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Use of descending thin layer chromatography for identification of cannabinoids

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ABSTRACT

The article describes a technique that uses descending thin layer chromatography (TLC) for identification of cannabinoids. The technique employs a partition system of two-dimensional descending TLC, in which toluene is used as the eluting solvent. The quantity of cannabinoids obtained by TLC has been confirmed by gas chromatography (GC). The technique presented in the article has proved useful for the analysis of cannabinoids.

Introduction

Samples of cannabis often include cigarettes, cheroots, cigars, dry plants, green dry plants, charas and smoking pot. Many techniques that use ascending thin layer chromatography (TLC) for analysis of cannabinoids have been described in the literature of recent years [1 - 12], but insufficient attention has been given to the use of the descending technique [13]. Chemical analysis of cannabis requires improved methods for the identification of cannabinoids.

Methods and materials

Thin layer chromatography

Cannabinol, cannabidiol, Δ^9 -tetrahydrocannabinol (THC) and Δ^8 -THC were obtained from the Narcotics Laboratory Section of the Division of Narcotic Drugs and were used as reference substances in this analysis. A 20 x 60 cm TLC aluminium roll precoated with silica gel F₂₅₄ (layer thickness 0.20 mm) was used with the necessary chromatography units for the analysis. The roll was impregnated with dimethylformamide and dried with a hair dryer for 10 minutes; 10 x 20 cm impregnated plates were used for both one- and two-dimensional TLC. Toluene was used as the eluting solvent for descending and the spots were visualized by spraying with methanolic fast B salt solution. The solvent was allowed to descend until its

front advanced 11 cm along the direction of 20 cm. The solvent reached the descending distance of 20 cm for approximately 80 minutes, while it reached 10 cm of descending distance for 23 minutes.

The geometrical form of the plates used in descending TLC are described below. A plate in the form of an isosceles triangle with sides 21 cm, 21 cm and 10 cm in length was used for slow partition descending chromatography as shown in figure I.

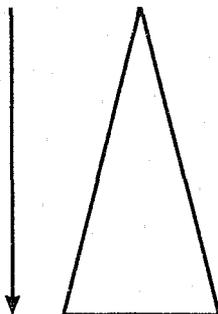


Figure I

Plate for slow partition descending chromatography

The geometrical form and the length of sides of the plate used for fast partition descending chromatography are shown in figure II.

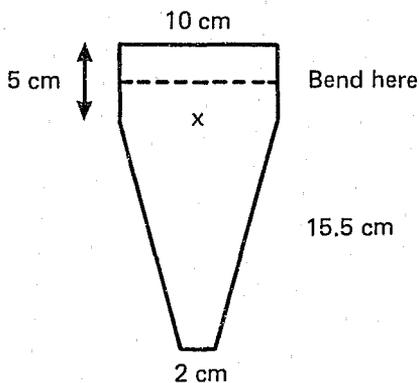


Figure II

Plate for fast partition descending chromatography

The geometrical form and the length of sides of the plate used for dual fast and slow descending chromatography are shown in figure III.

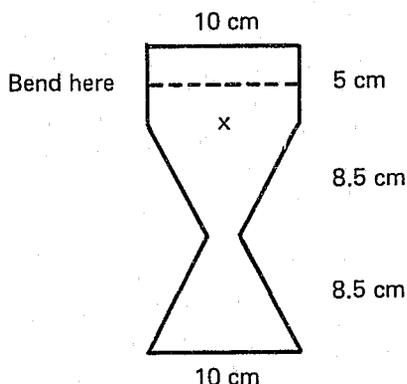


Figure III

Plate for dual fast and slow descending chromatography

Assignments of spots for cannabicyclol (CBC) and cannabichromene (CBCh) were based on published Rf data ([2], p. 145).

Gas chromatography

A suitable gas chromatograph was used. The glass columns (6 ft \times 0.08 in ID) were packed with 3 per cent OV-17 on Gas Chrom Q, 100–120 mesh; column temperature was 240°C and injector detector temperature 290°C; 6 μ l of a petroleum ether extract made up to 2 mg/ml were injected onto the column. By means of a recorder, relative retention times for CBC, CBCh, cannabidiol, Δ^8 -THC, Δ^6 -THC, Δ^9 -THC, cannabigerol (CBG) and cannabinal with respect to methadone hydrochloride were measured as 1.2, 1.5, 1.8, 2.1, 2.3, 2.5, 2.8 and 3.3, respectively. CBC, CBCh and CBG were identified on the basis of published data [2].

Results and discussion

The technique described above (see figures I, II and III) and one-dimensional descending chromatography were used in parallel to assist in the detection of 13 cannabinoids that were identified by two dimensional descending chromatography. The cannabinoids and the colours that identify them are the following: cannabitril ester of cannabidiolic acid (violet), cannabidiol (orange), cannabidivarol (orange-brown), cannabivarol (violet-brown), tetrahydrocannabivarol (crimson-brown), cannabiorcol (violet-brown), cannabinal (violet), Δ^9 -THC (crimson), Δ^8 -THC (crimson), Δ^6 -THC (orange-crimson), CBG (orange), CBCh (orange) and CBC (orange-crimson). The quantities of these cannabinoids obtained by two dimensional descending chromatography were comparable to the results obtained by GC.

Slow partition descending column chromatography can yield better results in the identification of cannabinoids when amberlite XAD 2 resin is used. When coiled fast partition columns in GC are replaced by a dual fast-slow chromatographic type of column it results in better separation of two similar substances which are analyzed, and such separation is better performed in dual than in fast partition chromatography. The advantage of two-dimensional partition descending TLC is that it shows more distinct cannabinoid spots than two-dimensional adsorption (that is, without impregnation) ascending TLC using toluene for elution in one direction (first front) and hexane:dioxane (9:1) for elution in the perpendicular direction (second front).

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