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## Note

# New approach to the visualization and identification of cannabinoids in plant material by thin-layer chromatography

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In the analysis of cannabinoids in plant extracts by thin-layer chromatography, the most suitable procedure for visualizing them is spraying with Fast Blue B salt[1]. Because many compounds related to this chromogen have been shown to be mutagenic or carcinogenic, it was thought that spraying the chromatogram, even in a hood, was risky and undesirable. Accordingly, a new procedure was developed in which the thin-layer plate was dipped prior to the application of the plant extract. This modified procedure produced some unusual, unanticipated results.

## EXPERIMENTAL

### *Plates*

Miniature thin-layer plates, approximately 5 cm high and up to 5 cm wide, were cut from Eastman Chromatogram Sheets coated with Silica Gel and Fluorescent Indicator.

### *Fast Blue B dipping solution*

Approximately 500 mg of Fast Blue B salt (Diazo Blue B) (ICN Pharmaceuticals) were dissolved in 20 ml of 70% ethanol. The solution was prepared fresh daily.

### *Cannabinoids*

Extracts were prepared from marihuana plant material (*Cannabis sativa*) by grinding approximately 5 g of plant material with 20 ml of light petroleum (b.p. 38–55°C) in a mortar and pestle. The plant extract was filtered through Whatman No. 1 filter paper and stored in a stoppered glass bottle at 4°C. Cannabinol (CNB) and $\Delta^9$-tetrahydrocannabinol (THC) standards were obtained from Applied Science Labs. (State College, PA, U.S.A.).

### *Dipping and spotting procedure*

The minature chromatographic sheets were dipped in the Fast Blue B salt solution to a depth of approximately 0.75 cm. Excess liquid was removed by touching the bottom edge to absorbent paper. The plates were dried with a hair dryer. Extracts and standards (*ca.* 0.5 $\mu$l) were spotted on the dipped portion of the plates using 2-$\mu$l

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pipettes. Using 3 ml of chloroform–light petroleum (60:40) in a 9 × 4 × 9 cm tank, the plates were developed by ascending chromatography to a height of 4 cm. With this procedure, no further visualization was necessary, since the cannabinoids had already reacted with the dye during their application.

## RESULTS AND DISCUSSION

Fig. 1 is a chromatogram produced by the usual procedure, *i.e.*, development followed by spraying with the chromogen. Purified THC (left) migrates as a single component, red after spraying. Cannabinol (center) also migrates as a single component, but is purple after spraying. A light petroleum extract of marihuana plant material partitions into four discrete components. After spraying, the leading component is orange and the rest appear red or red-purple. The major component in the mixture corresponds to THC.

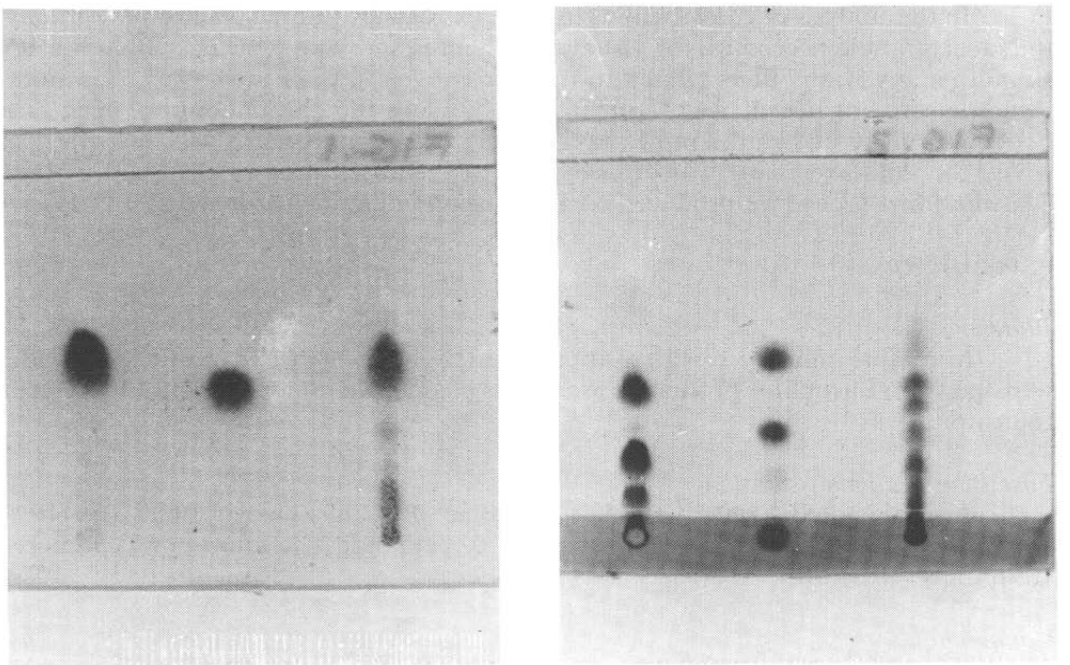

Fig. 1. Chromatogram of cannabinoids visualized by spraying with Fast Blue B solution. Tetrahydrocannabinol is at the left; cannabinol is at the center; extract of *Cannabis* is at the right.

Fig. 2. Chromatogram of cannabinoids developed on plates predipped in Fast Blue B solution. Compare with Fig. 1.

When the plates were predipped in the chromogen *prior* to spotting, the results seen in Fig. 2 were obtained. Instead of a single spot, THC separated into five separate components which are colored, in ascending order, red, red, faint pink, red, and orange. Similarly, when CNB was so treated, it separated into four discrete spots, with the leading spot colored orange and the other three purple. At least six components are found in plant extracts chromatographed in this fashion.

In order to rule out artifacts resulting from predipping in the chromogen, individual undipped plates (5 × 5 cm) were spotted in the corner with THC or CNB. After development to 4 cm, the plates were masked so that only the paths of the migrating compounds were exposed, and then sprayed with Fast Blue B solution. As

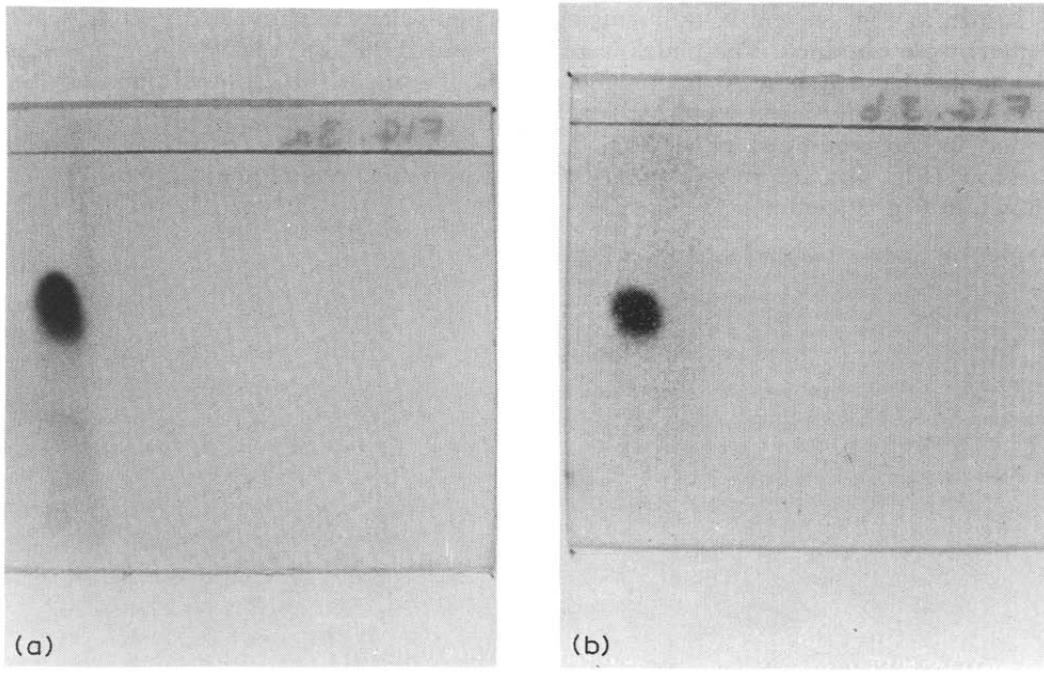


Fig. 3. One-dimensional chromatogram of THC (a) and CNB (b). After development, the plates were masked and only the path of migration was sprayed.

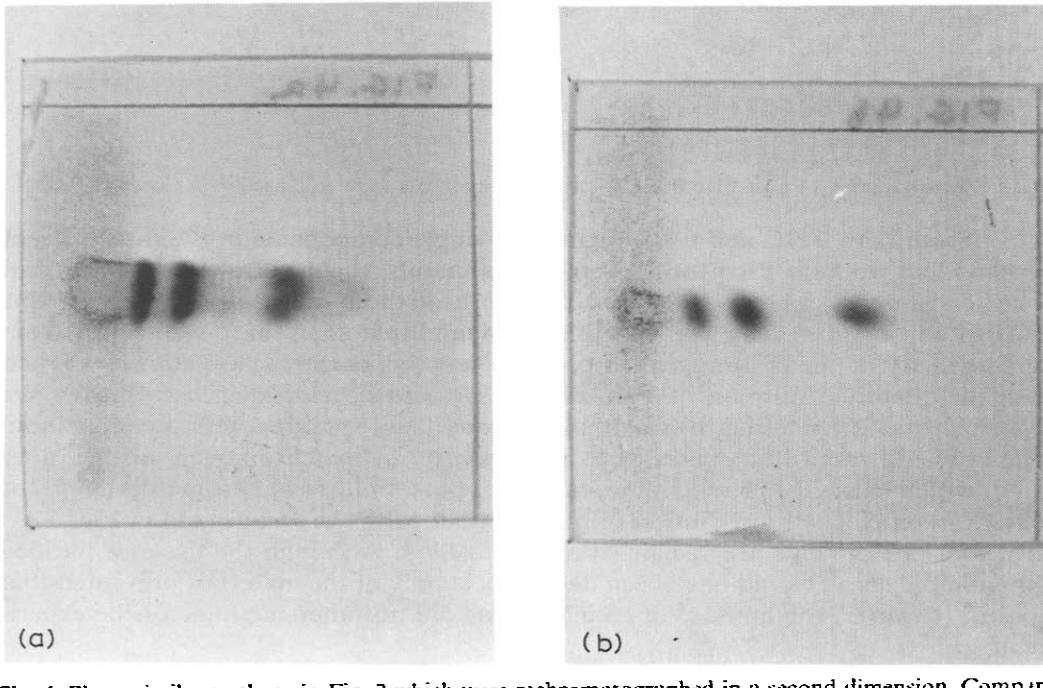


Fig. 4. Plates similar to those in Fig. 3 which were rechromatographed in a second dimension. Compare with Figs. 3 and 2.

is shown in Fig. 3a and b, only single spots equivalent to those in Fig. 1 (left and center) were obtained. The plates were then rotated 90° and rechromatographed in the same solvent system. As is shown in Fig. 4a, the single THC spot visualized by the *spray* separated into four bands, colored red, red, red, and orange, similar to the spots found in the predipped plate (Fig. 2, left). When CNB was treated in a similar fashion, (Fig. 4b), the purple spot separated into four components similar to that shown in Fig. 2 (center).

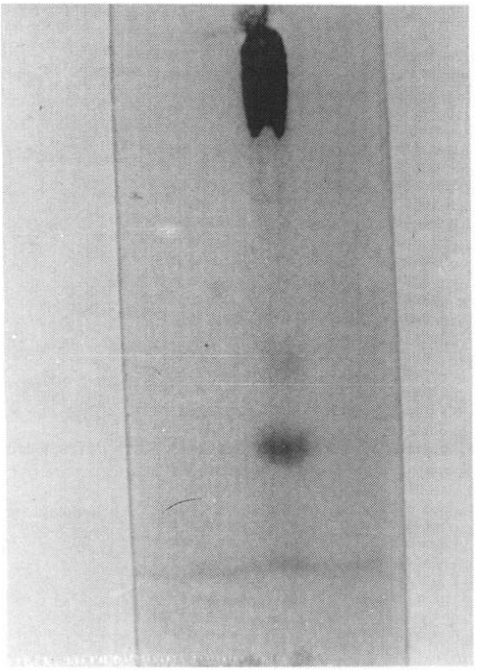

Fig. 5. Chromatogram of Fast Blue B salt solution. Components were visualized with *Cannabis* extract.

Since both THC and CNB migrate as single components in the three solvent systems that we have used for TLC of cannabinoids, (light petroleum–chloroform; toluene; benzene), we are persuaded that both of these materials are pure. If this assumption is valid, then the appearance of multiple spots may well result from heterogeneity of the chromogen. In order to test this question, a plate was spotted with the ethanolic solution of Fast Blue B salt and developed in acetonitrile. We reasoned that if Fast Blue B was homogeneous, then spraying with a cannabinoid extract would result in a single spot; heterogeneity of the chromogen, on the other hand, would cause the production of multiple spots. Following chromatography, the plate was sprayed with a solution of plant extract, with the results seen in Fig. 5. At least four components are visualized in this fashion. Although the use of a purified cannabinoid spray would have been desirable, supply of the materials prevented that option. However, the mixture of cannabinoids did not alter the logic of the experiment.

These results lead us to conclude that Fast Blue B is not homogeneous. The nature of the various components is not known and requires further elucidation.

REFERENCE

1 Egon Stahl, *Thin-Layer Chromatography*, Springer, New York, 1967, p. 716.