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Note

Studies in laboratory-use reagents

III. Simple thin-layer chromatographic system for the separation of cannabinoids

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Thin-layer chromatography (TLC) has been commonly used to support color and morphology tests for Cannabis in forensic laboratories. TLC is inexpensive, convenient and rapid and requires little training to perform and interpret the results.

Many TLC systems for Cannabis, including developing solvent, adsorbent and pre-treatment of the plates or adsorbent have been described in a review by Parker and Fiske[1]. Disadvantages of these systems include the use of mixed solvents for development, poor resolution of the major components of Cannabis, instability of the pre-treated plate and requirements for pre-saturation of the development chamber.

The best resolution of the components occurs with plates pretreated with dimethylformamide[2,3], silver nitrate[4,5] or secondary amines[6]. However, the dimethylformamide-pretreated plates gave good resolution only with multiple development. The silver nitrate-impregnated plates gave excellent resolution, but darkened on storage and, consequently, were not suitable for use at a later time. Diethylamine-impregnated silica gel plates were found to be useful even after several weeks of storage. However, the period of usefulness of the plates was not determined.

This note describes a simple method for the preparation and storage of silica gel sheets impregnated with a tertiary amine, triethylamine, and their use with benzene as the developing solvent for the separation and detection of the major cannabinoids.

METHODS AND MATERIALS

Baker-flex®*, Silica Gel IB2, Flexible TLC Sheets (20 × 20 cm) (J. T. Baker) were pre-dipped in a 20% triethylamine solution in benzene, in a stainless-steel dipping tank (A. H. Thomas, Philadelphia, Pa., U.S.A.). The sheets were air-dried for 15 min, individually wrapped in aluminum foil, and stored in a drawer.

For the assessment of cannabinoids, 1 g of ground sample was triturated with 10 ml of chloroform, filtered and evaporated to dryness. The residue was dissolved in 0.20 ml of chloroform. One microliter of this extract was spotted 1.5 cm from the

* Baker-flex® is a registered trademark of J. T. Baker, Phillipsburg, N.J., U.S.A.

bottom of the freshly unwrapped plate using Drummond microcaps (A. H. Thomas). The spots were air-dried as heat can cause decomposition. The chromatogram was developed in benzene in an unsaturated tank 8½ in. wide × 4 in. deep × 9 in. high (Kontes Glass). The time for a 10-cm development was about 45 min. The dried sheet was sprayed with a freshly prepared aqueous solution of 0.1% Fast Blue B salt (Sigma, St. Louis, Mo., U.S.A.).

RESULTS AND DISCUSSION

Various amines were tested as impregnating agents in the form of a 20% solution in benzene, including aniline, butylamine, propylamine, dibutylamine, diethylamine, piperidine, triethylamine and tributylamine. Only triethylamine gave good resolution combined with long-term storage capability.

The triethylamine-impregnated sheets gave R_F values of 0.75, 0.66 and 0.38 for 1 μg of cannabidiol (CBD), Δ^6-tetrahydrocannibinol (THC) and cannabinol (CBN) standards, respectively. Colors with Fast Blue B were orange for CBD, red for THC, and violet for CBN. The plates required no heating for development of color. The colored spots were stable indefinitely.

TABLE I

R_F VALUES FOR 1 μg OF CANNABINOIDS ON TRIETHYLAMINE-IMPREGNATED SHEETS AFTER VARYING STORAGE PERIODS

Storage time	*CBN*	*THC*	*CBD*
15 min after dipping	0.38	0.66	0.75
4 h	0.35	0.66	0.70
1 day	0.35	0.64	0.73
5 days	0.37	0.67	0.73
5 weeks	0.31	0.55	0.62
10 weeks	0.34	0.60	0.65

A study was undertaken to determine the period of usefulness of the triethylamine-impregnated plates. The results are shown in Tables I and II. As can be seen, the R_F values of the standards decrease slightly after ten weeks of storage of the aluminum foil-wrapped plates in a drawer at room temperature but results show that

TABLE II

R_F VALUES RELATIVE TO CBN (R_F = 100) AFTER VARIOUS STORAGE PERIODS OF THE IMPREGNATED PLATES

Storage time	R_F	
	THC	*CBD*
15 min after dipping	174	197
4 h	188	200
1 day	183	209
5 days	181	197
5 weeks	186	200
10 weeks	176	191

the R_F differences relative to CBN are virtually unchanged for at least ten weeks of storage.

Extracts of various plant materials that have been reported to be used either to dilute or to substitute for marijuana[7], were subjected to chromatography along with authentic marijuana. Binaca® (Ciba-Geigy, Summit, N.J., U.S.A.), a commercial product which gave a positive Duquenois–Levine test, was also run by directly spotting 1 μl of the condensed spray. The chromatograms are shown in Fig. 1.

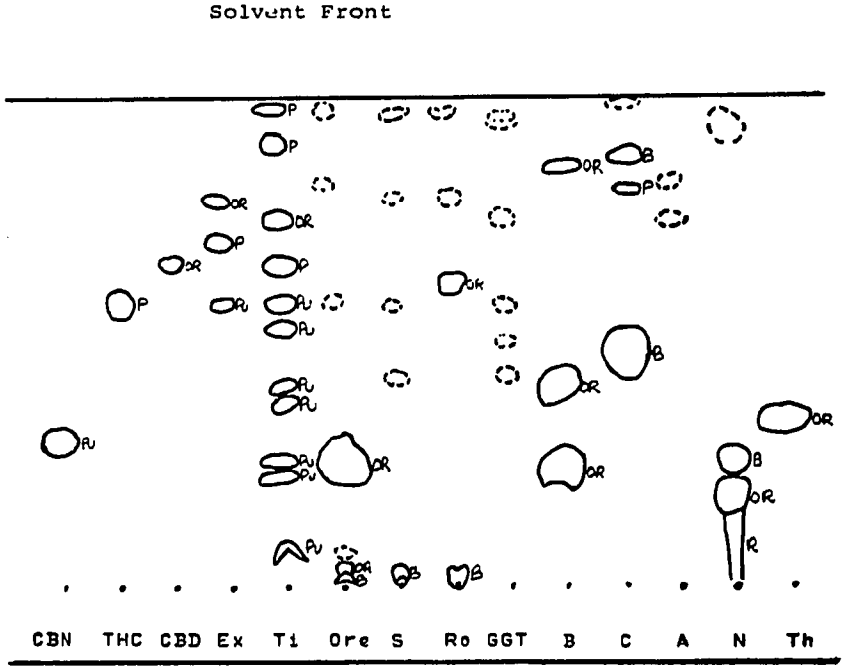

Fig. 1. Chromatography, with benzene, of standards and samples on triethylamine-impregnated silica gel. CBN = Cannabinol; THC = tetrahydrocannabinol; CBD = cannabidiol; Ex = marijuana extract; Ti = marijuana tincture; Orc = oregano; S = sage; Ro = rosemary; GGT = Gunpowder green tea; B = Binaca®; C = cloves; A = alfalfa; N = nutmeg; Th = thymol. P = Pink; Pu = purple; Or = orange; B = brown. A spot shown as a dotted line was colored yellow or green before spraying with Fast Blue B and did not change color after spraying.

None of the materials thus tested have spots that are a combination of similar R_F values and similar colors to that of marijuana. Cloves give a brown spot in the region of the major cannabinoids; rosemary gives an orange color similar to CBD, but at a slightly lower R_F. Gunpowder green tea has three green and yellow spots in the area of the major cannabinoids, but these spots could be seen before the spraying with Fast Blue B. Caraway seeds and menthol give no spots anywhere on the plate and thus are not shown in Fig. 1.

In summary, the described procedure has been shown to be an excellent technique for detecting and identifying the constituents of the marijuana. Plates prepared by this method can be used after storage for extended periods of time.

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