Separation and Identification of Cannabis Components by Different Planar Chromatography Techniques (TLC, AMD, OPLC)

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Abstract

The use of cannabis is illicit in numerous countries, and the increasing consumption has led to a multiplication of scientific studies. New methods of planar chromatography such as automated multiple development (AMD) and optimum performance laminar chromatography (OPLC) techniques can be used as a substitute for the traditional thin-layer chromatography for the identification and quantitation of the Indian hemp components. Each method offers its own advantage: high resolution with neither diffusion nor spot stretching for AMD and speed, efficiency, and the possibility of working in the semipreparative mode for OPLC.

Introduction

Because of the ever-increasing use of cannabis, it has become necessary to dispose of a whole range of efficient methods for the identification of its components and particularly for the characterization of the "narcotic compound" Δ^9-tetrahydrocannabinol (Δ^9-THC). Analyses can be carried out from plants or biological fluids. In urine, blood, and saliva samples, Δ^9-THC and major metabolites, such as 11-nor-Δ^9-THC-9-carboxylic acid, can be often required (1). Cannabinoids can be detected by numerous and various analytical methods, including immunoassays (EMIT, enzyme-linked immunosorbent assay, fluorescence polarization, and radioimmunoassay) (2,3,4,5,6), planar chromatography techniques [classical thin-layer chromatography (TLC), optimum performance laminar chromatography (OPLC), and automated multiple development (AMD)], gas chromatography–mass spectrometry (GC–MS) (7,8,9), and high-performance liquid chromatography (HPLC)–MS (10). Generally, there is a good quantitative correlation between these methods and few discrepancies even in the borderline region, especially if the cutoffs through immunoassay techniques are low, in spite of the different metabolites cross reactivities.

Moreover, a wide variety of methodologies have been recommended for the determination of marijuana samples or cannabis plants: TLC (11), OPLC (12), HPLC (13), GC, and GC–MS (14,15), capillary electrochromatography (16), time-resolved fluoroimmunological method (17), and immunoassay (18). Most of these techniques require heavy and costly instruments and a lot of time.

Planar chromatography is a suitable method to simultaneously screen numerous samples directly from plants. It has become a modern technique with the commercialization of numerous adsorbents and new appliances with automated development chambers such as OPLC and AMD, which are interesting alternatives to classical TLC.

OPLC opens up the possibility of analyzing an important number of samples in a very short time. Moreover, this method can be used in the semipreparative mode to purify products by direct elution thanks to the fact that migration is linear in correlation with time. *h*Rf values are reproducible, and each compound is eluted at a defined time.

AMD presents the best resolution without any spot diffusion and without oxidation because the microchamber is saturated with, for instance, methanol under a nitrogen atmosphere.

The aim of this study was to compare the performances of the TLC, AMD, and OPLC techniques for the identification and quantitation of the cannabis components.

Experimental

The standard solutions of Δ^9-THC, Δ^9-THC, and cannabinol (CBN) were purchased from Sigma-Aldrich Chimie (Saint Quentin Fallavier, France). Because cannabidiol (CBD) is not available, it was isolated from cannabis resin by OPLC in the semipreparative mode (see the Semipreparative OPLC applied to isolation of standard CBD section).

All the standard solutions were prepared in 0.5 mg/1 mL in

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methanol. Cannabis resin (0.1 g) was extracted by shaking at room temperature for 20 min with 10 mL of hexane. The filtrate was evaporated to dryness and the residue dissolved in 1 mL of toluene. A hemp sample (0.5 g) was extracted for 10 min with 20 mL of hexane. After filtration, the extract was evaporated under vacuum and the residue dissolved in toluene. All solvents were purchased from Carlo Erba Reactifs (Val de Reuil, France) and then distilled. A Linomat IV (Camag, Muttenz, Switzerland) was used for sample applications. A TLC-MAT Desaga (Bionisis, Le Plessis-Robinson, France), OPLC 50 (Bionisis), and AMD (Camag) were used for the chromatographic studies. TLC and AMD were performed on 10- × 20-cm plates (precoated silicagel HPTLC F_{254}) (Merck Art. 11764) (VWR International SAS, Fonterlaysous Bois, France). OPLC was performed on an HTSorb BSLA 011 and HT Sorb BSLA 003 (Bionisis). The chromatograms were derivated by spraying with Fast Blue salt B reagent (19).

For classical TLC and TLC-MAT, the eluent used was hexane–diethyl ether (80:20, v/v). For AMD, the elution gradient was acetone (100) (bottle 1), diisopropylether (100) (bottle 2), hexane (100) (bottles 3–6), and migration during 20 steps. For OPLC, the eluent was isooctane–diethyl ether (90:10, v/v). The external pressure was 50 bars, flow rate 100 µL/min, flash volume 75 µL, eluent volume 1000 µL, and the migration time 607 s.

The GC–MS instrumentation used consisted of a Hewlett-Packard system (HP 5890 series II GC with an HP5989A quadrupole MS) (Palo Alto, CA). An HP-5MS 15-m × 0.25-mm × 0.25-µm capillary column and a helium (99.99%) carrier gas at a flow rate of 1.3 mL min^{-1} were used. The injector temperature was maintained at 250°C, and all injections were made in the splitless mode. The GC oven temperature was held at 50°C for 1 min and then programmed to 250°C at 10°C min^{-1} and maintained for 10 min. The GC–MS transfer line was maintained at 280°C, electron ionization at 70eV, and the mass spectrum scanned from *m/z* 35–450. Chromatographic data were acquired using HP Chemstation software (Hewlett-Packard).

Table I. *h*Rf and Colors with Fast Blue Salt Reagent of Cannabinoides

Cannabinoids	*h*Rf TLC-MAT	*h*Rf AMD	*h*Rf OPLC	Colors with Fast Blue salt B
CBN	59	73	23	violet
Δ^9-THC	66	76	28	purple
CBD	73	79	34	orange-red

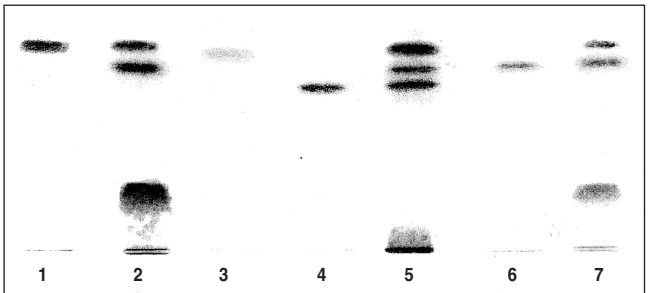

Figure 1. TLC-MAT of cannabinoids: (1) 1 µL CBD, (2) 7 µL cannabis extract, (3) 2 µL Δ^8-THC, (4) 1 µL CBN, (5) 2 µL cannabis resin, (6) 3 µL Δ^9-THC, and (7) 7 µL cannabis extract.

Results and Discussion

TLC

Comparison of various eluents used in TLC for the separation of cannabinoids

TLC is a suitable method for screening different samples.

In the literature, the eluents that are mostly used are eluent A, isooctane–ethyl acetate–acetic acid (30:10:1, v/v/v) (20); eluent B, petroleum ether–diethyl ether (90:10) (21); eluent C, acetone–methylene chloride–diisopropyl ether–hexane (1:1:3:20, v/v/v/v) (22); eluent D, toluene–chloroform–methanol (100:10:1, v/v/v) (23); eluent E, hexane–dioxane (90:10, v/v) double migration (24); and eluent F, hexane–diethyl ether (80:20, v/v) (19).

The eluents A and B result in a clean separation between Δ^9-THC and CBN but not between Δ^9-THC and CBD.

With eluent C, the main cannabinoids are separated, but the spots are stretched.

The best results were obtained with eluent F, hexane–diethyl ether (80:20, v/v) (Table I), which allowed a clean separation of Δ^8-THC, Δ^9-THC, CBN, and CBD (Figure 1).

The separation of cannabinoids Δ^9-THC, CBN, and CBD by classical TLC is not easy because these derivatives possess chemical structures with very close substitutes. Besides, the molecular weights of Δ^9-THC and CBD are the same (314.47), and the molecular weight of CBN is very close (310.44).

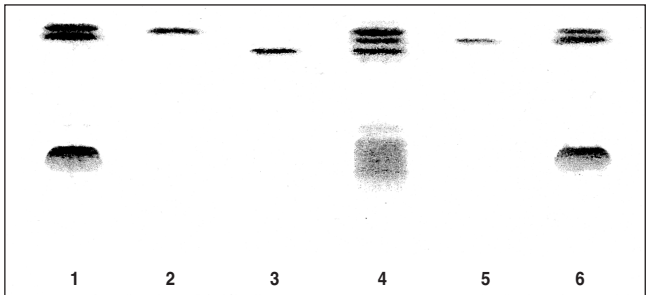

Figure 2. AMD of cannabinoids: (1) 7 µL cannabis extract, (2) 1 µL CBD, (3) 1 µL CBN, (4) 2 µL cannabis resin, (5) 3 µL Δ^9 THC, and (6) 5 µL cannabis extract.

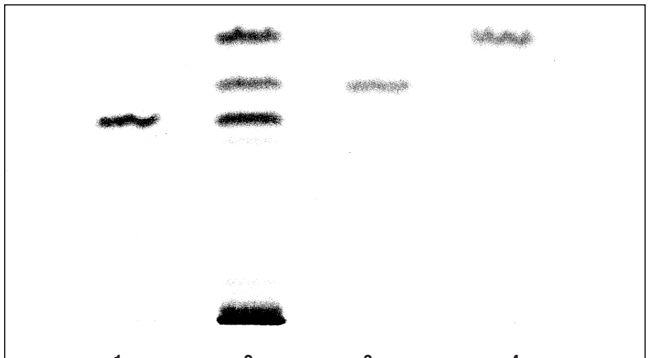

Figure 3. OPLC of cannabinoids: (1) 1 µL CBN, (2) 2 µL cannabis resin, (3) 3 µL Δ^9-THC, and (4) 1 µL CBD.

The analysis of the chromatograms revealed two different groups of cannabinoids, a first group, the least polar, composed of CBD, CBN, and Δ^9-THC (upper *h*Rf values) and a second one, which consisted in many compounds with lower *h*Rf.

The detection limit with the Fast Blue salt reagent was 0.25 µg for Δ^9-THC, CBD, and CBN.

Different eluents were tested: isooctane, heptane, hexane, and pentane–diethyl ether (90:10, v/v). The comparison between these four alkanes showed that the separation capability decreased when the carbon-bearing chain lengthened.

After this traditional TLC study, these compounds were studied with modern planar chromatographic methods such as AMD and OPLC, with the aim of optimizing their separation and identification.

AMD

The "universal gradient" *n*°1 with methanol, methylene chloride, and hexane is far too polar. Therefore, it was necessary to decrease the polarity by replacing methanol with methylene chloride.

First, two gradients were tested. Elution gradient 1A was methylene chloride (100), methylene chloride (100), methylene chloride–hexane (50:5), hexane (100), hexane (100), and hexane (100) during 25 steps.

Elution gradient 1B was diethyl ether (100)–hexane (50:50), hexane (100), hexane (100), and hexane (100).

These two eluents are interesting for revealing most of the constituents of cannabis but do not separate Δ^9-THC with CBN and CBD very clearly.

In AMD, the best separation of the three interesting compounds was realized in high-performance TLC with the elution gradient 1C acetone (100), diisopropylether (100), hexane (100), hexane (100), and hexane (100) during 20 steps (Table I) (Figure 2).

The visualization of the chemical constituents was accomplished by spraying Fast Blue salt B reagent (19).

The different cannabinoids were identified by their *h*Rf and the color of the spots (purple for Δ^9-THC, orange-red for CBD, and violet for CBN).

OPLC

Analytical OPLC

Applying the classical TLC eluent hexane–diethyl ether (80:20, v/v), OPLC gave clear separation, but the different stripes took a "zigzag" shape because the viscosity of the eluents was too low and the silicagel plates were not homogeneously permeated deep inside their structure. To solve this problem, the viscosity was increased by replacing hexane with a higher homologous such as isooctane, which does not change the elution power of the eluent but increases inner pressure, leading to higher *h*Rf values, thus improving considerably the shape of the stripe—the best results were obtained with isooctane–diethyl ether (90:10, v/v) as eluent (Table I) (Figure 3). Moreover, hexane–diethylether (80:20, v/v) used in the semipreparative mode offers the advantage of evaporating easily because of its low viscosity.

Semipreparative OPLC applied to isolation of standard CBD

CBN and Δ^9-THC were obtained from Sigma-Aldrich. Because obtaining CBD was not possible, this compound was isolated from cannabis resin by OPLC in the semipreparative mode. In the literature, few works have reported this technique. First of all, this method had been tested on opium (25) and xanthines from tea leaves extracts (26). In the case of cannabis extract, two series of compounds are shown. The first one has an *h*Rf above 50, which is easily and quickly carried out, and the second one has an *h*Rf below 50. For the latter, it was necessary to increase the eluent polarity. The aim of this work was to obtain pure compound from the resin of cannabis by coupling the chromatograph with a fraction collector. The extract was applied inline with Linomat IV and eluted with hexane–diethyl ether (90:10, v/v). The migration of the eluent was performed during the time required to begin the elution process. Because OPLC allows for linear migration in correlation with time, it was able to determine the instant when CBD was collected. Thanks to OPLC, it was possible to obtain pure CBD.

The advantage of OPLC compared with TLC in the semipreparative mode is that no scraping and eluting of bands are necessary. In OPLC, the components can be eluted from the plate and obtained pure by coupling with a fraction collector.

Every elution fraction was evaluated by analytical TLC with hexane–diethyl ether (80:20, v/v) as eluent. After derivation by Fast Blue salt B reagent (19), four fractions were obtained, giving only one spot in TLC. The control of the structural study performed by GS–MS analysis allowed the identification of a compound present in the sample as being CBD (Figure 4).

Structural analysis of CBD

From chromatographic data obtained by GC–MS, the organic compounds were identified by computer. Standard reference mass fragmentograms listed in the National Institute of Standards and Technology (NIST) library were compared with the specific results obtained here.

The total ion chromatogram obtained showed one major compound. A search in the database spectral library indicated that this substance might very likely be "2-[3-methyl-6-(1-methylethenyl)-cyclohex-2-en-1-yl]-5-pentyl benzene-1,3-diol (Figure 1). The identified compound is also known under the synonym CBD. This assumption is in full agreement with the mass spectrum of CBD investigated by Baptista (27).

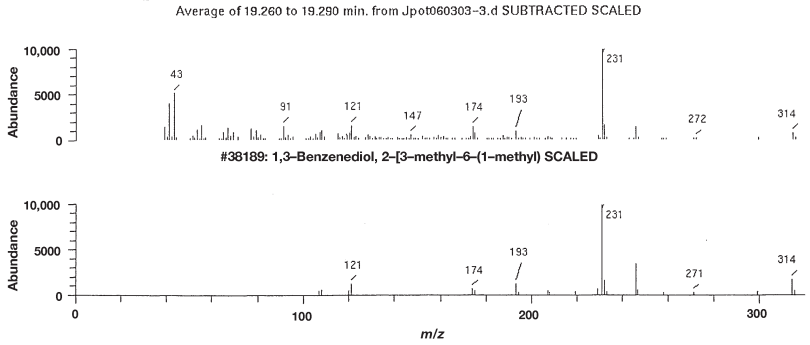

Figure 4. (Top) MS–GC of CBD obtained from cannabis hemp by semipreparative mode OPLC and (Bottom) MS of CBD from NIST library.

Furthermore, the chromatograms showed other substances with low concentrations, which can be assigned as aliphatic and ethylenic hydrocarbons. Unfortunately, these compounds cannot be identified with certainty because of their poor mass spectra resolution.

Conclusion

The modern planar chromatography techniques are reliable because they are automated and inexpensive, they allow a better resolution than classical TLC, and can potentially replace slower and more costly methods (GC–MS), thus increasing the productivity of the laboratory thanks to their ability to analyze several samples at the same time (up to 20).

Therefore, with traditional TLC, it was possible to separate D^9-THC, CBD, and CBN from cannabis resin and Indian hemp herb, but this method did not offer a clean separation of the most polar compounds; four spots for cannabis extracts with classical TLC and eight spots for the resin could be obtained with AMD.

AMD offers high resolution without any stretching of spots, the focalization of which gives the possibility of making dosage by scanner densitometry.

These two modern techniques, OPLC and AMD, are reproducible because they are completely automated. They can provide interesting information about the composition of different samples of Indian hemp and open up the possibility to determine the geographical origin of different samples.

The benefits of OPLC compared with TLC are numerous, namely efficiency, reproducibility, small consumption of developing eluent, and shorter analysis delay. Consequently, the spots have a more regular shape and diffusion and stretching is not as pronounced as in TLC. Another major advantage of OPLC compared with TLC is the possibility to extend this method to semipreparative chromatography, in which no scraping and eluting of bands are necessary because the components can be drained from the plate and obtained pure by coupling OPLC to a collector.

An additional benefit of planar chromatographic techniques versus HPLC and GC lies in the fact that it is possible to detect in the cannabis samples other addictive products belonging to different chemical classes (e.g., alkaloids, like opiates and derivatives; cocaine; and nicotine) mixed in a single cannabis sample by using specific reagents (e.g., iodoplatinate or Dragendorff, in the case of alkaloids).

TLC, OPLC, and AMD can also supply interesting information in regards to the composition of various samples of hemp and offer the possibility of determining its origin.

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