



High Performance Thin Layer Chromatographic Detection of Cannabis in Forensic Interest

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ABSTRACT

Cannabis, also known as Marijuana, and other forms of cannabis plant (bhang, ganja, and Charas) are very frequently submitted to forensic laboratories under THC narcotic drug and psychotropic substances act 1985. In routine cases the identification of Cannabinoids in marihuana is achieved unequivocally by the 'three-parameter approach [Morphology, color tests and thin-layer chromatography (TLC)] as suggested by Coutts and Jones'.

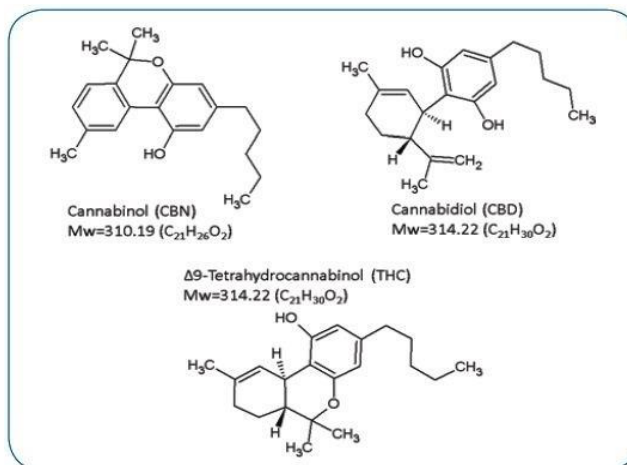
Cannabis is one of the most commonly abused drugs worldwide. Detection of cannabis is a great challenge for forensic scientists. Although the instrumental methods are sensitive as they are also expensive and there are limitations to their use in routine forensic work owing to the large number of samples (involving urine samples) to be handled. In this study, HPTLC method was found to be high- throughput, sensitive, reproducible and cost-effective compared to other methods. In this study we report 10% NaOH followed by p-Anisidine reagent and ammonium metavanadate as a new, specific chromogenic reagent for HPTLC detection of Cannabis.

Keywords: Forensic science; HPTLC; Cannabis; Narcotic drug; Psychotropic substances

INTRODUCTION

Cannabis or Hashish or hemp is an annual plant, known by the name: *Cannabis sativa* (F. Cannabinaceae). Generally, two varieties are grown: the fiber type which is cultivated mainly for fiber production and the drug type which is cultivated to provide the narcotic drug known by the name: *Marijuana (Marihuana) or Hashish*.

It contains tetrahydrocannabinol (delta-9-THC) and cannabinol (CBN), Cannabidiol also isolated (CBD). CBN is not present in the fresh plant and it is a measure of aging: as the sample ages, THC content declines and decomposes to CBN, which means that fresh plants contain all a Cannabinoids in their acidic form (THA-acid or THCA, CBD-acid or CBDA etc.).



Scheme 1: Cannabis.

Cannabis

Chemical constituents of forensic significance: Cannabis and other forms of cannabis plant (bhang, ganja, and Charas) are very frequently submitted to forensic laboratories under the narcotic drug and psychotropic substances act 1985 (Scheme 1). In routine cases the identification of Cannabinoids in marihuana is achieved unequivocally by the ‘three-parameter approach’ [Morphology, color tests and thin-layer chromatography (TLC)] as suggested by Coutts and Jone [1-3].

The presence of cannabinoids is usually detected using color tests [2], high performance liquid chromatography (HPLC) [3-5] gas chromatography (GC) and commercially available immunoassay based cassettes [6-10]. Following screening tests for cannabinoid detection, it is necessary to perform confirmatory tests using advanced techniques such as GC-MS, fluorescence polarization immunoassay [11], enzyme immunoassay [12] high performance thin layer chromatography (HPTLC) [13] and HPLC, which provide additional scope for quantitative monitoring of drugs during forensic analysis. Although the instrumental methods are sensitive, they are expensive and there are limitations to their use in routine forensic work owing to the large number of samples (involving urine samples) to be handled. In this study we found that HPTLC method was found to be high -throughput, sensitive, reproducible and cost-effective compared to other methods.

A number of chromogenic reagents such as Duquenois reagent [14], Fast Blue Salt B [15], 1-nitroso-2-naphthol [16], Fast Blue Salt 2 B [17] and 2-hydrazono-2, dihydr3methylbenzothiazolehydrochloride (HMBT) [18,19], have been reported for the detection of Cannabinoids. Although Fast Blue Salt B as a chromogenic reagent seems to be the most commonly used reagent, its safety is questionable because of its potential carcinogenicity [20]. In a search for an alternative chromogenic reagent, P-Anisidine reagent in combination with ammonium metavanadate was found to be suitable for the detection of cannabinoids in marijuana.

MATERIALS AND METHODS

All the solvents used were of analytical-reagent grade. Distilled water was used throughout.

Sodium hydroxide: 10 gram sodium hydroxide pallets dissolved in 100 mL distilled water.

Spray Reagent

Solution (a): saturated aqueous ammonium metavanadate

Solution (b): Dissolve 0.5 g. p-Anisidine in 2 mL. H₃PO₄, dilute up to 100 ml. with ethanol and filter.

For the extraction of cannabis, the cannabis sample (bhang, ganja or Charas) was extracted with chloroform, the extract was filtered and evaporated to dryness and the residue was dissolved in chloroform for spotting. Reference standard (cannabinoids) was available in our laboratory.

High Performance Thin Layer Chromatography

Chromatography was performed on 10cm×10cm silica gel60 F₂₅₄ HPTLC glass plate [Merck], A camag [Switzerland], linomat IV Applicator was used to apply 10 µl cannabis extract (Exhibits sent by police authority under NDPS act) along with the standard reference solutions of cannabinol and cannabidiol pure reference standards dissolved in ethanol, on the plate, which was developed by the ascending technique in a presaturated chamber. After a run of about 10 cm, the plate was removed and allowed to dry at room temperature. It was sprayed uniformly with freshly prepared sodium hydroxide solution followed by solution (a). While plate is still wet, spray with solution (b). Immediately various colors appeared on plate. The R_F values of cannabinoids with respect to standards are given in Table I. Three solvent systems-(a) toluene: ethyl acetate: acetone (90:10:10), (b) ethyl acetate: methanol: ammonia (8.5:1:0.5), (c) petroleum ether: diethyl ether (4:1) were used.

UV Spectra of THC, GCMS spectra of THC, CBD, CBN

The cannabis extract was spotted on a TLC plate, the plate was developed with either of the above solvent systems and one of the resolved spots was made visible by spraying ammonium metavanadate followed by p-anisidine solution. An equal area of silica gel layer was scraped off from a distance equal to the R_F value of A^c-tetrahydrocannabinol (A^c-THC), treated with 5 ml of ethanol and the solution mixed thoroughly. The solution was centrifuged and the UV spectrum of the supernant liquid was recorded. Same procedure has been done for GC-MS analysis for cannabinol, cannabidiol, and THC (Figure 1).

RESULT

This study was done to develop a new chromogenic spray reagent for detection and separation of cannabis plant in forensic interest, this spray reagent can be routinely used to standardized protocol for detection of cannabinoids in the urine samples of person with cannabis abuse.

To further characterize the components of cannabis, the cannabis standard was subjected to HPTLC analysis in different solvent systems. Based on preliminary experiments, it was found that the cannabinoids exhibited different R_f values in different solvent systems (Table 1). We selected THC, CBN and CBD as major cannabis constituents for detection of cannabis. HPTLC based separation of cannabinoids was done using Toluene: ethyl acetate: acetone (9:1:1) solvent system. Figure 2 shows the chromatogram.

Most of the cannabinoids have a phenolic group with the ortho and para positions free. As phenols couple in the para position with diazonium salts, the cannabinoids also undergo similar reactions with ammonium metavanadate-p-anisidine reagent to yield colored products. The cannabis extract gave approximately ten spots, a greenish yellow, yellow, violet spot corresponding to CBN, CBD and THC (Figure 3).

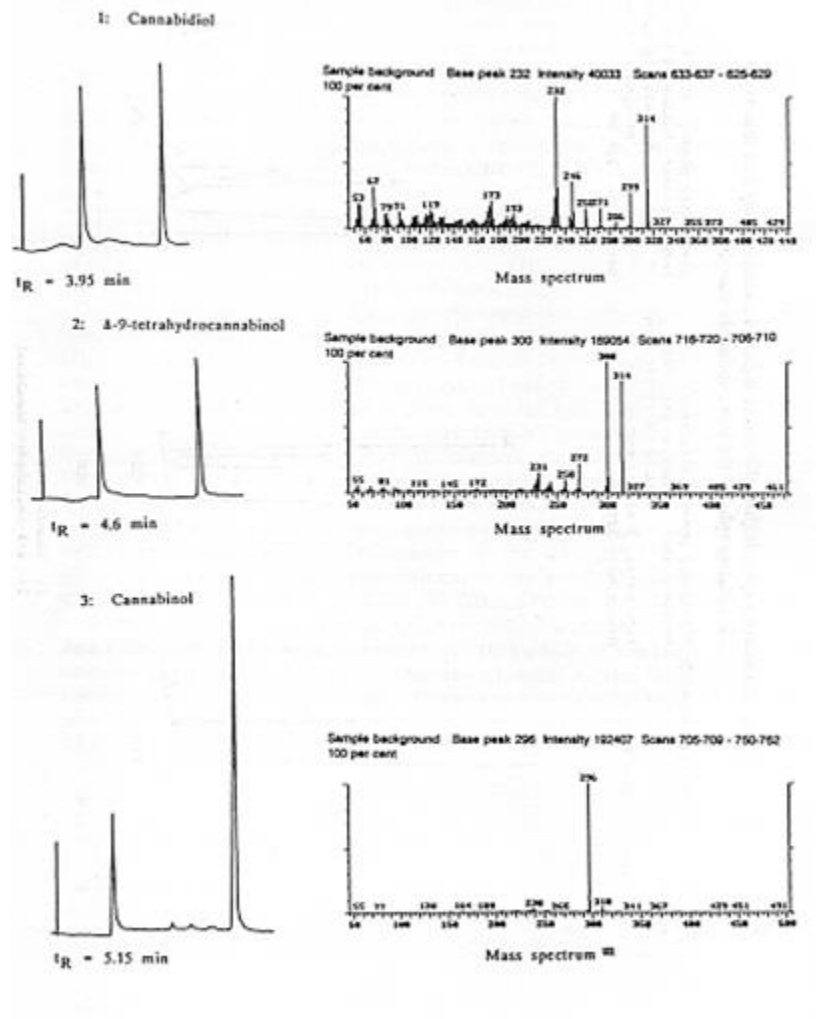


Figure 1. Identification of the three main constituents of cannabis using gas chromatography combined with mass spectrometry

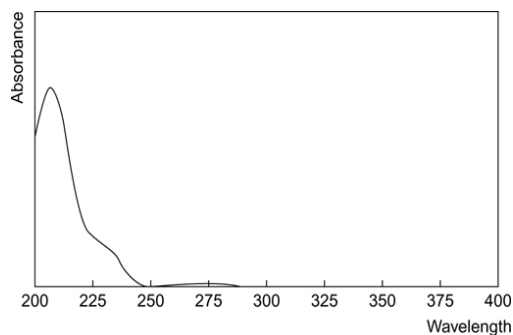


Figure 2. Chromatogram

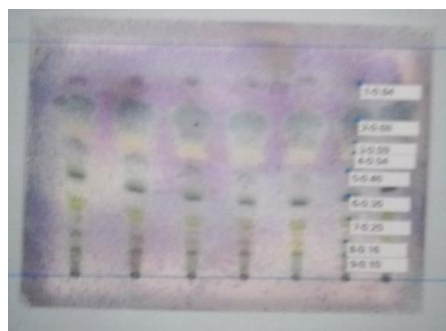


Figure 3. The cannabis extract gave approximately ten spots, a greenish yellow, yellow, violet spot corresponding to CBN, CBD and THC

Table 1. Rf values of cannabinoids in solvent systems

Rf values of cannabinoids in solvent systems: (a) Toluene: ethyl acetate: acetone (9:1:1) (b) ethyl acetate: methanol: ammonia (8.5:1:0.5) (c) petroleum ether: diethyl ether (4:1) plate sprayed with ammonium metavanadate -p-anisidine				
Sr No.	Constituent	RF in a	RF in b	RF I
1	CBN	0.59	0.27	0.28
2	CBD	0.84	0.36	0.44

DISCUSSION

Although cannabis tests, based on commercially available ready-to use cassettes are available, it has limited utility. Because it is relatively expensive and has limited sensitivity threshold, and gives only qualitative and not absolute quantification. Most of the commercial kits clearly state that the test provides only a preliminary result and more specific alternative testing method should be used to confirm the immunoassay result [9]. This could be by either HPTLC or GC/MS or HPLC [6-9]. In routinely testing, on TLC RF value is not accurately recorded 30. However, HPTLC plate gives good visualization. In this study, it was observed that the HPTLC based detection is best solution in forensic interest samples. It is a cost-effective, highly sensitive, and accurate up to the final data analysis and reporting only one hour is required). Further, with specific standards, and less time-consuming method. (Following extraction, for 20 samples, starting from sample application) quantification is possible which could help to correlate the progress in rehabilitation/detoxification with the levels of cannabis in the urine samples.

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