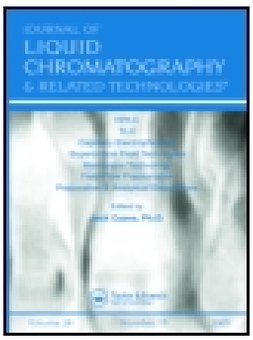


Taylor & Francis
Taylor & Francis Group

Journal of Liquid Chromatography & Related Technologies

ISSN: 1082-6076 (Print) 1520-572X (Online) Journal homepage: https://www.tandfonline.com/loi/ljlc20

Thin layer chromatography in the analysis of cannabis and its components and synthetic cannabinoids

Joseph Sherma & Fred Rabel

To cite this article: Joseph Sherma & Fred Rabel (2019): Thin layer chromatography in the analysis of cannabis and its components and synthetic cannabinoids, Journal of Liquid Chromatography & Related Technologies, DOI: 10.1080/10826076.2019.1663529

To link to this article: https://doi.org/10.1080/10826076.2019.1663529

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
https://doi.org/10.1080/10826076.2019.1663529

Thin layer chromatography in the analysis of cannabis and its components and synthetic cannabinoids

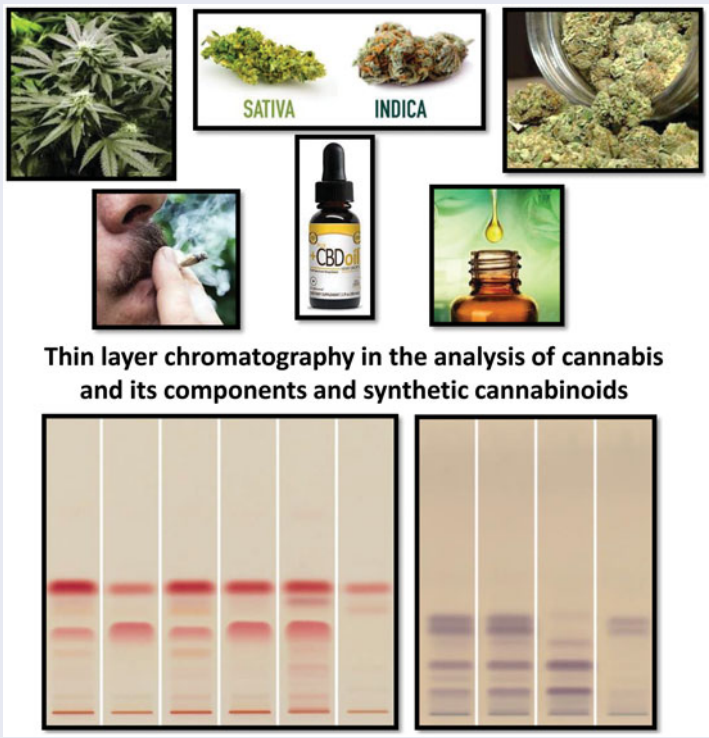
Joseph Sherma[a] and Fred Rabel[b]

[a]Department of Chemistry, Lafayette College, Easton, PA, USA; [b]ChromHELP, LLC, Woodbury, NJ, USA

ABSTRACT

Cannabis has been used as a medicinal plant for thousands of years. There are now over 700 varieties of cannabis that contain hundreds of compounds, including fatty cannabinoids that are the main biologically active constituents and volatile terpenes that have distinct odors. This is a selective review that includes important examples of the analysis and study of cannabis and its components and synthetic cannabinoids by thin layer chromatography (TLC) related to its medical and recreational uses. The TLC methods described in this review complement the more expensive and difficult to perform and sustain high performance liquid chromatography (HPLC), HPLC/mass spectrometry (HPLC/MS), gas chromatography (GC), and GC/MS methods. These TLC methods are especially valuable and often sufficient for use in resource-limited countries. Since this is apparently the first review devoted only to the TLC of cannabis in the literature, even earlier TLC references have been included for completeness.

GRAPHICAL ABSTRACT



KEYWORDS

Cannabidiol; cannabinoids; cannabis; CBD; CBN; drug analysis; hashish; hemp; HPTLC; marijuana; sample prep; tetrahydrocannabinol; TLC; Δ^9-THC

Introduction

Cannabis has been used as a medicinal plant for thousands of years. There are now over 700 varieties of cannabis that contain hundreds of compounds, including fatty cannabinoids that are the main biologically active constituents and volatile terpenes that have distinct odors. The two most prevalent and commonly known cannabinoids in the

CONTACT Fred Rabel f.rabel@comcast.net ChromHELP, LLC, 136 Progress Ave., Woodbury, NJ 08096, USA.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ljlc.

cannabis plant are Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD). THC is rapidly distributed to fatty tissue like the brain, and it is metabolized by the cytochrome P450 system to psychoactive 11-hydroxy-THC. Cannabis and cannabinoids have been used to treat a variety of medical conditions and are legal in many states in the United States for this purpose. A summary of the history, pharmacology, and medical uses of cannabis has been published.[1] This is a selective review that includes important examples of the analysis and study of cannabis and its components and synthetic cannabinoids by thin layer chromatography (TLC) related to medical and recreational uses, which are also becoming increasingly legalized in many states in the United States and in other countries, as well as use of illegal products. This is the first review devoted only to the TLC of cannabis in the literature.

Cannabis is a genus of flowering plants in the Cannabaceae family, which consists of three primary species: *Cannabis sativa*, *indica*, and *ruderalis*. Hemp is a term used to classify varieties of Cannabis that contain 0.3% or less THC content by dry weight, which are generally non-toxicating and are harvested to produce a variety of industrial, food, medicines, and other products. Marijuana (also spelled marihuana in many publications) is used to classify varieties that contain more than 0.3% THC and can induce psychotropic or euphoric effects in a user. Hemp-derived CBD is legal if it contains 0.3% of THC or less, while CBD derived from marijuana is illegal and classified as a controlled substance regardless of the percentage of THC; the molecular structure and associated pharmacology of CBD derived from both sources are identical.[2] Marijuana and hashish have been differentiated as plant material derived from dried pieces of the cannabis plant, mainly flower buds, for the former and from compressed resin of flowers of the plant for the latter. Male and female *Cannabis sativa* varieties are usually defined as pollen producing or bud producing, respectively.

TLC is a well-known technique currently used for cannabis analysis,[3] and the methods described in this review complement the more expensive and difficult to perform and sustain high performance liquid chromatography (HPLC), HPLC/mass spectrometry (HPLC/MS), gas chromatography (GC), and GC/MS methods. They are especially valuable and often sufficient for use in resource-limited countries.[4] Advantages of HPTLC as listed by Badyal et al.[5] include capability to analyze crude samples containing multicomponents; easy separation and detection of colored compounds; parallel analysis of several samples on the same plate resulting in high output, speed, and low cost; two dimensional (2 D) separations are easy to perform; different modes of evaluation, allowing identification of compounds having various light absorption characteristics or colors; and minimized exposure risk of toxic organic effluents and reduction of disposal problems and environmental pollution.

Extraction methodology and techniques of TLC

Citti et al.[6] reviewed reported uses of solid-liquid extraction, cloud point extraction, and supercritical fluid extraction for recovery of cannabinoids from plant material. Extraction from biological samples by liquid-liquid extraction and solid phase extraction was covered in this review. TLC was discussed for qualitative/semiquantitative determination of cannabinoids in plants, and the validated HPTLC method published by Fischedick et al. (discussed below, ref. 42) for accurate and precise quantification. It was noted that identification is based on R_f values compared to authentic standard compounds, aqueous fast blue B solution applied to a developed plate by dipping or spraying is a selective reagent for cannabinoids, and polar silica gel and nonpolar C18 (octyldecylsilyl) reversed phase (RP) bonded silica gel plates (usually Merck RP-18) are used to obtain complementary opposite elution orders for confirmation of target analyte identity. RP-18 plates have been reported for many analyses as the optimum stationary phase for the required separation of samples, e.g., for the detection of THC, cannabidiol (CBD), cannabinol (CBN), and cannabichromene (CBC) in commercially available cannabis oils using acetonitrile mobile phase with basic fast blue B spray reagent.[7] Glass microfiber sheets (ITLC) were used for very sensitive screening 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in urine by development with chloroform-methanol-concentrated ammonium hydroxide (85:15:2) and detection by spraying with fast blue BB solution [0.5% in methanol-water (1:1)] to give a pink spot that can be seen on both sides of the sheet at very low levels.[8] The ITLC product was rather fragile, being a microfiber sheet impregnated with silicates, and is no longer marketed.

Use of fast blue BB rather than fast blue B and spraying with diethylamine were shown to give chromatograms that had brighter spot colors and could be preserved for a longer period of time.[9] Di-o-anisidine tetrazolium chloride has been reported as an alternative spray reagent to fast blue salts for detection of cannabinoids.[10] In another study, diazotized dapsone reagent was described as a spray for detection of cannabinoids as standards and from cannabis chloroform extract on 0.25 mm layers of silica gel G with *n*-hexane-acetone (85:15) or *n*-hexane-diethyl ether (8:2); LOD for CBN was 600 ng for CBN compared to 10 ng for fast blue salt B, and characteristic colors were different to aid in spot identification.[11] Other visualization reagents reported in cannabis analysis include 3-methyl-2benzothiazolinone (MBTH) and ceric ammonium sulfate.[12]

Single ascending mobile phase development is predominantly reported for TLC analysis, but double[13] or triple[14] mobile phase development has been used in certain cases improve sample resolution. 2 D TLC[15] on silica gel G plates developed with heptane-dichloromethane-butan-2-one (83:5:12) and with *n*-hexane-acetone (86:14) after 90° rotation separated 47 different cannabinoid spots from an acidic chloroform extract of the flowering tops of *Cannabis sativa* L.; five were identified as **Δ**9-THC, *trans*-Δ8-THC, CBC, CBN, and CBD using authentic controls based on migration distances in the two directions and characteristic colors when the plate was viewed under 254 nm UV light and then sprayed with 0.1% fast blue B salt in 45% ethanol. The authors favorably compared their method to the use of

amine-treated plates, thermo-micro TLC, multiple development, and layer impregnation [e.g., silica gel G impregnated with dimethylformamide (DMF)-carbon tetrachloride (6:4), and petroleum ether-diethyl ether (4:1) mobile phase].[16] However, silver nitrate impregnated silica gel plates have been successfully applied to cannabinoid separations in several studies cited below.[12]

TLC for the study of biological activity of cannabis

Cell suspension cultures of *Cannabis sativa* L., fam. Moraceae, were examined along with seeds and leaves of intact plants for active cannabinoids and other metabolites. No large amounts of any of the major cannabinoids were found in extracts of cells, but TLC demonstrated spots of other phenolic compounds having significant antimicrobial activity against *Bacillus magaterium*, *Staphylococcus aureus,* and *Escharichia coli.* TLC systems used for analysis of petroleum ether extracts were kieselguhr-carboxymethylcellulose plates impregnated with *N*-methylformamide/cyclohexane mobile phase, silica gel-silver nitrate impregnated plates/benzene, and silica gel G/hexane-diethyl ether (4:1) for petroleum ether extracts, and alumina G/acetone-methanol (1:1) or ethyl acetate-methanol-glacial acetic acid (84:24:3) and silica gel/chloroform-diethylamine (9:1) for basic and neutral fractions. Reagents applied to detect spots included fast blue B, Gibbs, vanillin, Nile blue, and potassium iodoplatinate.[17]

The antimicrobial activity of *Cannabis sativa against* methicillin-resistant *Staphylococcus aureus* MRSA was studied using methanolic extraction of leaves and evaluation of the presence of bioactive compounds using HPTLC fingerprinting. Quercetin, gallic acid, and catechin were identified and quantified on Merck silica gel 60 F (F designates the presence of a fluorescent phosphor in the layer) aluminum plates developed with *n*-butanol-glacial acetic acid-water (80:6:20) mobile phase. A CAMAG Linomat 4 was used for standard and sample solution application, and densitometric determination was carried out after derivatization with anisaldehyde-sulfuric reagent by scanning at 350, 289, and 270 nm, respectively. It was found that leaf extract of *Cannabis sativa* had potential for control of both hospital- and community-based MRSA.[18]

The article by Schurr et al. (ref. 59) in the PTLC section below is another example of published studies of biological activity of cannabis.

Analysis of male and female *cannabis sativa* varieties

Qualitative and quantitative analysis of male and female plants of *Cannabis sativa* were performed by TLC, GC, and MS. Four parts of each sex were compared: flowering tops, small upper leaves surrounding the flowers, large leaves from the lower part of the stem, and lower parts of stems. Cannabinoids were extracted with light petroleum by grinding with a pestle in a mortar, extracts were separated on silica gel G plates impregnated with 20% DMF in acetone by developing with the mobile phase 20% diethyl ether in light petroleum, and spots were detected by spraying with 0.2% fast blue B in 2 M aqueous sodium hydroxide. Male and female cannabis were shown to contain similar amounts of Δ1-THC and CBD in similar ratios.[19]

TLC separation, detection, and identification of cannabis

A report was published on the TLC qualitative determination of cannabis constituents present in plant material and quantitative analysis of urine by GC.[20] R_f values with 11 mobile phases on 0.3 mm silica gel G layers and colors and sensitivity of detection using 14 different reagents were tabulated for Δ^1-THC, Δ^6-THC, CBN, cannabinolic acid (CBNA), and Δ1-THC acetate.

Silica gel plastic sheets impregnated with silver nitrate by dipping in an ethanolic solution and one dimensional (1 D) development with isooctane-pyridine (8:2) in a closed, mobile phase vapor equilibrated tank separated six characteristic spots from a cannabis chloroform macerate, including Δ^1-THC, CBN, and CBD, that were detected with 0.1% fast blue B salt in 70% ethanol. 2 D TLC in an open tank with toluene mobile phase in both directions and exposure of the sheet to ammonia vapors before the second run gave increased resolution showing 19 separated spots from the extract.[21] The following year, the same author published a 1 D method with toluene mobile phase for analysis of cannabis constituents on silver nitrate impregnated sheets that he characterized as simpler, more rapid, and much more sensitive [limits of detection(LODs) 1-5 ng] than other available TLC procedures.[22]

The geographical origin of cannabis plants (hemp) were shown to be related to the occurrence in different parts of the plant of Δ^9-THC, CBD, CBN, and other components. Material from the plants were extracted by stirring with petroleum ether, and the extracts were subjected to 1 D TLC using silica gel plastic sheets developed with toluene or xylene mobile phase or 2 D TLC with a second development using toluene-diethylamine (98:2). Fast blue B salt spray reagent [0.1% in water-methanol (1:3)] gave orange, violet, and scarlet spot colors that were used with R_f values compared to reference standards for compound identification.[23]

The RIM (Rutgers Identification for Marijuana) test combines histochemical and TLC chromatography techniques and eliminates the need for a separate extraction step to obtain a suitable sample for TLC. Clear sample from the microscopic histochemical test was spotted onto a Merck 0.25 mm thick silica gel G layer with a micropipet, followed by development with benzene mobile phase and spraying with fast blue B reagent (0.2 g in 80% ethanol). Confirmation that a sample contains marijuana was appearance of spots of Δ^9-THC, CBD, and CBN with characteristic colors and R_f values relative to reference standards run on the same plate.[24] A total of 526 non-marijuana plant samples representing 427 different plant species were subjected to the RIM test, and although a few samples gave fast blue B reactive spots by TLC they were easily distinguishable

from marijuana cannabinoid spots, and no false positive results were exhibited.[25]

The dried flowering and fruiting tops of *Cannabis sativa* from eight different regions of northern India were analyzed qualitatively for CBN, CBD, and Δ^1-THC by TLC on silica gel G layers impregnated with DMF. Samples were extracted repeatedly with chloroform, and cannabinoid acids were decarboxylated by heating the extract in boiling toluene for 20 min. The mobile phase was diethyl ether-petroleum ether (1:4), and cannabinoids were detected using a 0.5% aqueous solution of fast blue B followed by 0.1 M sodium hydroxide.[26]

Silica gel plates were developed twice up to 15 cm with benzene-*n*-hexane-diethylamine (25:5:0.5) for separation of THC, CBD, and CBN extracted using methanol in a blender from *Cannabis sativa* L. A 0.1% aqueous solution of fast blue B salt provided red, yellow, and violet spots, respectively. Content of the components was obtained by scraping, elution with ethanol, and visible absorption spectrometry.[27]

TLC was used for separation and identification of cannabis constituents from smoke. Cannabis smoke was produced in a specially designed chamber and passed through a series of nonpolar to polar organic solvents and urine, saliva, and water samples.[28] Samples of the organic solvents were applied directly and ether extracts of aqueous samples to Merck neutral and alkaline (impregnated with 0.1 M aqueous sodium hydroxide solution) silica gel G plates that were developed with toluene-diethylamine (20:1) and *n*-hexane-ethyl acetate (9:1) respectively. Spots were detected with 0.2% brentamine (o-Dianisidine) fast B salt in 0.1 M NaOH solution (for developing neutral chromatograms and 0.2% aqueous brentamine fast B salt solution (for developing alkaline chromatograms) and identified by comparing migration to that of standard spots on the same plate.

Seventeen 17 different cannabinoids were successfully separated from chloroform extracts of cannabis plant leaves, flowering tops, and resin on 20 × 20 cm 0.25 mm silica gel G layers using *n*-hexane-acetone (87:13) mobile phase. Four spots were identified as Δ^9-THC, CBC, CBN, and CBD based on detection by viewing under 254 nm ultraviolet (UV) light and spraying with a solution of 0.1% fast blue B salt in 45% ethanol, which gave more compact, characteristically colored spots than an aqueous solution of the reagent.[29]

It was shown that laboratories without GC-MS technology can accurately identify Δ^9-THC, CBD, and CBN by TLC, HPLC-UV, and GC with a flame ionization detector (GC-FID) in cannabis samples. The TLC procedure included extraction by ultrasound mixing with hexane-chloroform (9:1), development of Merck silica gel 60 F plates with hexane-chloroform-dioxane (89:8.75:2.25), and measurement of spectra of separated spots by scanning from 200-300 nm with a CAMAG Scanner 2.[30]

The following TLC methods were described in detail for identification of cannabis products: 1 D normal phase (NP) TLC on silica gel G plates with 0.25 mm layer developed with benzene, benzene-*n*-hexane (6:4),or benzene-*n*-hexane-diethylamine (70:25:5) mobile phase; 2 D TLC on silica gel with *n*-heptane-dichloromethane-butan-2-one (83:5:12) development followed by *n*-hexane-acetone (86:14) after 90° plate rotation; RP TLC on Merck RP-18 HPTLC plates developed with acetonitrile-water (9:1); 45 mg of fast blue B salt dissolved in 20 mL 0.1 M sodium hydroxide or 0.5 g in 10 mL water-acetone (1:9) color spray reagent; and extraction of cannabis oil, resin, and plant samples with toluene.[31]

Separations of cannabigerol monomethyl ether (CBGM), CBD, cannabidivarol (CBDV), cannabidolic acid (CBDA), cannabigerol dimethyl ether (CBGD), cannabicyclol (CBL), **Δ**8-THC, Δ^9-THC, Δ^9-tetrahydrocannabivarol (Δ^9-THCV), cannabigerol (CBG), and CBN on precoated silica gel G plates was reported for the mobile phases methanol-dioxane-hexane (1:2:7), hexane-ethyl acetate (4:1), petroleum ether-diethyl ether (4:1), and hexane-diethyl ether (4:1). Spraying with 0.5% fast blue salt B followed by 0.1 M aqueous sodium hydroxide gave characteristic yellow, red, orange-violet, and violet colors that aided compound identification.[32]

The advantages and disadvantages of HPTLC, automated multiple development (AMD), and overpressured layer chromatography (OPLC; JC Scientific Co. Ltd, Hong Kong) were presented for the separation and identification of cannabis components as standards and in hexane extracts of resin and Indian hemp. Merck silica gel 60 F plates with hexane-diethyl ether (8:2) mobile phase, a Desaga MAT chamber, and fast blue salt B detection reagent were chosen as optimal conditions for HPTLC separation of Δ^8-THC, Δ^9-THC, CBN, and CBD. A 20-step mobile phase gradient with acetone, diisopropyl ether was used with the CAMAG AMD device, and isooctane-diethyl ether (9:1) mobile phase for analytical OPLC and hexane-diethyl ether (8:2) for semipreparative OPLC with collection of components by elution rather than the usual layer scraping and elution of bands.[33]

TLC data were collected for eight neutral and eight acidic major cannabinoids from *Cannabis sativa* plant material as well as two human metabolites of Δ^9-THC using an RP system [Merck C18 bonded silica gel F plates with methanol-5% acetic acid (19:1) mobile phase] and a polar system (Merck silica gel F with chloroform-methanol (19:1)]. Plates were developed in a saturated chamber, and general visualization of compounds was done by spraying with anisaldehyde-sulfuric acid and selective visualization with 0.5% fast blue B salt in water followed by 0.1 M sodium hydroxide. HPLC, GC, UV spectrometry, infrared (IR) spectrometry, and MS data were also presented.[34]

A manual of TLC methods for cannabis and its products for use in national drug analysis laboratories was published by the United Nations (UN).[35] Ultrasonic extraction of herbal cannabis, resin, and oil using petroleum ether was specified for neutral and free cannabinoids and toluene or chloroform for cannabinoid acids. Silica gel plates spotted with extracts and appropriate standards were developed with petroleum ether-diethyl ether (8:2), cyclohexane-di-isopropyl ether-diethylamine (52:40:8), or *n*-hexane-dioxane-methanol (7:2:1) in a tank conditioned with mobile phase vapor for 30 min with paper on one side. Visualization was made by

spraying with fast blue B salt solution (50 mg/20 mL 0.1 M sodium hydroxide or with diethylamine followed by fast blue B salt solution (50 mg/1 mL water + 20 mL methanol). Plates were documented by storing in clear plastic bags or by densitometric scanning or photography.

Active pharma ingredients *Cannabis sativa*, *Embleia ribes, Myristica fragrans,* and *Piper longum* present in the ayurvedic herbal formulation Jatiphaldya were separated and identified by TLC on silica gel G layers with the micellar mobile phase 5% aqueous sodium dodecyl sulfate (SDS)-methanol (7:1) in a vapor saturated CAMAG twin trough chamber. Ethanol-water (4:1) extracts were spotted with a micropipet, and the detection reagents were iodine vapor, 1% vanillin in methanolic sulfuric acid, and anisaldehyde-sulfuric acid.[36]

The analytical monograph Cannabis flos flowers included a TLC identification method.[37] Flowers were ground and extracted by homogenization with ethanol. Merck silica gel 60 F plates were developed with petroleum ether (40–60 °C)-diethyl ether (4:1) in a saturated chamber, and the spray reagent was fast blue B aqueous ethanol solution. Bedrocan, Bedrobinol, Bedica, and Bedropuur varieties gave a red tetracannabidiolic acid (THCA) spot, and Bedolite and Bediol gave an orange spot of CBDA; the R_f values of all of these characteristic spots in sample solutions matched the R_f values of reference extracts chromatographed on the same plate.

HPTLC combined with image analysis was used for similarity assessment of 70 medicinal herbs, including *Cannabis sativa* L. hemp seeds. Silica gel 60 F plates, ethyl acetate-methyl ethyl ketone-98% formic acid-water (5:3:1:1) mobile phase, and Liebermann-Buchard and anisaldehyde detection reagents were employed. Image analysis based on Cannys' method was used to determine the spot sizes of each HPTLC image. A similarity search algorithm was capable of calculating spot size and R_f values. Similarity values were 75–96% for the selected herb chromatograms with those of the same herb in the database. It presented better results than principal components analysis (PCA), classification and regression trees (CART), and partial least squares discriminant analysis (PLS-DA). The extraction method was sonication-maceration with methanol, and a CAMAG TLC Sampler 4 (ATS 4), Automatic Developing Chamber (ADC 2), and TLC Visualizer were employed to carry out HPTLC and record chromatograms.[38]

The HPTLC Association website[39] contains NP and RP separations of cannabis suitable for fingerprinting. Herbal cannabis was sonicated with methanol-hexane (9:1) (NP) or methanol (RP) to prepare test samples chromatographed with reference standards. NP HPTLC was on Merck Si 60 F silica gel, the mobile phase was *n*-heptane-diethyl ether-formic acid (75:25:0.3), and fast blue salt was specified for spray or dip derivatization. RP HPTLC was on Merck Si 60 RP-18 F stationary phase with methanol-water-acetic acid (70:15:15) mobile phase and vanillin-sulfuric acid derivatization reagent. Figures 1 and 2 illustrate the NP and RP chromatograms, respectively.

TLC-densitometry quantification

TLC-densitometry methods were given for analysis of autopsy tissues (liver, kidney, spleen, stomach, and intestine) in a case of fatal poisoning by Δ^9-THC. *n*-Hexane extracts were chromatographed on 0.25 mm thick silica gel G layers by development with *n*-hexane-acetone (87:13) in a saturated chamber, and Δ^9-THC was identified by spraying with 0.1% fast blue salt B in 45% ethanol. Amounts found by scanning spots with a photoelectric densitometer were 3.75, 4.2, 1.2, 0.8, and 0.2 mg/100 g, respectively.[40]

Cannabinoids were separated, isolated, and quantified from methanol-chloroform (9:1) extracts by OPLC on Merck 60 F silica gel sheets with sealed edges. The pump was set to supply the toluene-dioxane (60:1) mobile phase at 0.3–0.7 mL/min resulting in linear velocities between 0.8 and 1.5 cm/min. Samples were applied by a CAMAG Linomat 3, and bands were visualized under UV light and with fast blue B reagent. The neutral cannabinoids were identified and quantified using a Shimadzu CS-920 scanner at 215 nm by comparison to standard compounds. On-line OPLC of *Cannabis satvia* L. extract with UV detection isolated fractions from which cannabinoid identity was confirmed by GC-FID.[41]

In an attempt to improve the sensitivity of quantification, a method for fluorescent dansyl derivatization of cannabinoids prior to separation on silica gel by development with isooctane-ethyl acetate-glacial acetic acid (30:20:1) was devised. The components could be observed after spraying with Triton X100-chloroform-*n*-hexane (1:20:80) and quantified by fluorescence densitometry at 340 nm. The method was applied to plasma samples using reference compounds, and LOD were estimated as 13, 20, and 40 ng/mL for CBD, Δ^9-THC, and CBN, respectively.[12]

An HPTLC-densitometry method was developed and validated according to International Conference on Harmonization (ICH) guidelines for quantification of Δ9-THC with the use of pure cannabinoid reference standards and two medicinal cannabis cultivars. Accuracy was determined by comparing results with those obtained from a validated HPLC method. The method was also useful for qualitative screening of the main neutral cannabinoids found in cannabis cultivars. Merck 20 × 10 cm silica gel 60 aluminum plates, chloroform mobile phase in CAMAG twin trough chamber, and a CAMAG ATS 4 applicator and TLC Scanner 3 controlled by winCATS software version 1.4.3 were employed.[42]

OPLC separation of Δ^9-THC, CBD, CBN, CBG, and CBC was achieved on silica bonded amino (NH_2) F plates using dichloromethane single component mobile phase. Employing bidirectional development (from both ends to the plate to the center), 30 samples were analyzed on a 10 x 20 cm plate within 4 min. Evaluation was performed by densitometry at 200 nm with a Desaga CD slit scanner. Plant samples were ultrasonically extracted with methanol-chloroform (9:1), and extracts and standards were applied with a 1 µL Hamilton syringe as 2 mm bands. OPLC with optimized, consistent mobile phase velocity can provide

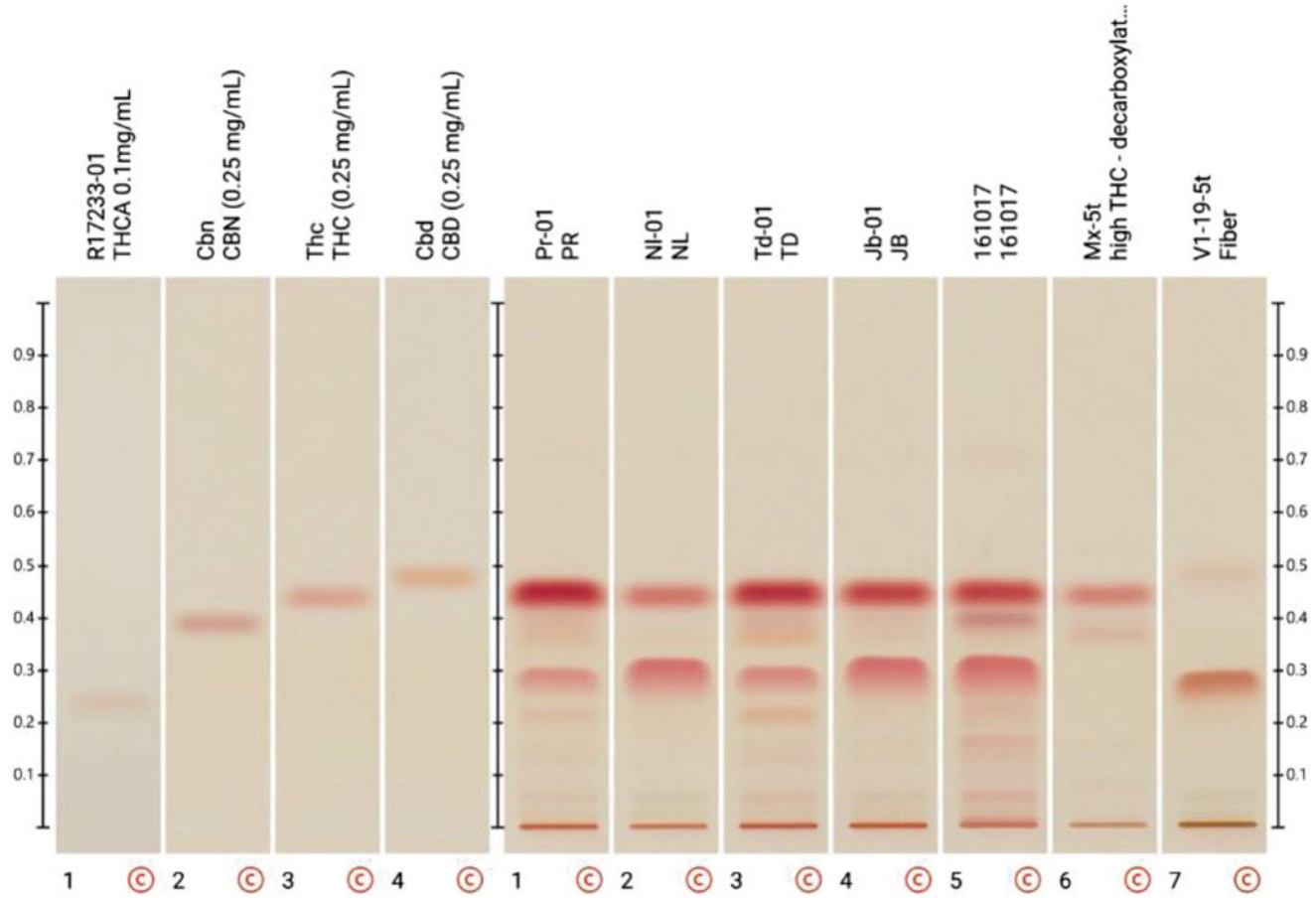


Figure 1. NP fingerprints of cannabis standards (Tracks 1–4), *Cannabis sativa* inflorescences (5–9), inflorescence decarboxylated (10), and fiber (11).

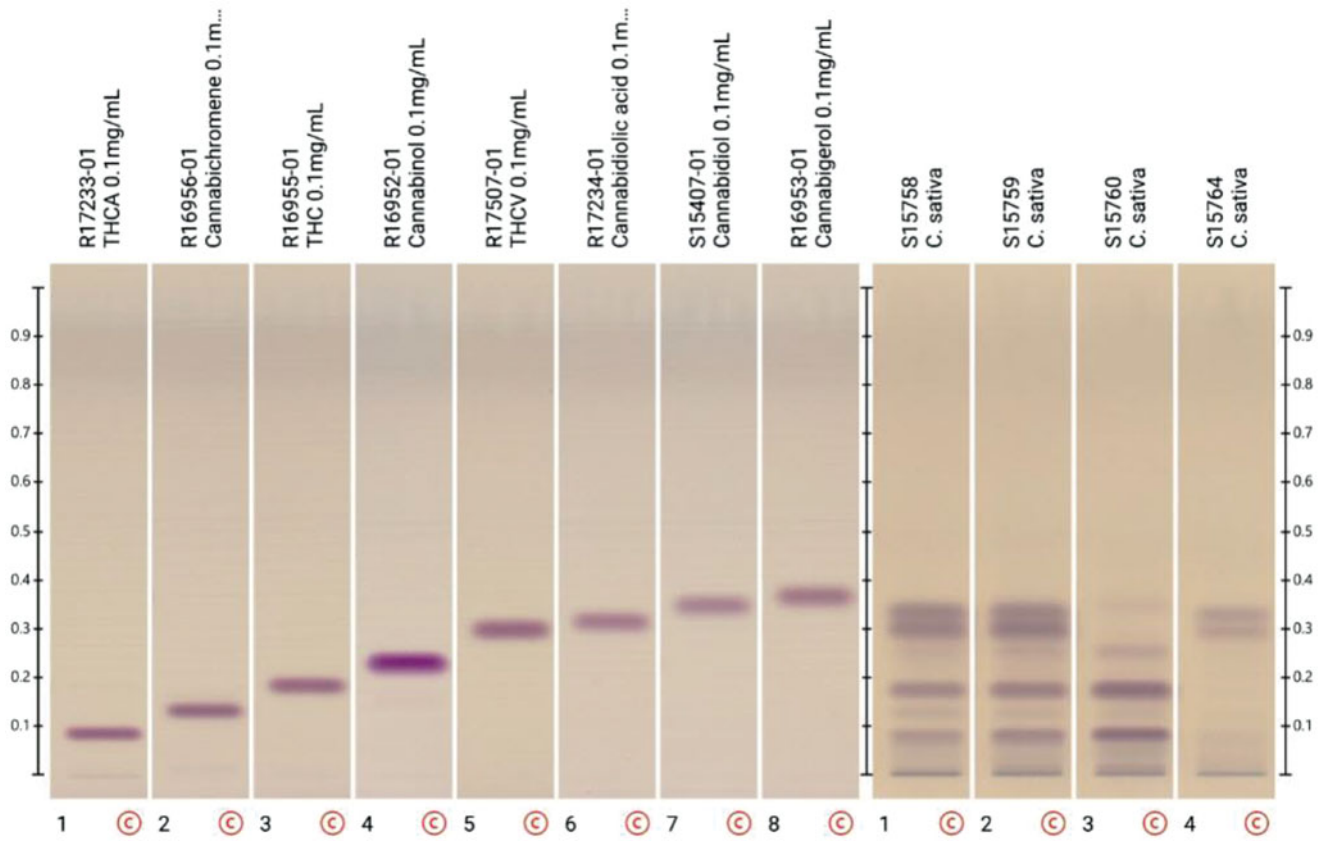


Figure 2. RP fingerprints of cannabis standards (Tracks 1–8) and inflorescences (9–12).

improved selectivity compared to capillary flow ascending plate development in some applications.[43]

THC, CBD, and CBN were quantified in marijuana seized by Chilean police using a HPTLC-densitometry procedure validated according to the UN Office on Drugs and Crime (UNODC).[44] Acetonitrile plant extracts and standard solutions were applied to Merck silica gel 60 F plates with a CAMAG ATS 4 Automatic Sampler, and development with mobile phase *n*-hexane-diethyl ether (8:2) was carried out in a CAMAG ADC 2 Automatic Developing Chamber without saturation. Areas of bands on dried plates were measured by absorption densitometry with a CAMAG TLC Scanner 4 at 206 nm, and the *in situ* spectrum of each peak was recorded in the 190–400 nm range. THC content in 15 extracts ranged from 4.8 to 26% (w/w).

TLC-Bioassay

Bioactivity profiling of hemp, flax, and canola seed cakes was performed by HPTLC combined with a group of bioassays, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH*) scavenging, acetylcholinesterase (AChE) inhibition, planar yeast estrogen (pYES), and antimicrobial *Bacillis subtilis* and *Aliivibrio fischeri* assays. These directed non-targeted analyses allowed discovery of previously unknown bioactive compounds in these oilseed cake extracts, which were characterized by TLC-ESI (electrospray ionization)-MS via the CAMAG elution head based TLC-MS interface. The demonstrated bioactivity of these compounds can guide their isolation for production of functional feed/food supplements. Extraction of polyphenols from defatted seed cake flours was by methanol-acetone-water (7:7:6) in an ultrasonic bath. Extracts were applied in bands to HPTLC silica gel 60 F plates using a CAMAG Automatic TLC Sampler 4 (ATS 4), and plates were developed with toluene-ethyl acetate-formic acid-water (15:30:5:3) in a CAMAG Automatic Development Chamber (ADC 2). The pYES bioassay was performed on Merck RP-18 W F plates (W designates a water tolerant layer) with *n*-hexane-toluene-ethyl acetate (8:3:2) mobile phase. Optional derivatization for detection of separated bands was performed by dipping the plate into 0.005 g/mL natural product reagent in methanol followed by 5% methanolic polyethylene glycol (PEG)-400 solution. Chromatograms were documented in daylight and under 254 and 366 nm UV light with a CAMAG TLC Visualizer or DigiStore Documentation system.[45]

Forensic analysis of cannabis

TLC was employed to attain detailed information on the age and origin of cannabis samples with significance in forensic analysis.[46] The absence of CBD and, if present, its ratio to THC in petroleum ether or chloroform extracts were the most useful criteria in origin assignment, and the apparent CBN content reflected the sample age. TLC on Chromogram silica gel polyester sheets with toluene mobile phase in a non-equilibrated tank allowed reproducible single sample analysis in 10 min and two six sample analyses in under 2 h. 2 D TLC involving development of one sample spotted in a corner of a plate with a second mobile phase providing a complementary separation mechanism [toluene-diethylamine (98:2)] at a right angle was used to obtain extra resolved diagnostic spots for confirmation of sample origin. For even better sample resolution, the unique method "doubled 2 D TLC" comprising application of one sample at one bottom plate corner, development of the plate to a center line of the plate with the first mobile phase, application of a comparison spot at the corresponding opposite bottom corner, turning of the plate through 180° and development of the second spot up to the same central line, then turning the plate back through 90° and development of both spots in their second dimension with the second mobile phase. Mobility of analytes was also changed for better resolution in 1 D and 2 D TLC by impregnation of silica gel plates with silver nitrate and DMF. Chromogram was the brand name given to the Kodak prepared flexible TLC plates with an especially thin layer (100 µm). This TLC plate worked best with sandwich chamber development, which is rarely used today. Kodak no longer manufacturers this plate, but this reference was kept in to demonstrate the TLC techniques described for use with other available TLC plates.

Dental deposits obtained using a toothpick covered with a cotton swab soaked in a few drops of petroleum ether or chloroform were analyzed to prove the consumption of cannabis. The swab was mechanically shaken for 30 min. in the presence of 5–10 mL of benzene, and the extracts were cleaned up on a microcolumn of Florisil eluted with benzene. TLC was on silica gel layers impregnated with DMF using cyclohexane mobile phase, and spots were located and differentiated by spraying with 0.5% fast blue B in water-acetone (1:1).[47]

A study to allow forensic chemists to know if materials obtained from local plants were able to produce false positive results for cannabis when examined by TLC and fast blue salt B visualization was carried out by analysis of petroleum ether extracts of leaves, flowers, and stems prepared from 118 species of plants indigenous to North Dakota USA. The TLC system consisting of silica gel G developed with chloroform-petroleum ether (6:4) produced chromatograms with no red, violet, or orange spots characteristic of cannabis, so these plants did not produce a false positive reaction.[48]

Analysis with no required detection reagent and fewer interferences in chromatograms was reported based on derivatization of cannabinoids prior to TLC. Derivatization was done by oxidative coupling of cannabinoids with 3-methyl-2-benzthiazolinone hydrazine in the presence of acidic ceric ammonium sulfate as the oxidant. Chloroform extracts of the reaction mixture were spotted on Merck silica gel 60 plates that were developed in an unsaturated tank with benzene-methanol (98:2) Characteristic colored patterns were obtained for cannabis and standard cannabinoid derivatives of Δ9-THC, CBN, CBD, and CBG in daylight. Certain spots were also detected as fluorescent spots under 254 and

366 nm UV light. Substances like henna, mace, and nutmeg did not interfere.[49]

Lillsunde and Korte[50] devised a simple and selective comprehensive system using TLC screening and GC-MS for confirmation. It was used in the National Public Health Institute, Department of Biochemistry, in Helsinki, Finland, for screening yearly about 2,000 urine samples in misuse, impaired driving, poisoning, and other forensic cases. About 300 drugs can be simultaneously detected, including 11-nor-Δ^9-carboxylic acid, which was extracted from urine with *n*-hexane-ethyl acetate (7:1) after glucuronide hydrolysis. TLC was carried out on Merck silica gel 60 aluminum plates developed with *n*-hexane—1,4-dioxane-methanol (35:10:5) and detection using 1% fast black K salt in water followed by 1% aqueous sodium hydroxide.

Recording of morphological features plus two thin layer chromatographic systems were used to determine cannabinoids present in forensic cannabis samples.[51] Merck silica gel 60 F plates developed with hexane-ethyl ether (4:1) or hexane-acetone (4:1) and detection with fast blue 2B in methanol-water (1:1) provided separation and identification of Δ^8-THC, Δ^9-THC, CBC, CBD, CBG, and CBN.

TLC and GC-MS MSD (mass selective detector) methods were devised to allow criminalists to confirm the presence of salvinorin A in a submitted plant material suspected to be the psychoactive herb *Salvia divinorum*. Thirteen other *Salvia* species and *Cannabis salvia* L were analyzed to test the methods. Methanol-chloroform (1:1) extracts and standard salvinorin A standard were applied onto Whatman K6 60 F silica gel plates (Miles Scientific equivalent: Catalog No. 43911) and separated by development with ethyl acetate-hexane (1:1) mobile phase. Salvinorins were detected as pinkish-purple spots and Δ^9-THC as a blue spot upon spraying with vanillin reagent.[52]

A TLC method was designed for analysis of herbal cannabis (marijuana), resin cannabis (hashish or charas), and liquid cannabis (hashish oil) for CBN, CBD, THC, and THCA by forensic chemists. Sample extraction was by stirring with petroleum ether, development of extracts and standards was with petroleum ether-diethyl ether (8:2)or cyclohexane-diisopropyl ether-diethylamine (52:40:8) on silica gel plates, and visualization of spots by spraying with fast blue B salt reagent (50 mg in 0.1 M sodium hydroxide solution). Quantification was by GC-FID with an OV-17, SE-30, or OV-1 column.[53]

Optimum mobile phases for detection of THC, CBD, and CBN in *Cannabis sativa* L on Merck silica gel 60 F 0.25 mm layer thickness glass plates were found to be petroleum ether-diethyl ether (8:2), *n*-hexane-toluene-diethylamine (15:5:1), and *n*-hexane-dioxane-methanol (7:2:1). Cannabis plants were extracted by shaking with toluene or petroleum ether, extracts and standards were applied with an autosampler, and bands were detected by spraying with fast blue B salt in 0.1 M sodium hydroxide. GC separation of the three compounds was also studied.[54]

The products of the colorimetric test reaction between fast blue BB salt and Δ^9-THC were identified by ESI(+)-FT-ICR-MS (positive mode electrospray ionization Fourier transform ion cyclotron resonance MS), CID-ESI(+)-MS/MS (collision induced dissociation tandem MS), and UV/visible (Vis)spectrometry. Silica gel TLC plates developed with cyclohexane-toluene-diethylamine (75:15:10) mobile phase and detection with fast blue BB salt revealed Δ^9-THC, CBN, and CBD as the three main cannabinoids in the forensic marijuana samples analyzed. A mechanism was proposed to justify the specificity of the colorimetric test for cannabinoid identification.[55]

An inexpensive, reliable, and fast cyclic voltammetry method was described to confirm TLC identification of illicit marijuana. Hexane ultrasonic extracts of marijuana samples and other fresh and dry leaves were applied to Sigma-Aldrich flexible backed silica gel F plates; development with hexane-acetone (9:1) separated Δ^9-THC, CBD, CBN, and other cannabinoids and components present in the plant matter; and fast blue B reagent detected the spots that were identified by comparison of R_f values to reference standard spots on the same plate. Δ^9-THC spots were scraped from plates and eluted with DMF for investigation by voltammetric analysis.[56]

Other papers cited in earlier and later sections of this review describe TLC analyses that were applied to samples that potentially can be of forensic interest.

Preparative thin layer chromatography (PTLC)

Cannabivarichromene, a new cannabinoid with a propyl side chain in Asian hashish extract, was separated from other cannabinoids by PTLC prior to determination of structure by GC-MS. Extracts were applied to Merck silica gel G F plates with 0.25 mm layer thickness that were developed with benzene in a chamber that had 10 mL of 25% ammonium hydroxide in a trough at the bottom. Alternatively, cyclohexane was the mobile phase for DMF impregnated plates. Visualization was under 254 nm UV light or by spraying with fast blue salt B solution. Chromatograms showed two unusual, intense cannabinoid spots besides the major components Δ9-THC, CBD, and CBN.[57]

A quantitative analysis procedure for CBDA, cannabichromenic acid (CBCA), THCA, and cannabigerolic acid monomethyl ether (CBGAM) was established using PLC and GC for a study of leaf age, season and sex of *Cannabis sativa* L. plant material. Benzene extracts were applied to Merck 20 × 20 cm silica gel PLC plates that were developed with benzene mobile phase. Bands were scraped, and the silica gel powder was eluted with methanol to recover the separated compounds prior to their GC analysis.[58]

A study using PTLC suggesting the presence of cannabinoids within cannabis extract capable of inhibiting mitochondrial monoamine oxidase of human brain and liver, especially the deamination of 2-phenylethylamine and benzylamine, emphasized the potential of cannabis as a source of therapeutically active agents. Silica gel G F PTLC plates were used with toluene-chloroform-methanol (100:10:1) mobile phase, and detection of separated fractions to be scraped was made by viewing under 254 nm UV light. Chloroform-toluene (10:1) eluents of the scraped fractions

were developed on silica gel G F analytical plates with the same mobile phase, and the presence of cannabinoids was confirmed as purple spots produced by spraying with black K salt ethanolic 1 mg/mL solution.[59]

The section below on designer drug analysis contains additional applications of PTLC.

Tlc-ms

TLC-MS using an Advion Plate Express extraction device and Compact mass spectrometer was shown to be applicable for cannabis detection in contraband material, detection and quantification of pesticide contaminants, and characterization of major cannabis components to provide adequately labeled products that informed consumers can choose from where it is legal to do so. Sample extracts were separated on Merck silica gel 60 F plates with petroleum ether (60–80 °C)-dioxane (8:2) mobile phase. The extraction device was operated with a 200 µL/min flow of methanol-formic acid (99:1) and an extraction head area of 1 × 2 mm.[60]

Analysis of urine

Marijuana use was determined by detecting Δ^9-tetrahydrocannabinol-11-oic acid in urine by employing double development TLC for identification based on its characteristic R_f value and color after spraying the plate with fast blue salt B. Sample preparation comprised enzymatic hydrolysis, extraction with anhydrous diethyl ether, and purification by treatment with $NaHCO_3$, followed by TLC in an alkaline and acidic mobile phase sequence. Analtech silica gel G plates were developed in turn with acetone-chloroform-triethylamine (80:20:1) and petroleum ether-diethyl ether-glacial acetic acid (50:50:1.5).[61]

A qualitative method developed for identification of the urinary metabolite 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid involved extraction onto a selective solid phase extraction material (Bond Elute-THC) and TLC. Urine samples were hydrolyzed with sodium hydroxide, extracted by SPE with elution using acetone, and treatment of the extract with dichloromethane and hexane to remove water prior to application with a capillary tube onto Merck silica gel 60 F plates. The mobile phase was ethyl acetate-methanol-water-concentrated ammonium hydroxide (12:5:0.5:1), and spots were visualized following spraying with 0.5% fast blue BB in methanol-water.[62] This SPE-TLC method [also called BPA (bonded phase adsorption)-TLC], together with RIA (radioimmunoassay) and GC-MS, was used to confirm the positive cannabinoid results of 100 samples analyzed by EMIT (enzyme multiplied immunoassay technique).[63]

Use of TLC for screening of abused drugs in urine was reviewed, including cannabinoids such as Δ^9-THC. Advantages cited were low equipment cost, rapid analysis, and simultaneous determination of several drugs and metabolites. Preliminary identification by TLC was confirmed using more specific analysis by GC-MS.[64]

Anys Technologies developed the Toxi-Gram THC II-Plus system for urine analysis in which a sample is applied to a disc type SPE column located in a hole in a TLC plate that is developed in *n*-heptane-acetone-glacial acetic acid (50:50:1), Visualization is achieved by dipping the plate into fast blue BB salt solution (0.1% in dichloromethane) and then exposing the plate to ethylamine vapor. Using this method, the THC metabolite 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid was identified in urine.[12]

Duquenois-Levine, fast blue B salt, and *p*-dimethylaminobenzaldehyde standard color tests were applied to the urine of 102 male patients suspected of cannabis abuse, and the results were confirmed by HPTLC. Extraction of urine was done with ethyl acetate-isopropanol (85:15) after alkaline hydrolysis at 60 °C and adjustment to pH 4 for 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid and with alkaline pH maintained for CBD and CBN. A CAMAG Linomat band applicator applied standard and sample solutions onto Merck silica gel HPTLC plates, and a CAMAG Automated Development Chamber (ADC) was used with ethyl acetate-methanol-concentrated ammonium hydroxide (85:10:5) or petroleum ether-diethyl ether (9:1) mobile phase. All tracks on the plate were scanned at 278 and 350 nm with a CAMAG TLC Scanner to obtain R_f values that were compared against the on-plate standard compounds and the built-in drug library in the scanner software to identify cannabinoid bands. Cannabis abuse was detected in 64 of the patients. The HPTLC method was shown to be high throughput, sensitive, reproducible, and cost effective compared to available commercial kits, and it could easily be adapted for quantitative analysis of the three target cannabinoids.[65]

Analysis of hair

Hair samples of marijuana users were qualitatively analyzed by TLC and quantitatively by GC-MS. Samples were extracted three times for 2 min each by sonication with methanol-2-propanol (1:1), and the combined extracts were spotted onto silica gel G F layers with a micropipet. Separation was made by development with *n*-hexane-toluene (1:1), and spots of Δ^9-THC, CBD, and CBN were visualized using fast blue B salt reagent and identified by comparison to co-spotted standards. Amounts of the cannabinoids in hair ranged from 0.25-2.82 ng/mg according to the validated GC-MS method.[66]

TLC in the analysis of synthetic cannabinoids

Designer drugs, which are also termed new psychoactive substances (NPSs), include compounds that are produced to mimic the effects of cannabis. This section covers the application of TLC in their analysis.

The cannabimimetic aminoalkyl napthoyl indole derivative JWH-018 was identified as an adulterant in an herbal product being sold in Japan for its narcotic effect after isolation by PTLC on Merck silica gel 60 preparative plates with 2 mm layer thickness. Methanol extraction of the herbal product was carried out by ultrasonication, and the mobile phase was hexane-acetone (4:1). The detected zone was

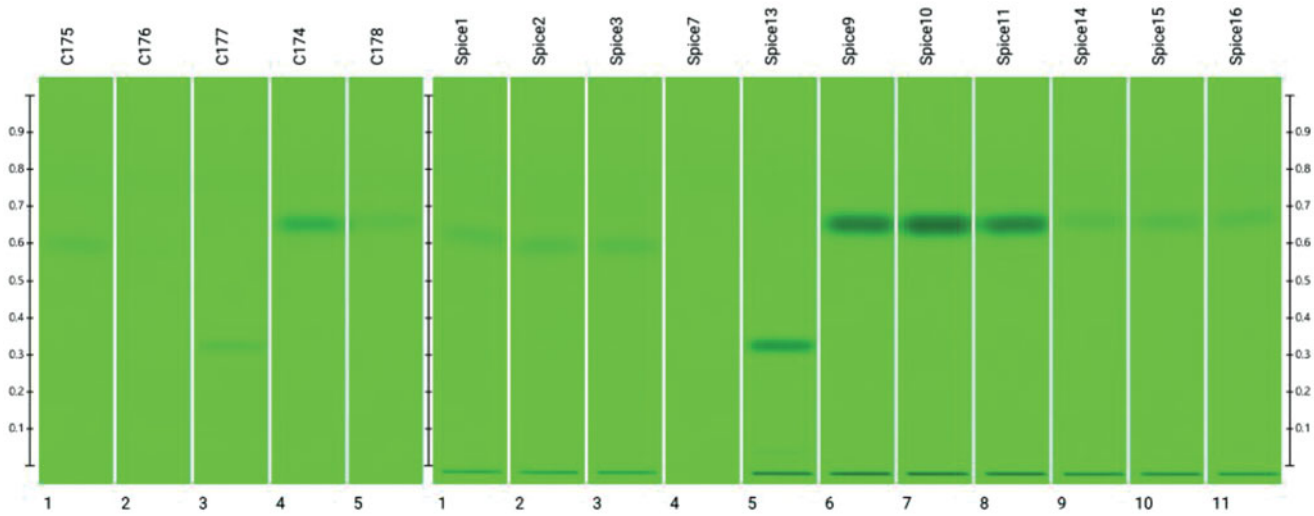


Figure 3. Spice sample chromatograms viewed under 254 nm UV light.

scraped from the plate and eluted with chloroform-methanol (2:1) to give fraction 1. Repeated PTLC of this fraction with hexane-chloroform (1:20) isolated JWH-018 as an off-white solid that was identified by GC-MS, ultra-performance column liquid chromatography (UPLC)-ESI-MS, direct analysis in real time (DART)-time of flight (TOF)-MS, and nuclear magnetic resonance (NMR) spectrometry.[67]

Merck silica gel PLC plates with 2 mm layer thickness were also used in the identification of the phenylacetyl indole JWH-251 and the naphthoylindole JWH-081 as designer drugs in illegal herbal products with the use of GC and UPLC coupled with MS and NMR spectrometry. The mobile phase was hexane-ethyl acetate (4:1) for separation of chloroform ultrasonic extract, and the portion of silica gel layer containing the target compound was scraped and eluted with chloroform-methanol (3:1) to obtain fractions 1 and 2. Each fraction was further purified by recycling preparative HPLC to give the two compounds for analysis.[68]

The separation and identification of synthetic cannabinoids in herbal incense blends available in the U.S. (called "K2") was accomplished using methanol and combined acid/base extraction of dried and crushed plant material (flowers, stems, and leaves); Whatman silica gel 60 LK6DF laned preadsorbent plates with 0.25 mm layer thickness; and toluene-diethylamine (9:1) and ethyl acetate-dichloromethane-methanol-concentrated ammonium hydroxide mobile phases. All 21 standard compounds studied exhibited UV absorption at 254 nm (fluorescence quenching), and some fluoresced white or yellow at 366 nm. In addition, the detection reagents fast blue B, fluorescamine, ninhydrin, 10% sulfuric acid, iodoplatinate, 50% nitric acid, mercuric sulfate, and 4-diethylaminobenzaldehyde were used to compare standards to zones in sample extract chromatograms. HPLC, HPLC-MS, and GC-MS applied to analysis of various commercially available products identified the synthetic cannabinoids JWH-018, JWH-019, JWH-073, JWH-081, JWH-200, JWH-210, JWH-250, CP47,487 (C = 8; cannabicyclohexanol), RCS-4, RCS-8, AM-2201, and AM-694, along with other noncannabinoic drugs including mitragynine (Kraton). Whatman plates are no longer available commercially, but plates equivalent to LKD6F are sold by Miles Scientific as Catalog No. 44911.[69]

Spice is an herbal mixture with added synthetic cannabinoids that is smoked for euphoria. Three cases of "Space" brand Spice use in the military were described, with TLC used for urine analysis in two of them giving negative results. Details of the TLC methodology were not given, but its value for forensic analysis was made clear.[70] Figures 3 and 4 show chromatograms of spice on HPTLC silica gel 60 F plates developed with toluene-acetone-diethylamine (85:10:3) mobile phase in a saturated (with filter paper, 33% humidity) CAMAG ADC 2 Automatic Developing Chamber and viewed under 254 and 366 nm UV light.

A research microcrystalline chemical advertised as the cannabimimetic compound [(*N*-methylpiperidin-2-yl)methyl]-3-(1-naphthoyl)indole (AM 1220), purchased via an internet trading platform, assayed as pure AM 1220 by GC-MS. However, when it was tested for purity by TLC, two zones were obtained, and after isolation by PTLC and high resolution MS and NMR spectrometry analysis, the azepane isomer of AM 1220 was confirmed to be present. Later, both substances were detected in several herbal mixtures purchased from different German internet shops. The crystalline drug substance was dissolved in ethanol, and the herbal mixtures were extracted by vortexing with ethanol. Purity testing of extracts was carried out on Merck silica gel 60 F aluminum plates developed with cyclohexane-diethylamine (9:1). Zones were detected after dyeing with iodoplatinate reagent. Isolation was done on Merck silica gel 60 F glass plates with the same mobile phase followed by scraping of zones detected on side lanes and elution with ethanol.[71]

The same PTLC layer, mobile phase, and detection method were used in the structural characterization of the synthetic cannabinoid 3-(1-adamantoyl)-1-pentylindole found in several herbal incense products purchased from a Dutch internet shop.[72] Samples were vortex extracted with ethanol, and the major band after PTLC of the extract was scraped and extracted for analysis by NMR spectrometry, high resolution MS, and GC-MS/MS. It was found that the

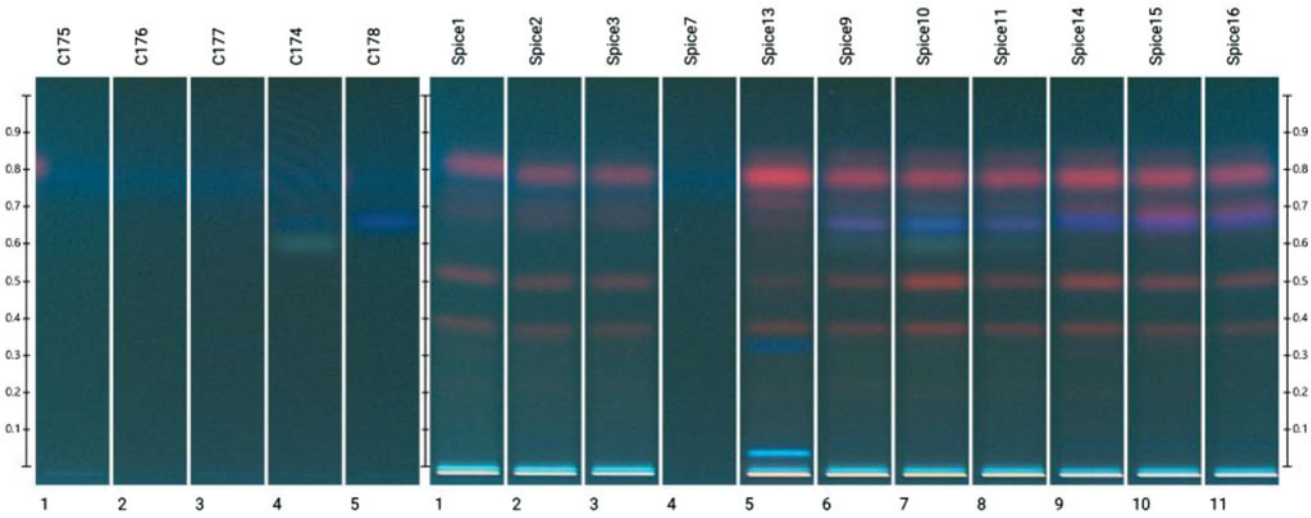


Figure 4. Spice sample chromatograms viewed under 366 nm UV light.

compound was mis-identified by GC-MS before applying techniques that provided more structural information.

A study was carried out to isolate and identify any synthetic cannabinoid disguised in claimed-to-be herbal scent bags known as "Funky Green Stuff", which are commercially available in Kuwait. Potential synthetic cannabinoids were extracted via percolating the content of the bag in methanol followed by filtration. The extract was chromatographed on Miles Scientific (formerly Analtech) 5 × 10 cm silica gel F plates with 0.25 mm layer thickness using the mobile phase toluene-methanol-diethylamine (80:15:5). The main separated compound was detected as an orange zone after spraying with Dragendorff reagent. Flash silica gel column chromatographic separation isolated this compound, and UV, IR, and NMR spectrometry identified it as AB-FUBINACA.[73]

Chromatograms of some synthetic cannabinoids are shown in Figures 5 and 6 on HPTLC silica gel 60 F plates developed with *n*-hexane-dioxane-methanol (7:2:1) (as above UN Solvent C[35]) viewed under 254 and 366 nm UV light

Summary and future prospects

AOAC International has launched the Cannabis Analytical Standards Program (CASP),[74] a program dedicated to developing standardized method performance requirements (SMPRs) for cannabis testing. So far, four SMPRs have been completed: cannabinoids in plant material, plant extracts, and chocolate (edibles), and pesticides in cannabis plant material. The Program will fully support the cannabis analytical community, including reference materials, proficiency testing, education, training, and ISO (International Organization for Standardization) 17025 accreditation as applies to the analysis of cannabis and its constituents, as well as hemp. The United States government still considers cannabis a Schedule 1 controlled substance at the federal level, but more than 30 individual states have been on their own in crafting regulatory cannabis analysis systems for medical programs or a recreational market. CASP is aimed at harmonizing state cannabis analytical standards and methods and developing globally-accepted consensus-based standards and testing methods that are established and as uniform as possible for use if the federal government moves to legalize cannabis. It is expected that TLC methods will be developed in the future to meet the AOAC SMPRs needed for use in the cannabis and hemp industries not only in the United States but worldwide, as well as the consensus methods being developed by organizations such as AOCS (American Oil Chemist's Society), USP (United States Pharmacopeial Convention), and ASTM International working with scientists and stakeholder communities.[75]

Sampling and sample processing procedures that ensure homogeneity, accuracy, and reproducibility will be required for these developed analytical methods. The mechanisms for sample processing were examined and compared for the optimum preparation techniques for targeted analytes in cannabis analysis, and cryogenic grinding was found to be one of the best methods for the preservation of volatile compounds. Further studies will be required to meet SMRP requirements of different cannabis determinative methods.[76]

TLC will continue to be used in conjunction with column chromatographic and spectrometric methods to identify new cannabinoids found in extracts of cannabis[e.g.,77] and to determine cannabis components or metabolites in urine as confirmation of legal or illegal usage.[e.g.,78,79] 11-nor-Δ^9-THC-9-carboxylic acid is the major urinary target metabolite analyzed by TLC to show cannabis usage, most often after an alkaline hydrolysis at 60 °C prior to acidification and extraction with solvents such as hexane-ethyl acetate or by SPE.[80]

Terpenes (terpenoids) present in cannabis affect the fragrance and flavor attributes of a product, which may be important in consumer preference. Other attributes of terpenes in cannabis products, including medicinal properties, have not yet been assigned.[81] TLC has been widely used to analyze terpenes in many types of plants used in traditional natural medicines, e.g., phytochemical secondary metabolites with potential to treat Alzheimer' disease.[82] However, only one TLC study of the analysis of terpenes in cannabis has been published, i.e., chloroform extraction, separation on silica gel 60 F plates with cyclohexane-acetone (1:1) mobile

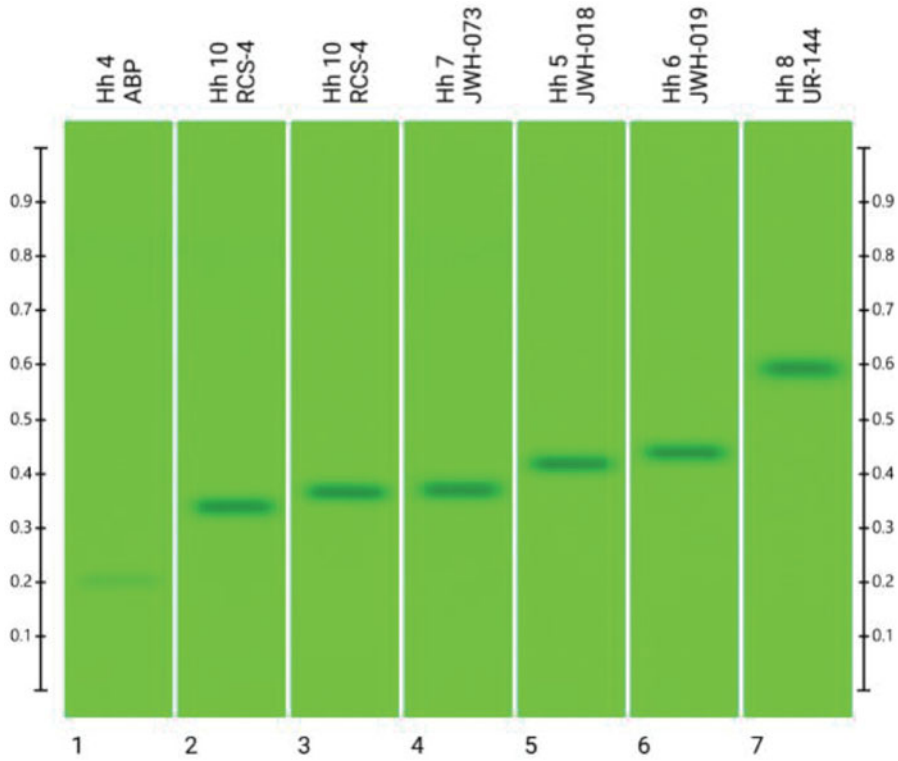


Figure 5. Synthetic cannabinoid chromatograms viewed under 254 nm UV light.

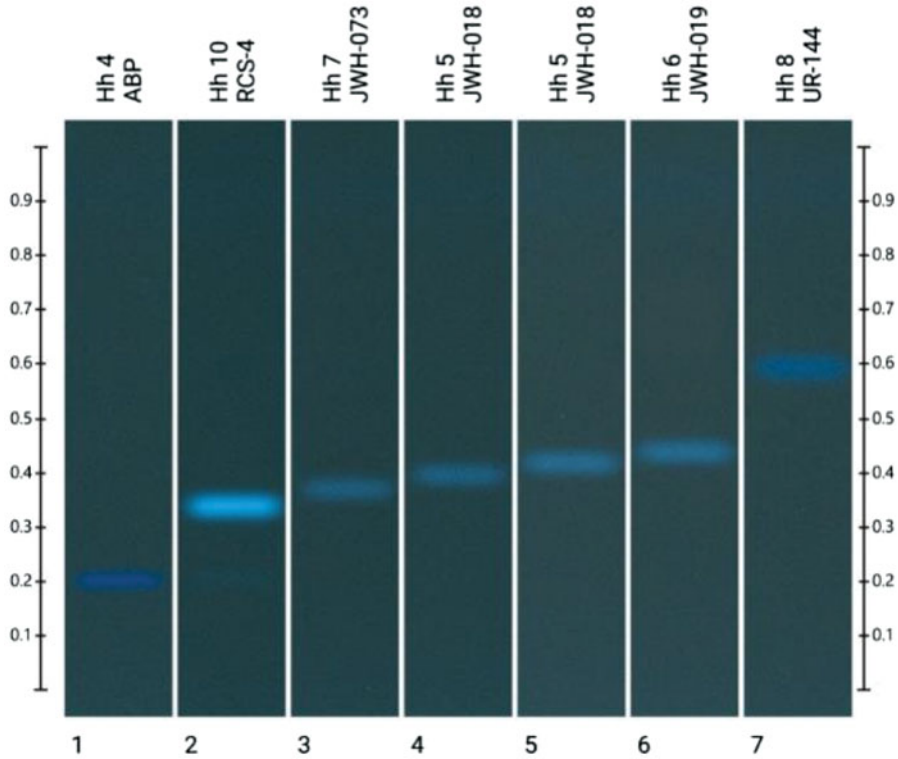


Figure 6. Synthetic cannabinoid chromatograms viewed under 366 nm UV light.

phase, and detection with vanillin reagent providing specific colors for different compounds.[83] Many more papers are expected to be published in the near future on cannabis terpene TLC analytical methods comprising separation, determination of biological properties by coupled effect EDA (TLC-direct bioautography), and identification of structures by online spectrometric methods such as TLC-MS mostly using commercial elution head interfaces as exploration of their contributions to flavor, scent, health, and nutrition values of cannabis plants and products accelerates.[84] Figures 7 and 8 show chromatograms of terpenes and terpenoids in fresh *Cannabis sativa* blossoms extracted by steam

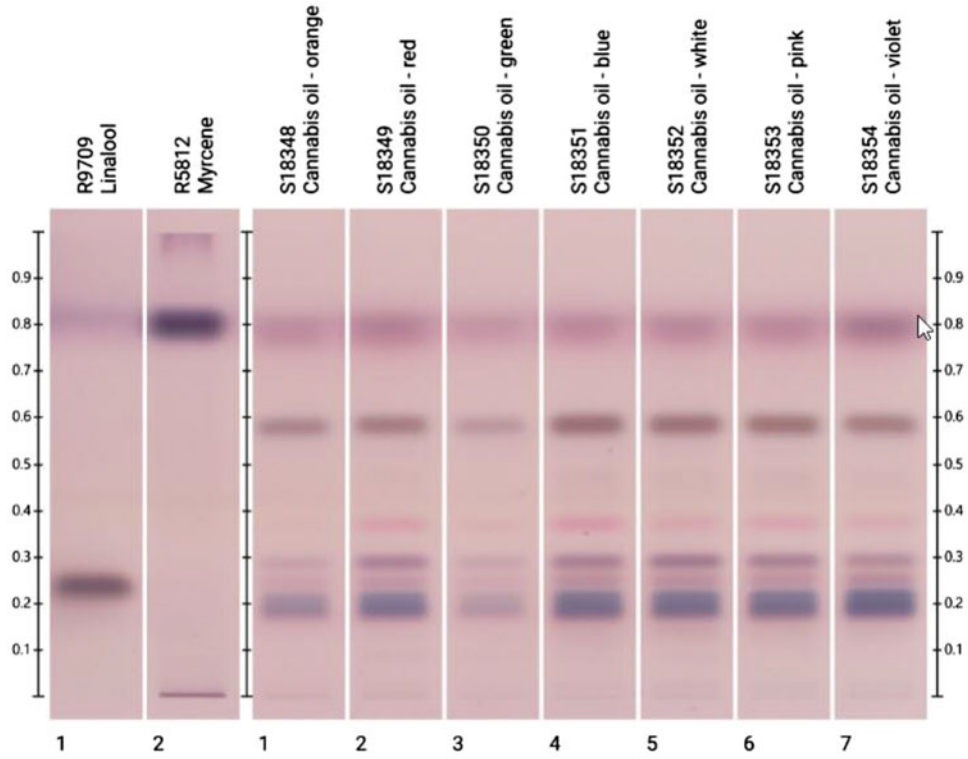


Figure 7. HPTLC chromatograms of terpenes and terpenoids viewed under white light after derivatization with anisaldehyde reagent. Left to right: track 1 linalool, track 2 mycene, tracks 3-9 *Cannabis sativa* blossom samples grown with different conditions.

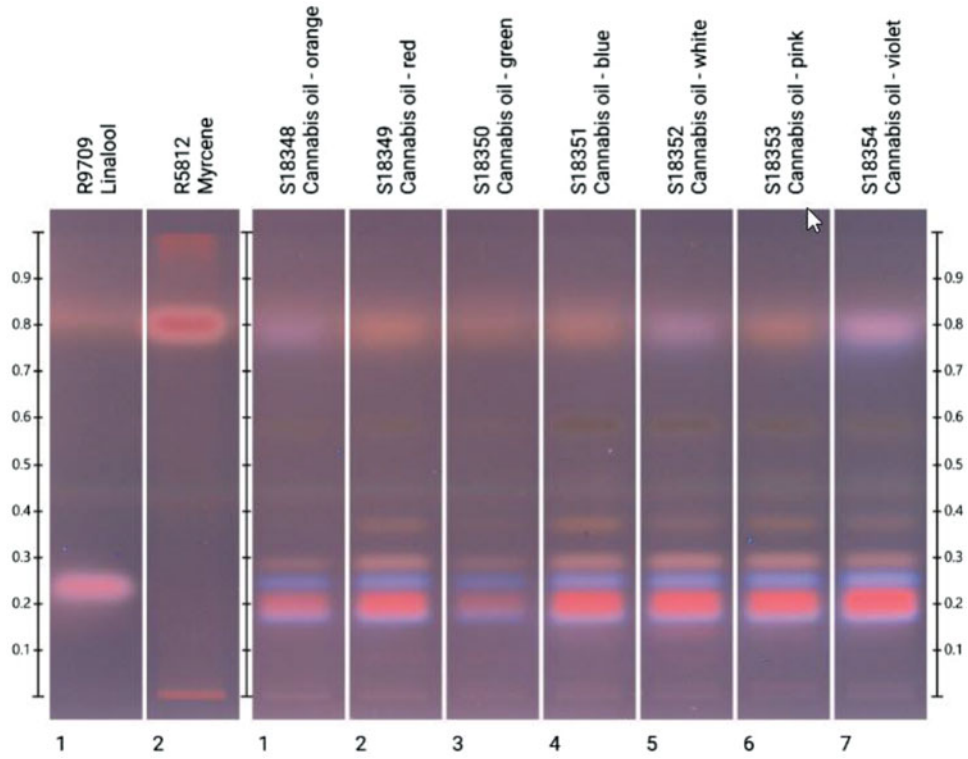


Figure 8. The plate shown in Figure 7 viewed under 366 nm UV light.

distillation, separated on silica gel 60 F plates using toluene-ethyl acetate (95:5) mobile phase with chamber saturation and 33% relative humidity, derivatized with anisaldehyde, and viewed under white light and 366 nm UV light, respectively.

Cannabis flavonoids (flavones, flavonols, flavanones, flavan-3-ols, isoflavones, and anthocyanidins) also affect flavors and aromas, but no TLC analyses of them have been published. Like terpenes, much analytical activity for their separation, identity, and biological activity is expected.

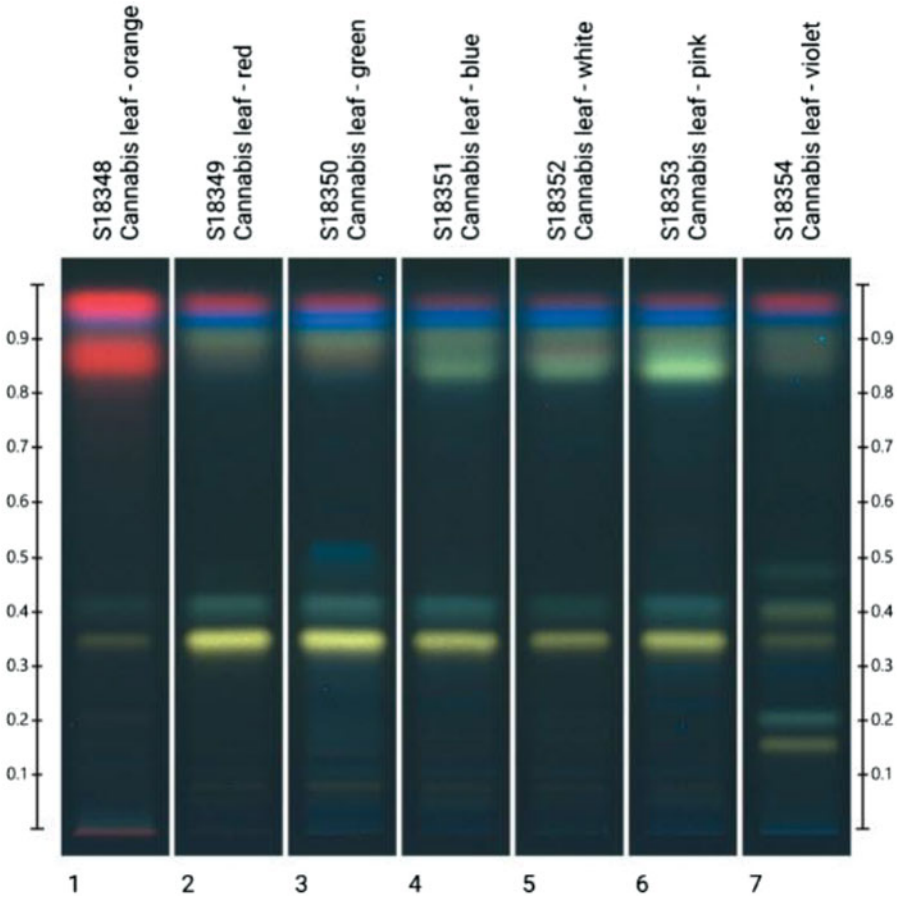


Figure 9. HPTLC chromatograms of flavonoids viewed under 366 nm UV light. Tracks 1-7, *Cannabis satvia* leaves grown under different conditions.

Chromatograms of flavonoids extracted with methanol from dried *Cannabis sativa* leaves, separated on silica gel 60 F plates by development with ethyl acetate-formic acid-water (80:10:10) with chamber saturation and 33% relative humidity, and detected with Natural Product reagent (2-aminoethyl diphenyl borate; NP) followed by a PEG overspray (as discussed above, where now the PEG is sprayed on, an alternative to dipping the plate; such subsequent treatment is used to either stabilize the visualization reaction or make it more intense) are illustrated in Figure 9.

Acknowledgements

The authors thank Karen F. Haduck, Lafayette College Interlibrary Loan Department, for supplying copies of many of the papers cited in this review, without which it could not have been written. Dr. Melanie Broszat, Scientific Business Development Manager, CAMAG, Muttenz, Switzerland, provided in August, 2019, the unpublished photographs of HPTLC plates shown in Figures 1–9 with permission to use in this review.

References

[1] Klumpers, L. E.; Thacker, D. L. A Brief Background on Cannabis: From Plant to Medical Indications. *J AOAC Int.* **2019**, *102*, 412–420. DOI: 10.5740/jaoacint.18-0208.

[2] Hemp vs Marijuana: the Difference Explained (2019 Update) https://cbdorigin.com/hemp-vs-marijuana/ (accessed on May 25, **2019**).

[3] Role of Chromatography in Cannabis Testing, https://bioinfoinc.com/chromatography-cannabis-testing (accessed on July 5, **2019**).

[4] Garcia Fernandez, J. C. Role Played by Narcotics Laboratories in the Campaign against Drug Abuse and Drug Trafficking: A View from a Developing Country. *Bull. Narc.* **1984**, *36*, 3–13.

[5] Badyal, P. N.; Sharma, C.; Kaur, N.; Shankar, R.; Pandey, A.; Rawal, R. K. Analytical Techniques in Simultaneous Estimation: An Overview. *Austin J. Anal. Pharm. Chem.* **2015**, *2*, 1037/1–1037/14.

[6] Citti, C.; Braghiroli, D.; Vandelli, M. A.; Cannazza, G. Pharmaceutical and Biomedical Analysis of Cannabinoids: A Critical Review. *J. Pharm. Biomed. Anal.* **2018**, *147*, 565–579. DOI: 10.1016/j.jpba.2017.06.003.

[7] Yotoriyama, M.; Ishiharajima, E.; Kato, Y.; Nagato, A.; Sekita, S.; Watanabe, K.; Yamamoto, I. Identification and Determination of Cannabinoids in Both Commercially Available and Cannabis Oils Stored Long Term. *J. Health Sci.* **2005**, *51*, 483–487. DOI: 10.1248/jhs.51.483.

[8] Kaistha, K. K.; Tadrus, R. Semi-Quantitative thin-layer mass-screening detection of 11-nor-delta 9-tetrahydrocannabinol-9-carboxylic acid in human urine. *J. Chromatogr.* **1982**, *237*, 528–533. DOI: 10.1016/s0021-9673(00)97646-5.

[9] De Faubert Maunder, M. J. Preservation of cannabis thin-layer chromatograms. *J. Chromatogr.* **1974**, *100*, 196–199. DOI: 10.1016/s0021-9673(00)86057-4.

[10] Neumeyer, J. L.; Schultes, R. E.; Musto, J. D.; Woodland, L. Evaluation of Chemical Tests on Vouchered Specimens of Cannabis. *J. Psychoactive Drugs.* **1988**, *20*, 459–462.

[11] Mali, B. D.; Parulekar, P. P. Diazotized Dapsone as a Reagent for the Detection of Cannabinoids on Thin Layer Chromatography Plates. *J. Chromatogr.* **1988**, *457*, 383–386. DOI: 10.1016/S0021-9673(01)82088-4.

[12] Raharjo, T. J.; Verpoorte, R. Methods for the Analysis of Cannabinoids in Biological Materials: A Review. *Phytochem. Anal.* **2004**, *15*, 79–94. DOI: 10.1002/pca.753.

[13] Hemphill, J. K.; Turner, J. C.; Mahlberg, P. G. Studies on Growth and Cannabinoid Composition of Callus Derived from Different Strains of *Cannabis Sativa*. *Lloydia.* **1978**, *41*, 453–462.

[14] Forrest, J. E.; Heacock, R. A. A Chromatographic Comparison of the Constituents of Nutmeg and Mace (*Myristica Frarans* Houtt.) with Those of Marihuana and Hashish (*Cannabis Sativa* L). *J. Chromatogr.* **1974**, *89*, 113–117. DOI: 10.1016/S0021-9673(01)84170-4.

[15] Tewari, S. N.; Sharma, J. D. Two-Dimensional Thin Layer Chromatography of Ganja (*Cannabis Sativa* L.). *UNODC-Bull. Narc* **1983**, *35*, 63–67.

[16] Turner, C. E.; Hadley, K. W. Chemical Analysis of *Cannabis Sativa* of Distinct Origin. *Arch. Invest. Med. (Mex)* **1974**, *5*, 141–150.

[17] Veliky, I. A.; Genest, K. Growth and Metabolites of *Cannabis Sativa* Cell Suspension Cultures. *Lloydia* **1972**, *35*, 450–456.

[18] Chakraborty, S.; Afaq, N.; Singh, N.; Majumdar, S. Antimicrobial Activity of *Cannabis Sativa*, *Thuja Orientalis*, and *Psidium Guajava* Leaf Extracts against Methicillin-Resistant *Staphylococcus aureus*. *J. Integr. Med.-JIM* **2018**, *16*, 350–357. DOI: 10.1016/j.joim.2018.07.005.

[19] Ohlsson, A.; Abou-Chaar, C. I.; Agurell, S.; Nilsson, I. M.; Olofsson, K. Cannabinoid Constituents of Male and Female *Cannabis Sativa*. *UNODC-Bull. Narc* **1971**, *23*, 29–32.

[20] Parker, K. D.; Wright, J. A.; Halpern, A. F.; Hine, C. H. Preliminary Report on the Separation and Quantitative Determination of Cannabis Constituents Present in Plant Material, and When Added to Urine, by Thin Layer and Gas Chromatography. *UNODC-Bull. Narc.* **1968**, Issue 4, 10–14.

[21] Grlic, L. Some New Possibilities of separation of Cannabinoids by Means of Thin Layer Chromatography on Silica Gel Plastic Sheets. *Acta Pharm. Jug.* **1970**, *20*, 35–39.

[22] Grlic, L. A Highly Sensitive Chromatographic detection of cannabis Constituents by Means of Silica Gel Sheets Treated with Silver Nitrate. *J. Eur. Toxicol.* **1971**, *23*, 43–45.

[23] de Faubert Maunder, M. J. A Comparative Evaluation of the Δ9-Tetrahydrocannabinol Content of Cannabis Plants. *J. Assoc. Public Anal. (JAPA)* **1970**, *8*, 42–47.

[24] Segelman, A. B. *Cannabis Sativa* L. (Marijuana) III. The RIM Test: A Reliable and Useful Procedure for the Detection and Identification of Marijuana Utilizing Combined Microscopy and Thin Layer Chromatography. *J. Chromatogr.* **1973**, *82*, 151–157.

[25] Segelman, A. B.; Segelman, F. P. *Cannabis Sativa* L. (Marijuana) VII. The Relative Specificity of the RIM Test. *J. Chromatogr.* **1976**, *123*, 79–100. DOI: 10.1016/s0021-9673(00)81105-x.

[26] Krishnamurty, H. G.; Kaushal, R. Analysis of Indian Marihuana. *Indian J. Pharm.* **1974**, *36*, 152–154.

[27] Poddar, M. K.; Ghosh, J. J.; Datta, J. Micromethod for the Estimation of Cannabis Components. *Sci. Cult.* **1975**, *41*, 492–495.

[28] Dahiya, M. S.; Jain, G. C. Analysis and Identification of *Cannabis Sativa* L. Constituents from Smoke by Thin Layer Chromatography. *Indian J. Criminol.* **1977**, *5*, 96–101.

[29] Tewari, S. N.; Sharma, J. D. Separation and Identification of Cannabinoids from *Cannabis Indica* L. by Thin Layer Chromatography. *Pharmazie* **1979**, *34*, 54.

[30] Debruyne, D.; Albessard, F.; Bigot, M. C.; Moulin, M. Comparison of Three Advanced Chromatographic Techniques for Cannabis Identification. *Bull. Narc.* **1994**, *46*, 109–121.

[31] Vollner, L.; Bieniek, D.; Korte, F. Review of Analytical Methods for Identification and Quantification of Cannabis Products. *Regul. Toxicol. Pharmacol.* **1986**, *6*, 348–358. DOI: 10.1016/0273-2300(86)90003-6.

[32] Hanus, L. Our Experience with Identification and Separation of Compounds from Marijuana (*Cannabis Sativa* L.) by Thin Layer Chromatography. *Acta Univ. Palacki. Olomuc. Fac. Med.* **1987**, *116*, 15–24.

[33] Galand, N.; Ernouf, D.; Montigny, F.; Dollet, J.; Pothier, J. Separation and Identification of Cannabis Components by Different Planar Chromatography Technique (TLC, AMD, OPLC). *J. Chromtogr. Sci.* **2004**, *42*, 130–134. DOI: 10.1093/chromsci/42.3.130.

[34] Hazekamp, A.; Peltenburg, A.; Verpoorte, R.; Giroud, C. Chromatographic and Spectroscopic Data of Cannabinoids from *Cannabis Sativa* L. *J. Liq. Chromatogr. Relat. Technol.* **2005**, *28*, 2361–2382. DOI: 10.1080/10826070500187558.

[35] Laboratory and Scientific Section, United Nations Office on Drugs and Crime Vienna, Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products, Revised and Updated. 2009, United Nations, New York, NY.

[36] Mohammad, A.; Sharma, S.; Bhawani, S. A.; Singh, R. B. Identification and Separation of *Cannabis Sativa*, *Embleia Ribes*, *Myristica Fragans* and Piper *Longum* from Organic Extract on Silica Gel Surface with Anionic Micellar Solvent Syatem: Application in Ayurvedic Medicine. *Tonutraj.* **2010**, *3*, 112–118. DOI: 10.2174/1876396001003030112.

[37] Monograph Cannabis Flos Version 7.1, November 28, 2014, Federal Institute for Drugs and Medical Devices (BfArM), Bonn, Germany.

[38] Ebrahimi-Najafabadi, H.; Kazemeini, S. S.; Pasdaran, A.; Hamedi, A. A Novel Similarity Search Approach for High Performance Thin Layer Chromatography (HPTLC) Fingerprinting of Medicinal Plants. *Phytochem. Anal.* **2019**, 1–10. DOI: 10.1002/pca.2823.

[39] HPTLC Association Website, https:www.hptlc-association.org (accessed on July 31, **2019**).

[40] Tewari, S. N.; Sharma, J. D. Detection of delta-9-tetrahydrocannabinol in the organs of a suspected case of cannabis poisoning. *Toxicol. Lett.* **1980**, *5*, 279–281. DOI: 10.1016/0378-4274(80)90073-9.

[41] Oroszlan, P.; Verzar-Petri, G.; Mincsovics, E.; Szekely, T. Separation, Quantitation and Isolation of Cannabinoids from *Cannabis Satvia* L. by Overpressured Layer Chromatography. *J. Chromatogr.* **1987**, *388*, 217–224. DOI: 10.1016/S0021-9673(01)94481-4.

[42] Fischedick, J. T.; Glas, R.; Hazekamp, A.; Verpoorte, R. A. Qualitative and Quantitative HPTLC Densitometry Method for the Analysis of Cannabinoids in *Cannabis Sativa* L. *Phytochem. Anal.* **2009**, *20*, 421–426. DOI: 10.1002/pca.1143.

[43] Szabady, B.; Hidvegi, E.; Nyiredy, S. Determination of Neutral Cannabinoids in Hemp Samples by Overpressured-Layer Chromatography. *Chromatographia* **2002**, *56*, S165–S168. DOI: 10.1007/BF02494131.

[44] Daffau, B. E.; Alcaman, K. Analysis of Three Main Cannbabinoids in Seized Marijuana by Densitometric High Performance Thin Layer Chromatography. *J. Planar Chromatogr.-Mod. TLC* **2019**, *32*, 343–346.

[45] Teh, S.-S.; Morlock, G. E. Analysis of Bioactive Components of Oilseed Cakes by High Performance Thin Layer Chromatography-(Bio)Assay Combined with Mass Spectrometry. *Chromatography*. **2015**, *2*, 125–140.

[46] De Faubert Maunder, M. J. The Forensic Significance of the Age and Origin of Cannabis. *Med. Sci. Law.* **1976**, *16*, 78–90. DOI: 10.1177/002580247601600203.

[47] Noirfalise, A.; Lambert, J. Proof of the Consumption of Cannabis. *UNODC-Bull. Narc* **1978**, Issue 3, 65–67.

[48] Rao, N. G. S.; Poklis, A.; Khalil, S. K. W.; Schermeister, L. J. Examination of North Dakota Plants by a Thin Layer Chromatographic Method Used to Identify Cannabis. *Quart. J. Crude Drug Res.* **1978**, *16*, 22–28. DOI: 10.3109/13880207809083249.

[49] Lavanya, K.; Baggi, T. R. An Improved Thin Layer Chromatographic Method for the Detection and Identification of Cannabinoids in Cannabis. *Forensic Sci. Int.* **1990**, *47*, 165–171. DOI: 10.1016/0379-0738(90)90210-P.

[50] Lillsunde, P.; Korte, T. Comprehensive Drug Screening in Urine Using solid-phase extraction and combined TLC and

GC/MS identification. *J. Anal. Toxicol.* **1991**, *15*, 71–81. DOI: 10.1093/jat/15.2.71.

[51] Hauber, D. J. Marijuana Analysis with Recording of Botanical Features Present and without the Environmental Pollutants of the Duquenois-Levine Test. *J. Forensic Sci.* **1992**, *37*, 13356J–131661. DOI: 10.1520/JFS13356J.

[52] Jermain, J. D.; Evans, H. K. Analyzing *Salvia* D*ivinorum* and Its Active Ingredient Salvinorin a Utilizing Thin Layer Chromatography and Gas Chromaography/Mass Spectrometry. *J. Forensic Sci.* **2009**, *54*, 612–616. DOI: 10.1111/j.1556-4029.2009.00999.x.

[53] Teotia, A. K.; Kumar, S. Screening, Identification and Quantitation of Cannabis. *Asian J. Res. Chem.* **2009**, *2*, 401–403.

[54] Kuila, D. K.; Lahiri, S. C. Search for Suitable Mobile Phase in TLC Analysis of Different Drugs of Forensic Interest and Their Gas Liquid Chromatographic Experiment. *J. Indian Chem. Soc.* **2007**, *84*, 69–73.

[55] dos Santos, N. A.; Souza, L. M.; Domingos, E.; Franca, H. S.; Lacerda, V., Jr., Beatriz, A.; Vaz, B. G.; Rodrigues, R. R. T.; Carvalho, V. V.; Merlo, B. B.; et al. Evaluating the Selectivity of Colorimetric Test (Fast Blue BB Salt) for the Cannabinoids Identification in Marijuana Street Samples by UV-Vis, TLC, ESI(+)FT-ICR MS and ESI(+). *MS/MS. Forensic Chem.* **2016**, *1*, 13–21. DOI: 10.1016/j.forc.2016.07.001.

[56] Balbino, M. A.; de Oliveira, L. S.; Eleoterio, I. C.; Oiye, E. N.; Ribeiro, M. F. M.; McCord, B. R.; Ipolito, A. J.; de Oliveira, M. F. The Application of Voltammetric Analysis of Δ9-THC for the Reduction of False Positive Results in the Analysis of Suspected Marijuana Plant Matter. *J. Forensic Sci.* **2016**, *61*, 1067–1073. DOI: 10.1111/1556-4029.13059.

[57] de Zeeuw, R. A.; Vree, T. B.; Breimer, D. D.; van Ginneken, C. A. Cannabivarichromene, a New Cannabinoid with a Propyl Side Chain in Cannabis. *Experientia* **1973**, *29*, 260–261. DOI: 10.1007/bf01926461.

[58] Kushima, H.; Shoyama, Y.; Nishioka, I. Cannabis. XII. Variations of Cannabinoid Contents in Several Strains of *Cannabis Sativa* L. with Leaf-Age, Season and Sex. *Chem. Pharm. Bull.* **1980**, *28*, 594–598. DOI: 10.1248/cpb.28.594.

[59] Schurr, A.; Rigor, B. M. Cannabis Extract, but Not Δ1-Tetrahydrocannainol, Inhibits Human Brain and Liver Monoamine Oxidase. *Gen. Pharmac.* **1984**, *15*, 171–174. DOI: 10.1016/0306-3623(84)90104-6.

[60] Eikel, D.; Prosser, S. J.; Henion, J. D. Using Compact Mass Spectromtry for Detection and Quantitation of Cannabis-Related Compounds. *Specrtoscopy* **2015**, *13*, 20–27.

[61] Kanter, S. L.; Hollister, L. E.; Musumeci, M. Marijuana metabolites in urine of man. X. Identification of marijuana use by detection of delta 9-tetrahydrocannabinol-11-oic acid using thin-layer chromatography . *J. Chromatogr.* **1982**, *234*, 201–208. DOI: 10.1016/s0021-9673(00)81793-8.

[62] Kogan, M. J.; Newman, E.; Willson, N. J. Detection of Marijuana Metabolite 11-nor-Tetrahydrocannabinol-9-Carboxylic Acid in Human Urine by Bonded Phase Adsorption and Thin Layer Chromatography. *J. Chromatogr.* **1984**, *306*, 441–443. DOI: 10.1016/S0378-4347(00)80913-5.

[63] Verbey, K.; Mule, S. J.; Alrazi, J.; Lehrer, M. One Hundred EMIT Positive Cannabinoid Urine Samples Confirmed by BPA/TLC, RIA, and GC/MS. *J. Anal. Toxicol.* **1986**, *10*, 79–80.

[64] Eskridge, K. D.; Guthrie, S. K. Clinical Issues Associated with Urine Testing of Substances of Abuse. *Pharmacotherapy* **1997**, *17*, 497–510.

[65] Sharma, P.; Bharath, M. M.; Murthy, P. Qualitative High Performance Thin Layer Chromatography (HPTLC) Analysis of Cannabinoids in Urine Samples of Cannabis Abusers. *Indian J. Med. Res.* **2010**, *132*, 201–208.

[66] Taufik, M.; Marpaung, H.; Kaban, J.; Wirjosentono, B. Analysis of User's Hair Cannabinoid of Narcotic Type of Marijuana (*Cannabis Sativa* L.) Using GCMS Technic. *Ajbls*. **2016**, *4*, 1–10. DOI: 10.11648/j.ajbls.20160401.11.

[67] Uchiyama, N.; Kikura-Hanajiri, R.; Kawahara, N.; Goda, Y. Identification of a Cannabimimetric Indole as a Designer Drug in a Herbal Product. *Forensic. Toxicol.* **2009**, *27*, 61–66. DOI: 10.1007/s11419-009-0069-y.

[68] Uchiyama, N.; Kawamura, M.; Kikura-Hanajiri, R.; Goda, Y. Identification and Quantitation of Two Cannabimimetric Phenylacetyl Indoles JWH-251 and JWH-250, and Four Cannabimimetric Naphthoylindoles JWH-081, JWH-015, JWH-200, and JWH-073 as Designer Drugs in Illegal Products. *Forensic. Toxicol.* **2011**, *29*, 25–37. DOI: 10.1007/s11419-010-0100-3.

[69] Logan, B. K.; Reinhold, L. E.; Xu, A.; Diamond, F. X. Identification of Synthetic Cannabinoids in Herbal Incense Blends in the United States. *J. Forensic Sci.* **2012**, *57*, 1168–1180. DOI: 10.1111/j.1556-4029.2012.02207.x.

[70] Bebarta, V. S.; Ramirez, S.; Varney, S. M. Spice: A New "Legal" Herbal Mixture Abused by Young Active Duty Military Personnel. *Subst. Abus.* **2012**, *33*, 191–194. DOI: 10.1080/08897077.2011.637610.

[71] Kneisel, S.; Bisel, P.; Brecht, V.; Broecker, S.; Müller, M.; Auwärter, V. Identification of the Cannabimimetic AM-1220 and Its Azepane Isomer (*N*-Methylazepan-3-yl)-3-(!-Napthhoyl)Indole in a Research Chemical and Several Herbal Mixtures. *Forensic Toxicol.* **2012**, *30*, 126–134. DOI: 10.1007/s11419-012-0137-6.

[72] Kneisel, S.; Westphal, F.; Bisel, P.; Brecht, V.; Broecker, S.; Auwärter, V. Identification and Structural Characterization of the Synthetic Cannabinoid 3-(1-Adamantoyl)-1-Pentylindole as an Additive in "Herbal Incense. *J. Mass Spectrom.* **2012**, *47*, 195–200.

[73] Alsoud, A. R. A.; Al-Tannak, N.; Bojabarah, H.; Orabi, K. Y. AB-FUBINACA, a Synthetic Cannabinoid in "Funky Green Stuff. *Int. J. Pharm. Pharmaceut. Sci.* **2015**, *7*, 111–115.

[74] The AOAC Cannabis Analytical Science Program (CASP) <https://cannabisindustryjournal.com/feature_article/spotlight-on-aoac-new-leadership-new-initiatives-in-cannabis-food/> (accessed on May 24, **2019**).

[75] Audino, S. Can Analytical Testing Quality Be Assured in the Cannabis Industry? Cannabis Sci. Technol. **2018**, *1*, 1–4.

[76] Atkins, P. L. Sample Processing and Preparation Considerations for Solid Cannabis Products. *J. AOAC Int.* **2019**, *102*, 427–433. DOI: 10.5740/jaoacint.18-0203.

[77] Shoyama, Y.; Kuboe, K.; Nishioka, I.; Yamauchi, T. Cannabidiol Monomethyl Ether. A New Neutral Cannabinoid. *Chem. Pharm. Bull.* **1972**, *20*, 2072. DOI: 10.1248/cpb.20.2072.

[78] Andersen, J. M.; Nielsen, E.; Schou, J.; Steentoft, A.; Worm, K. A Specific Method for the Demonstration of Cannabis Intake by TLC of Urine. *Acta Pharmacol. Toxicol. (Copenh)* **1971**, *29*, 111–112. DOI: 10.1111/j.1600-0773.1971.tb00590.x.

[79] Quraishi, R.; Jain, R.; Chatterjee, B.; Verma, A. Laboratory Profiles of Treatment-Seeking Subjects with Concurrent Dependence on Cannabis and Other Substances: A Comparative Study. *Int. J. High Risk Behav. Addict.* **2013**, *2*, 107–111. DOI: 10.5812/ijhrba.10993.

[80] Simpson, D.; Braithwaite, R. A.; Jarvie, D. R.; Stewart, M. J.; Walker, S.; Watson, I. W.; Widdop, B. Screening of Drugs of Abuse (II): Cannabinoids, Lysergic Acid Diethylamide; Buprenorphine, Methadone, Barbiturates, Benzodiazepines, and Other Drugs. *Ann. Clin. Biochem.* **1997**, *34*, 460–510. DOI: 10.1177/000456329703400502.

[81] Booth, J. K.; Bohlmann, J. Terpenes in *Cannabis Sativa* - From plant genome to humans. *Plant Sci*. **2019**, *284*, 67–72. DOI: 10.1016/j.plantsci.2019.03.022.

[82] Wojtunik-Kulesza, K. A.; Targowska-Duda, K.; Klimek, K.; Ginalska, G.; Jozwiak, K.; Waksmundzka-Hajnos, M.; Ciesla, L. Volatile Terpenoids as Potential Drug Leads in Alzheimer's Disease. *Open Chem.* **2017**, *15*, 332–343.

[83] Jiang, Z.; Kempinski, C.; Chappell, J. Extraction and Analysis of Terpenes/Terpenoids. *Curr. Protoc. Plant Biol.* **2016**, *1*, 345–368. DOI: 10.1002/cppb.20024.

[84] Atkins, P. Beyond Potency: The Importance of Terpenes. *Cannabis Sci. Technol.* **2019**, *2*, 22–27.