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## Twin-film chromatography of gibberellins

This communication describes how gibberellins $A_1$–$A_9$ and $A_{13}$ can be separated on a single chromatogram on two adjacent thin films of silica gel and kieselguhr (twin-films). The method is based on the report by MACMILLAN AND SUTER[1] that the solvent system benzene–acetic acid–water (8:3:5 v/v) separates gibberellins $A_1$–$A_3$ and $A_8$ on kieselguhr and gibberellins $A_4$–$A_7$ and $A_9$ on silica gel. Thus if the gibberellins are applied to the kieselguhr of a kieselguhr–silica gel twin-film and a chromatogram developed such that the solvent migrates first through the kieselguhr and then the silica gel these nine gibberellins and gibberellin $A_{13}$ are separated on one chromatogram. The less polar gibberellins $A_4$–$A_7$ and $A_9$ migrate through the kieselguhr near or with the solvent front and are separated on the silica gel.

*Experimental*

To make twin-films the usual procedure for making thin-films is followed but a perspex partition is fitted with water-tight joints across the width of the spreader (Shandon Scientific Co.) dividing it into two compartments. One is filled with a slurry of Merck Silica Gel G and the other with a slurry of Merck Kieselguhr G (20 g silica gel or kieselguhr in 40 ml water for five 20 × 20 cm films of 300 $\mu$ thickness). The bottom edge of the partition is milled to a point to ensure that when the slurries leave the spreader, they meet, forming two adjacent films with a sharp interface. The films are activated at 120° for 40 min.

The solvent system benzene–acetic acid–water (8:3:5 v/v) which forms two phases, is best mixed, separated into two phases, stored, and used in a constant temperature room or incubator (15–30°). The twin-film with the gibberellins applied onto the kieselguhr is equilibrated (overnight at 15–25°, or for 6–8 h at 30°) with the vapour from the bottom phase. To do this, kieselguhr is scraped off the edge of the plate below the gibberellins and the plate stood in a small volume of the bottom phase in a sealed tank. The use of filter paper around the sides of the tank to aid saturating the atmosphere with vapour is inadvisable as it later causes unequal development of the chromatogram across the plate. After equilibration, the top phase is carefully added through a funnel and tube onto the bottom phase until it reaches the kieselguhr when it acts as migrating solvent. The plate is removed from the tank when the solvent has nearly reached the upper free edge of the silica gel, approx. 50 min after starting development.

The gibberellin spots can be located by spraying the dry plate with 5 % sulphuric acid in ethanol, when, after different periods of time at 120°, they fluoresce under U.V. light (360 m$\mu$)[1]. The time taken to maximum fluorescence, the colour of the fluorescence, the $R_F$ value of each gibberellin and the section of the twin-film where each gibberellin is located are noted in Table I.

*Discussion*

On kieselguhr in the solvent benzene–acetic acid–water (8:3:5 v/v) gibberellins $A_1$, $A_2$, and $A_3$ partially overlap, but even so they can be differentiated from each other by their fluorescence colours and the times taken to fluoresce after treatment

TABLE I

TWIN-FILM CHROMATOGRAPHIC PROPERTIES OF GIBBERELLINS

| *Gibberellin* | *Solvent A* $R_F$ | *Solvent B* $R_F$ | *Section of twin-film* | *Fluorescence colour* | *Time taken to max. fluorescence (min)* |
|---|---|---|---|---|---|
| $A_1$ | 0.09 | 0.21 | KG | blue | 10 |
| $A_2$ | 0.13 | 0.28 | KG | purple | 10 |
| $A_3$ | 0.06 | 0.14 | KG | blue–green → blue | 2 → 5 |
| $A_4$ | 0.89 | 0.93 | SG | purple | 5 |
| $A_5$ | 0.59 | 0.67 | SG | blue | 10 |
| $A_6$ | 0.47 | 0.56 | SG | blue | 10 |
| $A_7$ | 0.82 | 0.88 | SG | yellow | 2 |
| $A_8$ | 0.00 | 0.02 | KG | blue | 10 |
| $A_9$ | 1.00 | 1.00 | SG | purple | 5 |
| $A_{13}$ | 0.40 | 0.49 | SG | purple | 10 |

Solvent A = benzene–acetic acid–water (8:3:5 v/v;) solvent B = benzene–acetic acid–propionic acid–water (8:2:1:5 v/v); SG = silica gel; KG = kieselguhr.

with 5 % sulphuric acid. However by replacing one part of the acetic acid by propionic acid (see footnote to Table I) they can be completely separated from each other. In this system the less polar gibberellins run closer together nearer the solvent front but remain separate. The $R_F$ values of gibberellins entering the silica gel film are dependent upon the distance the gibberellins travel through the kieselguhr relative to the length of the chromatogram. In obtaining the data in Table I the length of the chromatograms was 16 cm of which 5.5 cm were on kieselguhr. These are the recommended conditions for use with the solvent system containing propionic acid but for use with the other solvent system the length of the chromatogram on kieselguhr should be reduced to 4.5 cm. In addition to separating the first nine gibberellins and gibberellin $A_{13}$, gibberellins $A_{10}$ and Lupinus 1 (gibberellin $A_{18}$)[2] are also expected to be separated in these systems (by extrapolation from the report by CAVELL *et al.*[3]) but gibberellins $A_{11}$, $A_{12}$, and $A_{15}$ are expected to migrate at the solvent front with gibberellin $A_9$.

The term "twin-film" has been coined because the author considers it more appropriate and in keeping with past nomenclature than the term "dual-band" introduced by ABBOTT AND THOMSON[4].

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