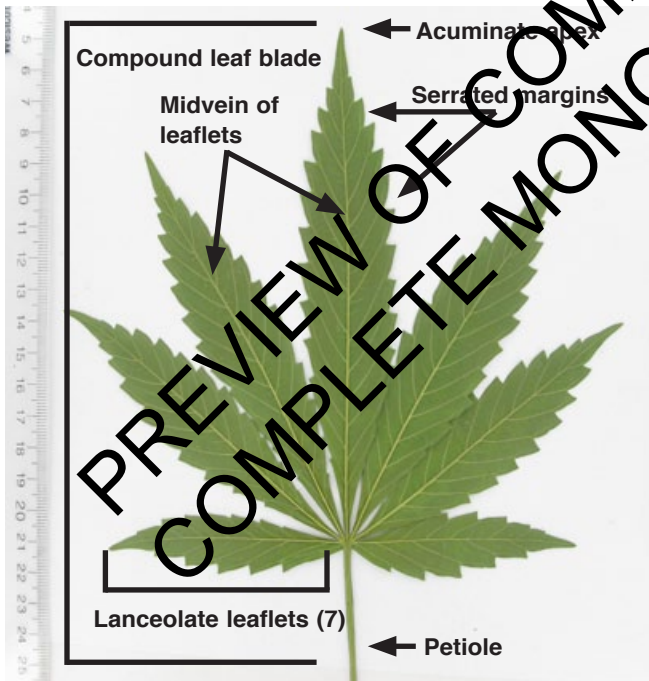
3c.

3d.

Figure 3 Botanical characteristics of cannabis leaf

3a. Adaxial (upper) surface of a typical cannabis leaf (9 leaflets).

3b. Adaxial (upper) surface of a typical cannabis leaf (5 leaflets).

3c. Adaxial (upper) surface of a typical cannabis leaf with morphological characteristics highlighted.

3d. Abaxial (lower) surface of a typical cannabis leaf.

Natural Contaminants and Adulterants

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

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

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

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

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

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

Qualitative Differentiation

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

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

Dispensaries should maintain strict quality control practices to ensure the purity and quality of their material by contracting for testing with independent labs that apply independently verified testing methodologies and transparent testing

standards. Individual growers and care givers producing medical cannabis for personal use should employ good agricultural practices (GAPs) to the extent possible in all aspects of growing, harvesting, drying, and storage.

Sustainability and Environmental Impact

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

In addition to the impacts of cannabis cultivation, the manufacture of butane extracts poses significant risk. A number of explosions and fires associated with butane cannabis extract production have been reported, some that have included injury. Some butane contains compounds that may not be desirable in finished products. Extraction with CO_2 (sub- or super-critical) is preferred by some as the one environmentally safe extracting option.

Documentation of Supply

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

Security (modified from OMC 2003)

a. The buildings in which cannabis is cultivated, processed, packaged and stored must be sufficiently secured, only allowing authorized personnel access to the buildings.

b. Personnel involved in the production process of cannabis must be authorized for that purpose by the employer.

c. Waste must be stored in such a way that the potential for theft is minimized.

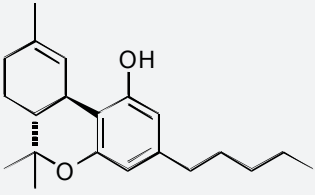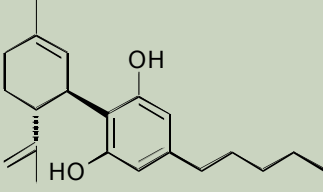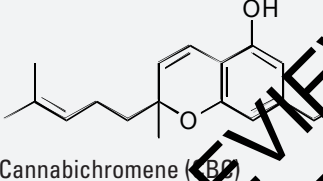| Growing and Harvesting Guidelines (OMC 2003) |
|---|
| **a.** Location of cultivation and the name of the supervising cultivator. |
| **b.** Details on crops previously grown at that location. |
| **c.** Nature, origin and quantity of the herbal starting materials. |
| **d.** Chemicals and other substances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

Suppliers and Dispensaries

Cannabis supplied by dispensaries should be as fully characterized as possible with traceability and a verifiable chain of custody to type of material, whether the plants were cultivated conventionally or organically, or was indoor or outdoor cultivated. Procedures should be implemented to ensure the absence of pesticides and raw material and finished product should be characterized as to its basic chemical profile (e.g., Δ^9-THC and/or CBD content). This information should be made available to patients upon request. Dispensary personnel should be appropriately trained in how to process and handle cannabis to ensure purity, maintain quality, and to morphologically identify material. The cannabis committee of the American Herbal Products Association (AHPA) has developed a set of draft guidelines outlining recommended practices for dispensaries and cultivators to follow (AHPA 2013a), and Americans for Safe Access (ASA) has developed a industry certification program for dispensaries and cultivators (ASA PFC).

CONSTITUENTS

To date, more than 750 different secondary metabolites have been identified in cannabis. The diversity of cannabis constituents encompasses numerous phytochemical classes, notably, cannabinoids, and a host of other secondary metabolites. These other compound classes include terpenoids, non-cannabinoid phenols, nitrogenous compounds, as well as other more common plant compounds, all of which are non-psychotropic. Cannabinoids are the most studied

Table 6 Structure and activity of primary phytocannabinoids

Compound	Putative Medicinal Action
 Δ^9-Tetrahydrocannabinol (Δ^9-THC)	**Primary psychotropic cannabinoid** Activates PPAR-y and TRPA1 at nano- and micromolar concentrations, respectively (Pertwee 2008). Analgesic via CB_1 and CB_2 agonism (active at ~20–40 nM) (Rahn and Hohmann 2009). Antiemetic (Haney et al. 2007; Hollister 1971; Machado et al. 2008). Anti-inflammatory, antioxidant (Hampson et al. 1998). Antipruritic, cholestatic jaundice (Neff et al. 2002). Benefits duodenal ulcers (Douthwaite 1947). Bronchodilatory (Williams et al. 1976). Muscle relaxant (Kavia et al. 2010). Reduces Alzheimer symptoms (Eubanks et al. 2006; Volicer et al. 1997).
 Cannabidiol (CBD)	**Non-psychotropic cannabinoid** Anandamide (AEA) reuptake inhibitor (De Petrocellis et al. 2011). Analgesic (Davis and Hatoum 1983). Anticonvulsant (Jones et al. 2010). Antidepressant in rodents (Deyo and Musty 2003). Anti-emetic (5HT1A agonist; 5 mg/kg ip) (Rock et al. 2010). Antifungal (ElSohly et al. 1982). Anti-inflammatory (Booz et al. 2011). Antagonizes effects of THC in humans (Pertwee 2008). Antioxidant (Hampson et al. 1998). Anxiolytic via 5HT1A agonism (Campos and Guimaraes 2008; Resstel et al. 2009; Russo et al. 2005). Decreases sebum/sebocytes proliferation (Biro et al. 2009). Effective against methicillin-resistant *Staphylococcus aureus* (MRSA) (Appendino et al. 2008). Increases adenosine A2A signaling (Carrier et al. 2006). Pro-apoptotic against breast cancer cell lines (Ligresti et al. 2006). Treatment of addiction (Xi et al. 2010). Treatment of psychosis (Russo et al. 2007).
 Cannabichromene (CBC)	**Non-psychotropic cannabinoid** Analgesic (weak) (Turner et al. 1980b). Anandamide reuptake inhibitor (weak) (De Petrocellis et al. 2008; Ligresti et al. 2006). Anti-inflammatory (Davis and Hatoum 1983). Antimicrobial (Turner and ElSohly 1981). TRPA1 agonist (De Petrocellis et al. 2008; Ligresti et al. 2006).

Standards Preparations

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

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

Standards Solution Stability

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

Reagent Preparation

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

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

Chromatographic Conditions

Stationary Phase:

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

Mobile Phase:

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

Sample Application

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

Development

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

Detection

Visualize the plates under UV 254 nm, then spray one set of the plates with the Fast Blue reagent and the other set of plates with the vanillin/H_2SO_4 reagent, followed by visu-

Table 7 R_f values for cannabinoid standards

Phytocannabinoid	R_f
CBC	0.21
Δ^9-THC	0.26
CBN	0.29
CBG	0.33
CBD	0.40
THCV	0.42
Δ^9-THCA	0.61
CBDA	0.70

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

alization under white light. For basic identification of the primary cannabinoids, either reagent can be used.

Results

See Table 7 and refer to the chromatograms provided (Figure 17–0).

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

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

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

Sample Preparation

Crude Cannabis

Test samples are dried for 24 h in a 35 °C forced ventilation oven. Dried samples are ground to a fine powder. 200 mg of the sample is weighed in a glass vial and extracted with 10 mL of a mixture of methanol/chloroform (v/v: 9:1)
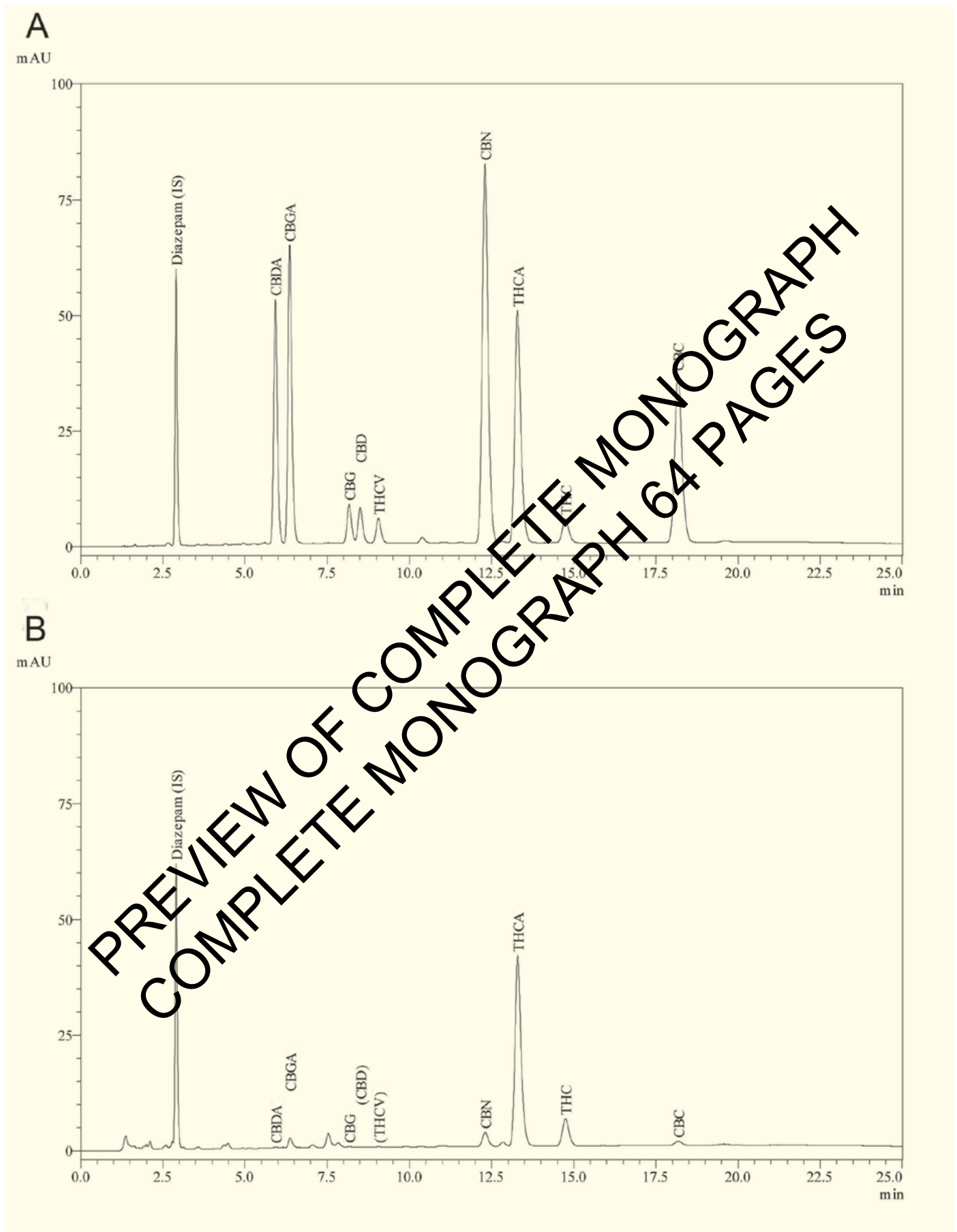
diode array detector. For routine use, a standard UV detector is suitable.

Run time:

30 min.

Post-run time:

6 min.

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

Quantitation

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

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

where:

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

  y = peak area of the invidivual cannabinoid;

  c = calculated y-intercept of the calibration curve;

  m = calculated slope of the calibration curve.

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

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

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

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

where:

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

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

V_{sample} = sample volume (mL);

D = dilution factor;

m_{sample} = sample mass (g).

Calibration Range

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

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

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

Sample Preparation

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

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

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

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

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

Chromatographic Conditions
Column:

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

Table 10 Pesticides commonly used in cannabis cultivation

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

ECD = Electron capture detector; FLD = Fluorescence detector; GC = Gas chromatography; HPLC = Liquid chromatography; IR = Infrared spectroscopy; MS = Mass spectrometry; NMR = Nuclear magnetic resonance; NPD = Nitrogen phosphorous detector.

* Natural pyrethrins are tolerance exempt; synthetic pyrethrins are not.

Analytical Methods [RAM]) or those of the Food and Drug Administration (FDA Pesticide Analytical Manual [PAM]), should be employed when appropriate. However, as these tests were developed for commodity food products, the amount of sample needed may be prohibitive to apply to the cannabis industry. Alternatively, the food testing QuEChERS screen uses smaller quantities and may be more applicable to a variety, though not all, of cannabis products (Schoen 2012, personal communication to AHP, unreferenced).

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

Before commercial use, any immunoassay should be validated against a standard testing methodology.

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

Solvent Residues

Limits on solvents used in the manufacture of botanical products are established by the International Conference on Harmonization (ICH) (ICH 2011), with exceptions made for ethanol and acetic acid in products formulated to contain these substances (e.g., tinctures and vinegars). According to the ICH guideline, solvents are categorized in three classes. Class 1 includes known carcinogens, toxic substances, and environmental hazards such as benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene, and 1,1,1-trichloroethane. These are to be avoided in the manufacture of herbal and/or pharmaceutical products. Class 2 and 3 solvents (Table 12) are distinguished based on their relative toxicity level. Limits established for permissible daily exposures (PDE) are determined individually for Class 2 solvents. Limits for Class 3 solvents are set at a

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