28 L. FISHBEIN AND J. FAWKES, *J. Chromatog.*, 22 (1966) 323.
29 L. ANDERSEN, *Finska Kemistsamfundets Medd.*, 70 (1961) 32.
30 D. L. GUMPRECHT, *J. Chromatog.*, 18 (1965) 336.
31 B. SMITH, *Acta Chem. Scand.*, 16 (1962) 843.

Received February 28th, 1966

J. Chromatog., 24 (1966) 245–250

Concentrating compounds by continuous horizontal thin-layer chromatography

In preparative TLC, the common technique is to place the sample in a small band on the starting line of the plate, and separate the components by single or multiple development, followed by removal of the strip of adsorbent containing one of the compounds, and extraction with a suitable solvent[1–8]. The thus isolated compound can then be identified or determined. Because of band widening during the elution process a relatively large amount of adsorbent must be removed, so that the solution obtained after extraction always contains finely divided adsorbent particles. In quantitative work this is a serious disadvantage, only partly overcome by high-speed centrifuging (15.000 r.p.m.). It is practically impossible to prepare a clear solution which is sufficiently free of background adsorbance for measurement in the U.V. region.

A second disadvantage in quantitative work is the presence of organic compounds in nearly all commercial adsorbents[9]. Dependent upon the elution system, these are more or less eluted, so that on extraction of the separated compounds the solution will be contaminated. It is therefore necessary to pretreat the plates by several elutions with chlorohydrocarbons (*e.g.* trichloroethylene). As the organic compounds are thus concentrated at one end of the plate, their interference in the final solution can largely be obviated in this manner.

The effect of finely divided particles of adsorbent can also be reduced substantially by drastic reduction of the amount of adsorbent required. This, at the same time, would lead to more rapid and more effective extraction.

In the following, a method is described which concentrates separated compounds on a very small surface area by two-dimensional chromatography. Thus only a very small amount of adsorbent need be extracted. Use, in this connexion, is made of the continuous horizontal TLC method according to BRENNER AND NIEDERWIESER[10,11]. Though this method has mainly been applied to the separation of compounds differing very little in R_F value, it appeared to be excellently suited to our purpose: the amount of adsorbent to be extracted could be reduced to 1/50–1/60 that of the amount in normal procedures on 20 × 20 cm plates.

Experimental

As shown schematically in Fig. 1, the set-up for continuous horizontal TLC consists of a chromatoplate covered partly by a second glass plate, kept 2 mm apart

by means of strips along the edge, the space between the plates forming the development chamber. A strip of chromatography paper serves as a wick to transfer the eluant from its reservoir to the lower plate over its full width. After the elution has proceeded to the end of the plate, which is exposed to the air, the solvent evaporates, so that the eluant can be fed continuously.

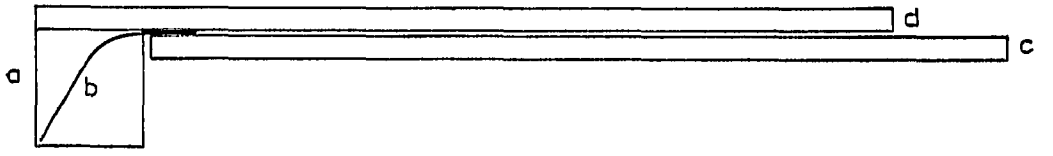


Fig. 1. Continuous horizontal layer chromatography. a = Solvent reservoir; b = paper wick; c = lower plate carrying the adsorbent layer; d = upper plate with edge strips (not drawn).

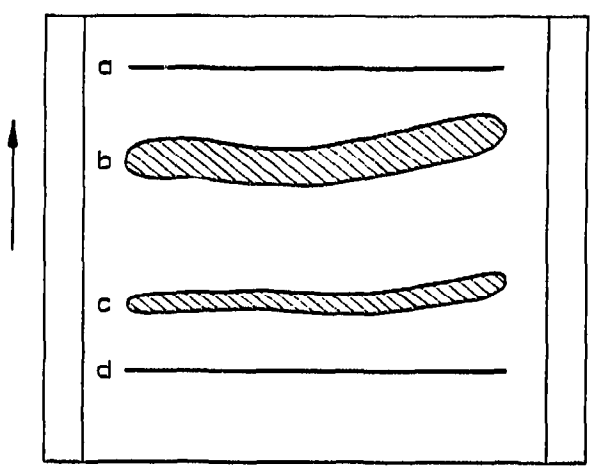
In our tests we used glass plates of 20 × 20 cm, the lower one covered with a 0.2 mm layer of Kieselgel G-HR (Macherey & Nagel). By way of illustration a mixture of 250 μg of Fettrot 7 B, 250 μg of dimethyl yellow, and 250 μg of indophenol was applied on a line 4 cm from the left side of the lower plate; the latter was covered with the upper plate to form an exposed strip of 2 cm. The paper wick was then positioned to reach to 1 cm from the starting line. The reservoir was filled with chloroform. Elution was started, and continued even after the front of the first band, which was about 12 mm wide, was outside the chamber. Because of evaporation of the chloroform, which can be speeded up by means of a hot air blower, the front of the band did not proceed any further, whereas the rear of the band was still moving. Finally, all the material was concentrated in a straight, very narrow band less than 1 mm wide (see Fig. 2).

Even with irregularities in the shape of the bands during the elution process, the compounds could be concentrated in a straight narrow band. After thus concentrating the first compound, the upper plate was shifted 2 cm backwards, so that a 4 cm wide strip of adsorbent was now exposed. Elution was then continued until the second compound was concentrated. Shifting of the upper plate must be done very carefully and without interruption of the elution process, which is unnecessary, however, if the upper plate is partly replaced by a number of small glass strips laid close together and of widths corresponding with the distances between the separated bands. In this case one strip is removed after concentration of the first compound, and so on.

After concentration of the two dyes elution was stopped, and the upper plate removed. The eluant in the layer was evaporated, and the adsorbent removed to within a distance of 0.5 cm on both sides of the narrow bands containing the concentrated compounds. The plate was then chromatographed at right angles in the same manner, now using a paper wick 1 cm wide. The result was that the compounds were concentrated on a very small area at the side of the plate, as shown in Fig. 3.

In this example the coloured regions are clearly visible. In the case where colourless compounds are to be concentrated, two possibilities arise, *viz.*:

(a) When the compounds show U.V. absorbance, a fluorescent adsorbent may be used and the free end of the lower plate exposed to U.V. radiation. As soon as a compound reaches the exposed area, fluorescence is extinguished. This procedure was followed in the separation and concentration of isomeric aromatic diamines.



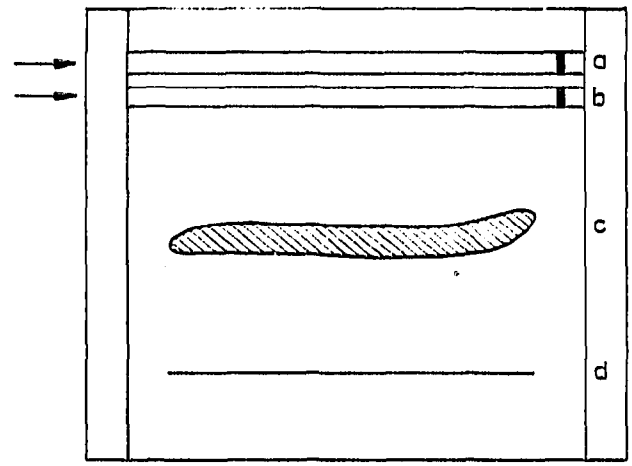


Fig. 2. Concentration in first direction. a = Concentrated in narrow band; b and c = not concentrated; d = start.

Fig. 3. Concentration in second direction. a and b = Concentrated on small surface areas; c = not concentrated; d = start.

(b) When the compounds to be separated are non-absorbing, it must previously be determined when they will leave the elution chamber and enter the free area. This can be done by running the compound together with a dyestuff under normal TLC conditions and, after development and coloration of the compound, measuring the distance between the spots. With the same dye as a reference in continuous TLC, the upper plate is shifted the moment the dye spot reaches a distance from the end corresponding with that determined in the preliminary test[12].

After scraping off the adsorbent the concentrated compounds can be separated by micro-extraction for identification or further analysis. By this technique solutions with very low background absorbance are obtained without centrifuging, while for the very small amounts of material to be extracted the desorption process is more quantitative.

Central Research Institute of AKU (Algemene Kunstzijde Unie N.V.) and Affiliated Companies, Arnhem (The Netherlands)

J. L. MULDER
G. J. VEENSTRA

1 H. HALPAAP, *Chem. Ing. Techn.*, 35 (1963) 488.
2 C. G. HONEGGER, *Helv. Chim. Acta*, 45 (1962) 1409.
3 C. G. HONEGGER, *Helv. Chim. Acta*, 46 (1963) 1772.
4 F. J. RITTER AND G. M. MEYER, *Nature*, 193 (1962) 941.
5 M. A. MILLETT, W. E. MOORE AND J. F. SAEMAN, *Anal. Chem.*, 36 (1964) 491.
6 H. SEEBOTH AND H. GÖRSCH, *Chem. Tech. (Berlin)*, 15 (1963) 294.
7 H. SCHILCHER, *Z. Anal. Chem.*, 199 (1964) 335.
8 R. D. BENNETT AND E. HEFTMANN, *J. Chromatog.*, 12 (1963) 245.
9 F. GEISS, A. KLOSE AND A. COPET, *Z. Anal. Chem.*, 211 (1965) 37.
10 M. BRENNER AND A. NIEDERWIESER, *Experientia*, 16 (1960) 378.
11 M. BRENNER AND A. NIEDERWIESER, *Experientia*, 17 (1961) 237.
12 B. DE VRIES AND G. JURRIENS, *J. Chromatog.*, 14 (1964) 526.

Received March 14th, 1966