seen that DES or F6060 can be separated equally well from the natural estrogens with the same solvent combination.

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A simple thin-layer chromatography of cannabinoids by means of silica gel sheets treated with amines

Thin-layer chromatography of cannabis extracts has been applied for detecting, isolating and determining various constituents of the hemp resin[1–4], for examining the composition of cannabis of various origins[1,5], as well as for forensic and toxicological purposes[6–11]. However, most techniques yielding a good separation are relatively complicated and time-consuming for simple and accurate routine testing, some of them requiring previous column purification of the extracts[3,4,10], others involving a multicomponent solvent system[6,7], the preparation of special plates[4,6,9], or their troublesome impregnation[1,2]. Some recent studies indicate that the presence of certain alkaline components in the solvent system, such as ammonia and diethylamine, may improve the separation of cannabinoids[4,7]. The possibility of using as solvent system simply one of the aromatic hydrocarbons has also recently been reported[3,5,12].

This note describes a highly sensitive technique for simple and rapid separation and detection of major cannabinoids, by means of silica gel pre-coated sheets previously dipped in diethylamine or dipropylamine and using toluene (or xylene) as a developing solvent. In this way, the advantages of both the presence of alkalies and of using one single solvent have been joined.

Method

Eastman Chromagram 6060 sheet (ethylene polyterephthalate film coated with

silica gel and polyvinyl alcohol) is dipped in diethylamine for 2–3 sec, allowed to dry at room temperature and cut into 6 cm long strips. A quantity of not more than 0.5 μl of chloroform or petrol ether cannabis macerate (estimated to contain not more than 1 % of the resin) is spotted 1 cm from the end of the strip. The spot diameter should not exceed 1 mm. The chromatogram is developed in toluene up to 4 cm from the starting line. The dried sheet is sprayed with a freshly prepared 0.1 % solution of Fast Blue B salt in 70 % ethanol.

Results and discussion

The examined samples of cannabis (six samples of American marihuana and one of hashish) showed three well separated spots: the orange spot at R_F 0.47, the red spot at R_F 0.36 and the violet spot at R_F 0.22. These spots have been identified by means of the reference substances as belonging to cannabidiol (CBD), tetrahydrocannabinol (THC) and cannabinol (CBN), respectively. In addition, in most of samples there was also an unknown spot at R_F 0.11, but this was sometimes not sharply separated from the violet area extending from the starting line. Before spraying the sheet with the reagent, CBN can be easily detected under UV light of 254 nm, while CBD and THC are detectable only if present in higher amounts (over 2 and 5 μg, respectively).

Consequently, by using this simple technique, at least three major components of cannabis (CBD, THC and CBN) can be easily detected and identified on a small strip with only 4 cm of solvent run.

Similar separation of cannabinoids is obtained if dipropylamine is used instead of diethylamine, with R_F values of 0.52 for CBD, 0.40 for THC and 0.21 for CBN. Xylene as a developing solvent yields also a good separation of the three major cannabis constituents, giving lower R_F values than toluene.

Sheets once treated with amine may be used even after several weeks. In time, changes in R_F values may occur. R_F values, of course, are here affected by many other factors and are not strictly reproducible. However, the observed spots were always dispersed to give R_F differences not lower than 0.1. It should be mentioned that Eastman Chromagram sheets with recent serial numbers gave better resolution than the older ones; this difference was more pronounced in sheets treated with dipropylamine.

The zones of the spots obtained can be easily cut out, eluted in ethanol and the eluate used for quantitative colorimetric assay. Spot size comparison method and densitometric assay may be applied as well.

In comparison with many of the previously reported techniques, the described micro procedure shows the following advantages:

(1) High sensitivity. As small quantities as $5 \cdot 10^{-8}$ g of both CBD and THC and $2 \cdot 10^{-8}$ g of CBN can be detected. This may be of importance in forensic and toxicological analyses.

(2) Rapidity. The whole procedure of detecting cannabinoids present in a crude extract requires less than 15 min. This may prevent eventual interconversion of cannabinoids during the analysis, so that their ratio as shown by chromatograms may correspond closely to that in the original extracts.

(3) Simplicity of the equipment. Chromatograms may be developed in small glasses or test tubes 7–8 cm long; as only one, poorly volatile solvent is used, previous saturation and sealing of the chamber may be neglected. This may be of importance

in field identification tests or in the rough estimation of the potency of cannabis samples.

(4) Preservation of record. As spots are fairly stable, the sheets can be easily kept in files for a lengthy period.

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