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Note

Preservation of cannabis thin-layer chromatograms

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It is frequently necessary to preserve cannabis thin-layer chromatograms for forensic or reference purposes. A major problem is preservation of the developed colours in their original bright condition. It was noted during routine screening of cannabinoid contents[1] that two-dimensional chromatograms frequently developed brighter and more stable colours, but the effect was not reproducible. These observations have been re-examined and three major factors have now been identified: other laboratories may establish their own parameters if a different chromatographic system is preferred. The three factors are chromogen basicity, residual solvent, and chromogen dyestuff.

MATERIALS AND METHODS

Conditions

The chromatographic conditions are essentially as previously described[1,2] and confirmed by Grlić[3].

Apparatus

Any convenient glass vessel with a lid to exclude draughts can serve as a tank. 250- and 400-ml beakers with Petri dish lids are most practical for 5 × 5 and 5 × 10 cm sheets, respectively.

Reagents

The sorbent used is Eastman "Chromagram 6061" (silica gel on polyester sheets without fluorescent indicator). Toluene or xylene can be used as the mobile phase. Keep in dark glass bottles and replace or redistil if a pronounced yellow background is produced on the chromatogram. Diethylamine is used as mordant/bleach reagent. Some improvement in colour intensity may be noted by adding up to 5% morpholine. As chromogen 0.5 g Fast Blue BB salt dissolved in 50 ml water is used, diluted to 100 ml with methanol. These concentrations are not critical and the prepared reagent is stable for several hours. Stability may be further improved by the addition of one drop of 2 *N* hydrochloric acid[4].

Recommended procedure

Spot sample solutions in multiples of 0.25 μl to 0.5 μl with a maximum spot diameter of 1 mm.

Develop the chromatogram to the desired degree of resolution using a solvent run of 4 cm for routine screening and up to 9 cm for higher resolution. Remove the chromatogram from the tank and allow to dry naturally, *e.g.*, at the back of a fume cupboard. Fill two separate spray guns with mordant/bleach and chromogen. Spray the chromatogram with diethylamine as soon as excess chromatographic mobile phase has evaporated (as observed by the disappearance of absorbent translucence). Do not overspray with diethylamine, nor hold the spraygun too close, to avoid excessive solvent evaporation. Immediately, spray the chromatogram with chromogen using a spray intensity that will dampen but not soak the sheet. In routine practice this procedure will usually produce chromatograms that are stable indefinitely. Where minor components must be seen with clarity, or it is known that the chromatograms must be stored indefinitely, it is advisable to continue spraying with chromogen to a point where the sheet is only just soaked. This is observed as spots of translucence. Then bleach out the yellow background colour by respraying with diethylamine, and allow the chromatogram to dry naturally in air. The observed colours are generally weak and insipid in tone at this stage and require up to an hour to develop their full brightness. Allow the chromatogram to dry in diffuse daylight, or in the dark, and store in the dark.

A more rapid colour development may be obtained by carrying the chromatogram through two or three further cycles of chromogen spray followed by bleaching. It is essential throughout to avoid over-wetting to prevent spot diffusion. It is also important to ensure that drying occurs with residual base (amine) present. Even in alkaline conditions it will be noted that initial colour development is considerably slower than with Fast Blue B, although the final colour is more intense. The reagent is ultimately capable of detecting samples too weak to respond to Fast Blue B.

On a routine basis, it may be found more convenient to transfer developed chromatograms from the developing chromatotank to a further tank containing a trough of diethylamine. This will allow, without excessive attention, toluene to evaporate from the sheets whilst absorbing diethylamine. After 10 to 20 min remove the chromatogram from the second tank and spray with chromogen as before. In either case, the chromatograms will keep in a "bright" condition indefinitely.

RESULTS AND DISCUSSION

Chromogen basicity

A number of published methods, derived from the work of Korte and Sieper[5], recommend the addition of sodium hydroxide to the aqueous solution of azo dye to obtain rapid colour development. It has been found difficult to avoid excessive wetting, with consequent spot diffusion. More control was achieved in neutral solutions by the addition of a high proportion of methanol[2]. However, direct addition of a base to the methanolic solution induced self-coupling, essentially complete in a minute, and rendered the reagent inoperative. Consequently, it is necessary to add a base separately.

A base is automatically added with the reversed-phase system of Korte and Sieper[5] and Beckstead and French[6], or in alkaline systems such as that of Aramaki

et al.[7]. Grlić[3] combined both approaches by pretreating his plates with an amine such as diethylamine or dipropylamine. It has been shown that such base-containing systems are unsatisfactory for precise identification of the major cannabinoids because of spot overlap[1]. This observation led to the conclusion that the base should be applied after the solvent run and before the chromogen spray. Any organic base will suffice as the mordanting agent. Diethylamine is preferred since it is available in quantity at low cost, and its low boiling point will allow it to be sprayed as a liquid and to diffuse as a vapour through the thin-layer chromatographic support. In use it is less objectionable than the majority of amines. More intense colours are observed if a proportion of a strong base, such as morpholine, is added.

Residual solvent

The time allowed for solvent evaporation from the chromatogram is very important. If the absorbent is thoroughly dried, poorer colours are observed. Colour development is best carried out before complete solvent evaporation from an essentially non-aqueous medium. It is presumed that the residual solvent molecules remain bound to the active sites on the support and allow a more effective competition between dye–cannabinoid complex and water molecules on the hydrophilic silica gel. In predominantly aqueous conditions, the dye complex may be precipitated from solution, rather than adsorbed, and thereby account for the observed colour differences.

Toluene or xylene are preferred mobile phases and have adequately low volatility and high hydrophobic properties for intense colour development. The best colour permanence was found when the chromogen spray was applied after evaporation of surplus solvent, but before total volatilisation. This was observed to be the period after the support loses its translucent appearance, and before the wave of secondary drying —seen as a white line— progresses appreciably down the chromatogram. At an ambient temperature of 20°, without forced air drying, this working period was found to be up to 5 min on "Chromagram" sheets, which is ample in practice.

Chromogen dyestuff

Fast Blue B salt is widely used as a chromogen. It develops colours of adequate intensity for most purposes, although batches can vary considerably. Nevertheless, the safety of this dyestuff is still sometimes questioned —essentially because of the risk of the diazonium salt containing residual, unreacted, potentially carcinogenic, free amine. Fast Blue RR is already used as an alternative[6,8] but has much lower sensitivity and response speeds than Fast Blue B.

A number of other alternative dyestuffs were screened for use in a field test reagent and many of these are suitable as cannabinoid chromogens. Fast Blue BB was found to be superior to Fast Blue B[9] both in sensitivity and improved vividness, although slower in response speed. Thornton and Nakamura[10] also investigated alternative dyes and selected Fast Blue BB (2B) as their preferred chromogen. They found that Fast Blues B and BB gave identical colours, and response speeds. The apparent inconsistency in the two results has been identified as differences between manufacturer's batches, and in the method of chromogen application.

Thus, Thornton and Nakamura[10] concluded that Fast Blue BB is less photosensitive than Fast Blue B because plates sprayed with the latter turned deep yellow and then light brown upon exposure to sunlight. It was found in this laboratory that

both dyes were approximately equally photosensitive and that supplies of Fast Blue B available in Great Britain did not exhibit this darkening except when applied in excess, or to thoroughly dried plates. Also, one batch of Fast Blue BB that was marginally more sensitive than another batch from the same supplier still exhibited this darkening phenomenon to a more marked degree than Fast Blue B when applied in excess. All dyes when applied in excess will develop, to some extent, an initial yellow background, before photodarkening.

For long-term storage of chromatograms the yellow background must be "bleached". This is achieved by over-spraying with extra base which incidentally improved the response speed. It also improves dye mordanting to the acidic sites on the silica gel to yield more vivid colours. It has been found that a single addition of the base only at this later stage is less efficient as a bleaching/mordanting agent than addition prior to the chromogen: the colours are less vivid.

Whilst, with the recommended procedure, Fast Blue BB initially yielded comparable colour intensity and speeds to Fast Blue B, the colour intensity improved considerably during the next hour or so. Drying preferably should be natural and in the dark. The ultimate sensitivity and colour clarity was significantly better than with Fast Blue B. Provided the chromatogram was stored away from acidic vapours, and in the dark, it will keep in its initial "bright" condition indefinitely: long-term storage trials were considered complete after 5 years.

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