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Two-dimensional thin-layer chromatography of ganja (*Cannabis sativa* L.)

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ABSTRACT

An efficient and reliable two-dimensional thin-layer chromatographic technique for separation and identification of cannabinoids present in cannabis resin (*Cannabis sativa* L.) is described. A total of 47 different cannabinoids were successfully separated and 5 of these, cannabinol, cannabidiol, cannabichromene, *trans*-delta-8-tetrahydrocannabinol and delta-9-tetrahydrocannabinol, were identified. A 0.1 per cent solution of Fast blue salt B in 45 per cent ethanol was employed as chromogenic reagent.

Introduction

The flowering tops of *Cannabis sativa* L., popularly known as ganja, represent the most widely found cannabis preparation. Its illicit trafficking and consumption have markedly increased throughout the world in recent years. Many workers have reported their work on cannabis using chromatographic methods. Grlic [1] preferred amine-treated thin-layer chromatography (TLC) plates whereas Chiesa, Rondina and Coussio [2] described a thermomicro TLC procedure for the identification of cannabinoids. Using multiple development and impregnation, Bertulli, Mosca and Pedroni [3] identified delta-9-tetrahydrocannabinol (THC) and cannabinol and some other researchers [4–10] also utilized TLC for their purpose. Mobarak, Zaki and Bieniek [11] and Fowler, Gilhooley and Baker [12] reported two-dimensional (2D) TLC of cannabis. The available literature reveals, however, that most earlier workers confined their efforts mainly to unidimensional TLC and very few of them employed 2D-TLC. The objective, therefore, was to develop a suitable 2D-TLC system which would enable separation and identification of a larger number of cannabinoids.

Experimental

An amount of 0.5 g of fresh ganja was taken in 50 ml chloroform with 10 drops of glacial acetic acid to make the medium faintly acidic. The mixture was kept for half an hour at room temperature and filtered. The

filtrate was evaporated at temperatures below 50° C to 0.5 ml and subsequently dried completely with a stream of hot air. The residue was then dissolved in 1 ml chloroform for spotting on TLC plates.

The glass plates (20 cm × 20 cm) were coated with a 0.25 mm thick layer of silica gel G slurry (30 g gel + 65 ml water), dried at room temperature and ultimately activated at 110° C for 40 min before use. The plates were spotted with 80 µg of cannabis resin in *n*-hexane at a common point 2.5 cm from the two sides of a plate on diagonal plane. A quantity of 5 µg each of 5 authenticated samples of cannabinol (6,6,9-trimethyl-3-pentyl-6H-dibenzo-(b, d)-pyran-1-ol), cannabidiol (3′-methyl-6′-prop-2-enyl-4-pentyl-1′,4′,5′,6′-tetrahydrodiphenyl-2,6-diol), cannabichromene (5-hydroxy-2-isohex-3-enyl-2-methyl-7-pentylchromene), *trans*-delta-8-tetrahydrocannabinol (6,6,9-trimethyl-3-pentyl-6a,7,10,10a-tetrahydrodibenzo-(b, d)-pyran-1-ol) and delta-9-tetrahydrocannabinol (6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydrodibenzo-(b, d)-pyran-1-ol) were also spotted separately at 2.5 cm above the plate edge near two adjacent corners but at points which were at least 12 cm from the point of sample application.

The TLC plates were first developed (by the ascending technique) in direction I, using a heptane/dichloromethane/butan-2-one (83/5/12 volume ratios) solvent system which was allowed to saturate the developing chamber for one hour. When the solvent front reached 12 cm, the plates were dried at room temperature, rotated 90° and redeveloped in direction II using *n*-hexane/acetone (86/14 by volume). The ambient temperature was 25° C.

These two-dimensionally developed plates were first viewed under short uv light (254 nm). The fluorescent colours of resolved spots were recorded and the plates were then sprayed with a 0.1 per cent solution of Fast blue salt B (3,3′-dimethoxybiphenyl-4-4′-bisdiazonium chloride) in 45 per cent ethanol as the chromogenic reagent. The different colours of resolved spots along with their respective migration distances in the two directions were recorded (see table and figure).

Results and discussion

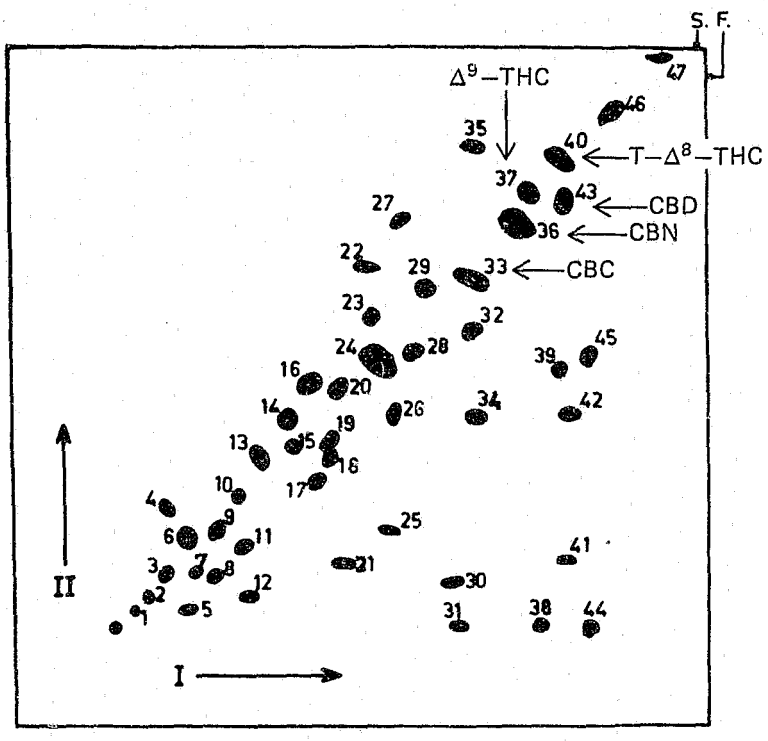
The 2D-TLC of ganja yielded clearly separated cannabinoid spots. The solvent systems used for development possessed excellent resolving properties and as many as 47 different cannabinoids were successfully separated (see table and figure). Of these, 5 major cannabis ingredients, i.e., cannabinol (CBN), cannabidiol (CBD), cannabichromene (CBC), trans-delta-8-tetrahydrocannabinol (T-Δ^8-THC) and delta-9-tetrahydrocannabinol (Δ^9-THC) were identified using authentic controls.

The spray of 0.1 per cent solution of Fast blue salt B in 45 per cent ethanol was found to be an extremely effective and specific chromogenic reagent and it gave distinct colours with different cannabinoids. The colours

Two-dimensional thin-layer chromatographic separation of ingredients of ganja (*Cannabis sativa* L.)

Reference No.	*Cannabinoid*[a]	*Spot migration (mm)*		*Colour of spot*[b]	
		Direction I	*Direction II*	*Under uv light (254 nm)*	*With Fast blue salt reagent*
1		3	3		Purple
2		6	6		Magenta
3		10	11	Red	Violet
4		10	24	Pale yellow	Faint violet
5		14	3		Pink
6		14	18		Violet
7		16	11	Sky blue	Orange
8		20	10		Purple
9		20	20	Red	Purple
10		25	27		Pink-orange
11		26	16		Purple
12		27	6	Red	Violet
13		29	35		Violet
14		35	43		Yellow
15		36	37		Pink
16		39	50	Red	Magenta
17		41	30		Pink
18		43	35		Brown
19		43	38		Blue-violet
20		45	49	Sky blue	Blue-violet
21		46	13		Pink
22		51	75		Pink
23		52	64		Pink
24		53	55		Violet
25		55	20		Blue-violet
26		57	44		Pink
27		58	84		Pink
28		60	57		Pink
29		63	70		Purple
30		68	9		Purple
31		69	0		Pink
32		72	61		Pink
33	CBC	72	72		Yellow
34		73	43	Dark	Purple
35		73	99		Pink
36		81	83	Sky blue	Pink
37	Δ^9-THC	84	90	Dark	Deep violet
38		80	0		Purple
39		90	50		Purple
40	T-Δ^8-THC	90	97	Dark	Magenta
41		91	14		Pink
42		91	44		Pink
43	CBD	91	88	Dark	Orange
44		96	0		Pink
45		96	56	Sky blue	Purple
46		101	106	Sky blue	Purple
47		111	117		Purple

[a] No entry means ingredient was not identified. [b] No entry means spot was colourless.



Figure

Two-dimensional thin-layer chromatographic separation and identification of ingredients of ganja (*Cannabis sativa* L.)

of CBN, CBD and T-Δ^8-THC were so distinct, prominent and dense that these ingredients could be recognized by their colours alone: deep violet, orange and magenta.

The proposed technique, besides giving the largest number of cannabinoids resolved on a single chromatogram, has the added advantage that it completely avoids plate impregnation and makes use of readily available materials. It was found to be sensitive, reliable and reproducible. The best results were observed at 25° C.

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