CHROM. 5443

Three-dimensional thin-layer chromatography on a single plate with constant thickness and unmodified adsorbent

Two-dimensional chromatography, where two successive developments in two directions at right angles to one another are made on the same plate[1,2], is a technique extensively used as well as so-called three- or multidimensional chromatography, in which the adsorbent is modified or the separation procedure and other factors are changed between both developments[3–5]. However, three consecutive developments run normally can actually be performed on the same plate in three directions, as is shown below, without any of the modifications inherent to multidimensional chromatography; such a procedure is particularly suitable for the quantitative separation of a product from small amounts of complex mixtures, because no losses can occur due to transfer of materials since the whole separatory process is carried out on the same plate.

Method

Precoated layers on glass, plastic or aluminum plates are preferably employed. Standard samples of the product to be separated can be spotted on ancillary zones marked on the plate and used for identification purposes after each development.

The pattern shown in Fig. 1 is marked on the plate with a pencil and the mixture and the control solution are applied on points *a* and *b*, respectively; the plate is then developed along the direction marked with an arrow in the figure until the front reaches the level of zone F-1; a rather polar developing system is used in order to remove the polar components of the mixture and make better use of the plate in the following developments; at the same time the plate is washed. Indeed, a system slightly polar with respect to the mixture of components may be employed, but then the pattern marked on the plate must be accordingly modified.

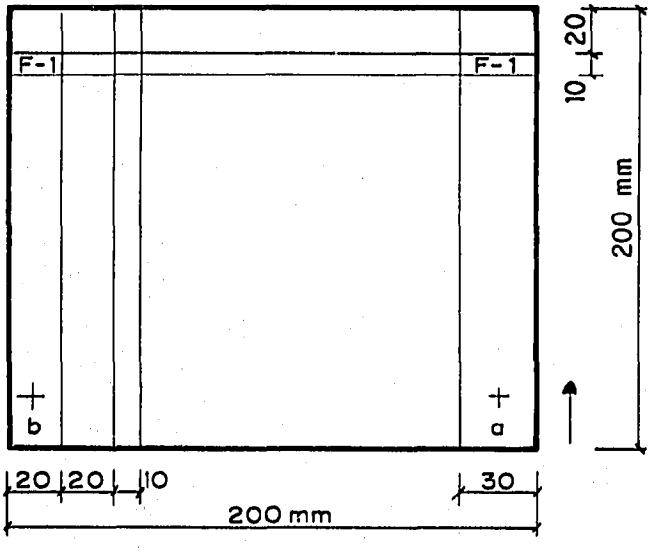

Fig. 1. Pencil-marked pattern. First development.

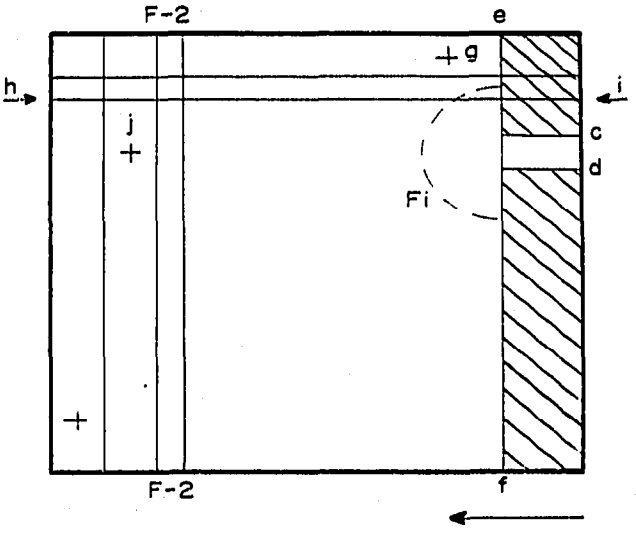

Fig. 2. Intermediate and second development.

After drying the plate in an air stream, the position of the control product is visualized and the lines *c* and *d* (Fig. 2) are marked at a distance apart depending upon the R_F of the product and the difficulty of its visualization. The sorbent in the shaded portion of the plate is scraped off; this portion can be visualized prior to this and used for the separation of any product included in it. Then the plate is developed again with the same solvent as before along the direction of the arrow until the front reaches a line such as F_1 in the figure so that the product is now approximately 2 cm above the line e–f. The plate is dried, cut along the line e–f and the adsorbent in the zone F-1 is removed. A new aliquot of the control solution is applied to the point *g* and the plate is developed with another system until the front migrates to the zone F-2. After drying the plate and visualizing the spot of the control product, the plate is cut along h–i and the adsorbent in F-2 is removed.

Finally, another aliquot of the control solution is applied to *j* (Fig. 3) and a further development is carried out along the direction of the arrow. The position of the product in the development zone of the mixture can be determined from the visualization of the control in its respective zone.

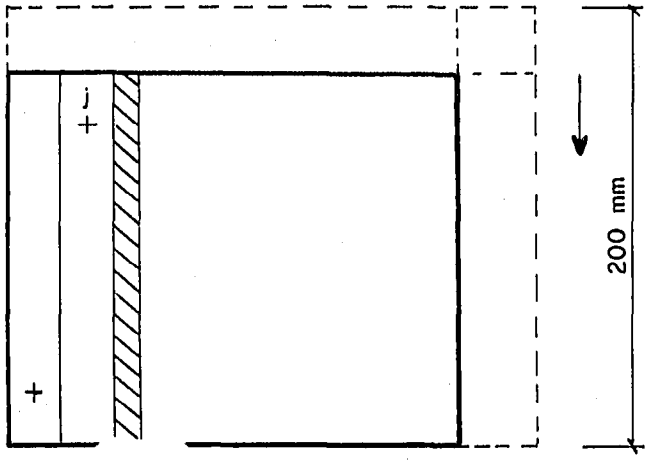

Fig. 3. Third development.

The vapours from the solvents employed in the preceding developments have an effect on the R_F values found each time which, therefore, are different from those which would be obtained in individual runs[6,7]; however, the plate can be activated between consecutive developments if the products to be separated are thermally stable.

Results

As a demonstration of this method we have tried the separation of benzyl orange from a mixture with diazine green, brilliant green, rhodamine B, dimethyl yellow, bromothymol blue, Congo red and brilliant yellow, all at a concentration of 1 mg/ml of ethanol–water (1:1). The chromatograms were carried out on 20 × 20 cm Merck plates (ref. 5715/0025) precoated with silica gel; 5λ aliquots of the mixture and control solutions were applied each time to the plate.

The following products were separated in the first development with ethanol: diazine green (R_F = 0.03), brilliant green (R_F = 0.09), rhodamine B (R_F = 0.54) and

a portion of dimethyl yellow (R_F = 0.68); the R_F of benzyl orange was 0.80, according to the control, and the lines c and d were marked at positions corresponding to R_F 0.81 and 0.71, respectively, and this band also included bromothymol blue, Congo red and brilliant yellow. In the second development with acetone bromothymol blue (R_F = 0.50) and the remnants of dimethyl yellow were separated; the R_F of benzyl orange was 0.05. In the third run with acetone–ethanol (3:1) the latter product was completely separated (R_F = 0.48) from Congo red (R_F = 0.10) and brilliant yellow (R_F = 0.18). The three steps of the process were carried out in glass cuvettes 22 × 6 × 22 cm lined with saturated paper.

The separated colours were identified by means of their R_F values determined independently. After the third development brilliant yellow and benzyl orange can be characterized if necessary by exposing the plate to the vapours from aqueous solutions of NH_4OH and HCl. The former will have a reddish colour with NH_4OH and is violet with HCl and the latter reacts only with HCl to show up as a red spot.

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Received March 29th, 1971

J. Chromatogr., 60 (1971) 270–272

CHROM. 5440

The separation of fatty acids and their derivatives on urea-impregnated paper

Urea forms insoluble adducts with aliphatic compounds possessing a straight chain with at least eight methylene groups. The adducts of unsaturated compounds are usually unstable and branched derivatives are mostly non-adduct-forming. Various types of fatty acids have been separated on urea–Celite columns[1] on the basis of the different stabilities of their urea adducts. By using relatively non-polar solvent systems, fatty acids have been separated, on paper or thin layers impregnated with urea[2,3], into three fractions: (1) saturated fatty acids, remaining on the start; (2) branched fatty acids, with an R_F value of about 0.4–0.6, and (3) short-chain and unsaturated fatty acids, moving with the front. Waxes have also been separated by chromatography on thin layers impregnated with urea[4].

A separation using a polar solvent system and an excess of urea is reported in this paper. The mobility of various groups of fatty acids in this scheme renders it very suitable for the prefractionation of some complicated mixtures.