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Note

Thin-layer chromatography of cannabinoids

R. FOWLER*, R. A. GILHOOLEY and P. B. BAKER**
Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London SE1 9NQ (Great Britain)
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Many separations of cannabinoids by thin-layer chromatography (TLC) have been described and have recently been discussed by Crombie[1] and Mechoulam *et al.*[2]. In the main, only two types of thin-layer system have been found to give good results; the first is based upon modification of the absorbent properties of silica gel by the presence of bases and the second uses silica gel modified by silver nitrate. A simple system described by De Faubert Maunder[3], and still used in this Laboratory, whilst being valuable for preliminary screening, does not resolve tetrahydrocannabinol (THC) from tetrahydrocannabivarinol (THV) or cannabinol (CBN) from cannabivarinol (CBV); furthermore, a recent change by the manufacturers in the binder incorporated into the silica gel sheets used for this method resulted in a serious loss of resolution, thus making it necessary for a new system to be designed. For the same reasons a new two-dimensional thin-layer chromatography (2D-TLC) system has superseded that which was previously employed[4]. A modification of this new system has also been devised which provides more information on the acidic components of the extract.

MATERIALS AND METHODS

Sample preparation

The extracts for TLC were prepared by adding light petroleum (b.p. 40–60°) to the sample (approximately 1 volume of cannabis or cannabis resin to 1 volume of solvent) as previously described[3]. Approximately 0.5 μl of solution is used for analysis, keeping the spot diameter below 1 mm.

Solvent systems

Two solvent systems were devised for TLC of the cannabinoids:

(1) chloroform (ethanol-free)–1,1-dichloroethane (15:10);

(2) xylene (mixed isomers)–1,4-dioxan (19:1).

Ethanol-free chloroform was prepared by standing chloroform (AnalaR grade) over anhydrous granular calcium chloride for 24 h. The resolution using solvent system

* Present address: Boots Ltd., Nottingham, Great Britain.
** To whom correspondence should be addressed.

2 (used for 2D-TLC) was greatly improved by spraying the plate with diethylamine before development.

TLC procedure

Simple ascending TLC was carried out on 5 × 10 cm silica gel pre-coated plates with a layer thickness of 0.25 mm (Merck, Darmstadt, G.F.R., Art. 5719) in solvent system 1.

2D-TLC was carried out on 10 × 10 cm silica gel pre-coated plates with a layer thickness of 0.25 mm prepared by cutting 10 × 20 cm plates (Merck, Art. 5729).

The first development was made in solvent system 1, after which the plate was dried, sprayed with diethylamine and then developed a second time at right-angles to the first in solvent system 2. The amount of diethylamine was found not to be critical, provided that the plate was not soaked.

Modified 2D-TLC was carried out similarly to normal 2D-TLC except that after the first development, the plate was heated in an oven at 150° for 5 min, cooled, sprayed with diethylamine and then developed in solvent system 2.

Detection

After all of the TLC analyses described below, the plates were air-dried and sprayed with Fast Blue BB as previously described[5].

RESULTS AND DISCUSSION

The R_F values of the principal cannabinoids separated by the two solvent systems described are listed in Table I. Identification of the separated components was by direct comparison with standard substances or by mass spectrometry. In solvent system 1, good separation between cannabidiol (CBD), THC and THV is obtained; the ratios of the amounts of these three components varies widely with the origin of the sample and consequently this new system is of value in indicating the geographical origin of cannabis samples. Confirmatory evidence is subsequently obtained using other chromatographic techniques. Furthermore, as the sample ages, THC and THV are converted by a slow oxidation process to CBN and CBV, respectively; solvent system 1 separates these products from their parent compounds.

TABLE I

R_F VALUES OF THE CANNABINOIDS

Compound	*Solvent system*		*Colour (after detection)*
	1	*2**	
Cannabinoid acids	0.00–0.25	0.00–0.05	Red-orange
Cannabichromene	0.38	0.32	Purple**
Cannabigerol	0.41	0.39	Orange
THV	0.45	0.52	Red
CBV	0.50	0.47	Purple
THC	0.55	0.60	Red
CBN	0.60	0.39	Purple
Cannabidiol	0.63	0.65	Yellow

* After spraying with diethylamine.

** This colour changes to orange-brown after approximately 1 h.

It is often necessary to compare two or more cannabis samples to establish (or refute) a connection between them: 2D-TLC has proved very useful for the rapid and simultaneous screening of samples prior to detailed comparison by high-performance liquid chromatography (HPLC)[6]. When this new 2D-TLC system is used, resolution of about 15–20 components is achieved, which is far superior to previous methods. This enables detailed comparisons to be made between samples prior to quantitative analysis by other methods.

In both solvent systems 1 and 2, the principal cannabinoid acids (cannabidiolic acid, tetrahydrocannabinolic acid and tetrahydrocannabivarinic acid) run together at low R_F. During the heating stage of the modified 2D-TLC system, these acids decarboxylate to the cannabinoids on the plate. Therefore, after the second development and detection of the separated cannabinoids, qualitative information on the acids present in the original sample may be obtained. As during smoking these acids decarboxylate in a similar manner, this procedure gives some information on the "quality" of the cannabis being examined, which is not revealed by the unmodified system. The technique is also useful as an aid to the identification of constituents separated by HPLC, where no reference compounds are available. By examining such fractions by TLC any cannabinoid acids are readily distinguished from the cannabinoids themselves.

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