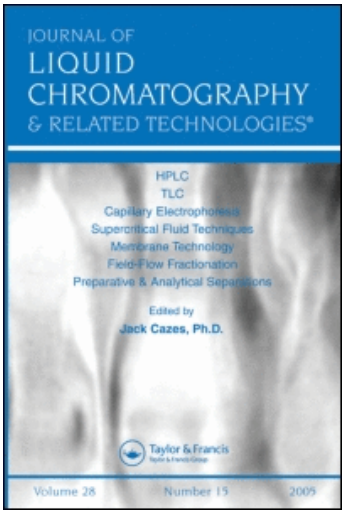


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Micropreparative TLC Separation of Large Sample Volumes. Frontal Elution Chromatography

E. Soczewinski[a]; J. Kuczmierczyk[a]; B. Psionka[a]
[a] Department of Inorganic and Analytical, Chemistry Medical Academy Staszica, Lublin, Poland

**To cite this Article** Soczewinski, E. , Kuczmierczyk, J. and Psionka, B.(1980) 'Micropreparative TLC Separation of Large Sample Volumes. Frontal Elution Chromatography', Journal of Liquid Chromatography & Related Technologies, 3: 12, 1829 — 1834
**To link to this Article: DOI:** 10.1080/01483918008064772
**URL:** http://dx.doi.org/10.1080/01483918008064772

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(12), 1829-1834 (1980)

# MICROPREPARATIVE TLC SEPARATION OF LARGE SAMPLE VOLUMES. FRONTAL + ELUTION CHROMATOGRAPHY

E. Soczewinski, J. Kuczmierczyk and B. Psionka

Department of Inorganic and Analytical Chemistry
Medical Academy
Staszica 6
20-081 Lublin, Poland

## ABSTRACT

It is demonstrated that mixtures containing few compounds can be applied as wide zones on the edge of the thin layer using a sandwich tank with glass distributor. The mixture is partially separated during application (frontal chromatography) so that subsequent elution accomplishes complete separation. Using 5 x 20 cm plates of silica, 0.5 mm thick, 1 - 3 ml samples were separated easily which permitted elution of 3 - 9 of separated compounds from a single plate.



## INTRODUCTION

In a previous paper (1) it was demonstrated that the sandwich tank with a special glass distributor for the developing solvent (2-4) permits application of the sample solution across the TLC plate as an even band. The method has been illustrated for a complex mixture containing eight components.

Mixtures to be separated are frequently much simpler (e.g., product and substrate or few byproducts). In these cases the sample size can be considerably scaled up for increased yield. However, the manner of application of large sample volumes on the thin layer is then of critical importance. Application in the form of single spots leads to formation of a series of microcircular chromatograms; streaking with a mechanical applicator produces two opposite frontal chromatograms with a central zone containing the most strongly retained component (5). The method of choice is to apply the sample solution from the edge of the layer (as is typical in column chromatography). As demonstrated in an earlier paper (1) this is easily carried out with a simple glass distributor which forms a horizontal flat pipette in contact with the edge of the adsorbent over the carrier plate.

## EXPERIMENTAL

The sandwich tank with glass distributor and the method of band application of sample have been described in earlier papers (1-4).

Glass carrier plates (50 x 200 x 1.2 mm) were covered with 0.5 mm layers of silica (Kieselgel 60-G, E. Merck, Darmstadt, F.R.G.), activated at 120°C and stored in a dessicator. All solvents were dried over wide-pore silica. Chloroform was extracted with water to remove alcohol. Two binary test mixtures were prepared: 4-aminoazobenzene and 4-dimethylaminoazobenzene as 0.02 M solutions in 10% or 15% (v/v) heptane solutions of methyl ethyl ketone; azobenzene and Sudan I as 0.2% (w/v) solution in 40% (v/v) heptane solution of chloroform.

The plates were predeveloped with the eluents (10% or 15% methyl ethyl ketone for the first test mixture and 40% chloroform for the second mixture) on the full distance to eliminate solvent demixing effects and to elute any impurities from the adsorbent. The supply of the eluent was then removed and, after the disappearance of the solvent under the distributor, the space between the margin of the carrier plate and the distributor (about 200 µl) was filled with the test mixture from a syringe in several portions. 0.5 to 3 ml samples were thus introduced into the layer to form starting bands 4-9 cm wide. Owing to differences in colours of the dyes, it was possible to also observe the front of the second most polar component. During the predevelopment and sample application, a wick of filter paper protruding from the chamber was pressed to the end of the layer to facilitate elution of impurities. After complete absorption of the sample solution the reservoir containing the eluent was connected again to the distributor and development was continued.

To determine the expenditure of eluent, the reservoir was occasionally weighed; this permited us to plot the movement of the bands against the solvent flow. After evaporation of the solvent, the coloured zones were transferred into small funnels whose narrow outlets were plugged with glass wool (6); they were then eluted with ethyl ether directly into agate mortars. After evaporation of the ether, they were mixed with potassium bromide to make pellets for infrared spectroscopy.

## RESULTS AND DISCUSSION

In the first series of experiments, aminoazobenzene and N,N-dimethylaminoazobenzene were separated (as model compounds for amine and the product of its methylation). In accordance with well known practice, the solutes were dissolved in the eluent. In Figure 1a-c, the formation and migration of the zones are represented for 0.5 ml, 1 ml and 2 ml sample sizes. It can be seen that very high capacity can be achieved for the thin layer; even when the starting band occupies more than half of the plate area (Fig. 1c) the separation is complete. The spec-

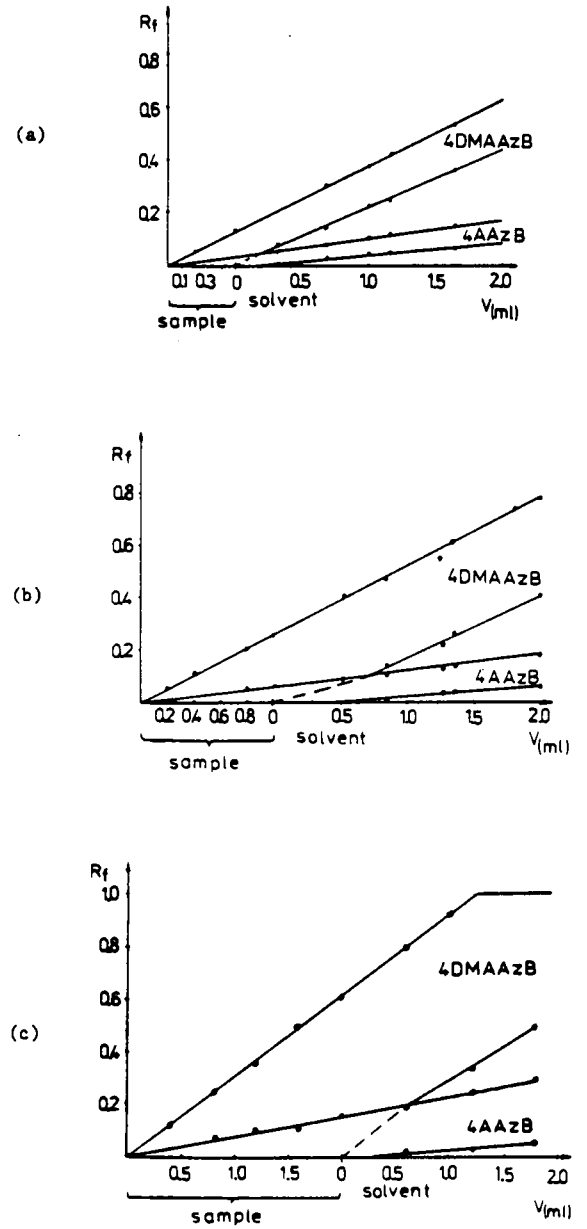


FIGURE 1

Formation and movement of zones of 4-aminoazobenzene and 4-dimethylaminoazobenzene on 5 x 20 cm layers of silica (0.5 mm) eluted with 10% heptane solution of methyl ethyl ketone. Sample volume: 2, 0.5 ml; b, 1 ml; c, 2 ml.

trum of eluted aminoazobenzene was practically identical to that of the initial compound while, for dimethylaminoazobenzene, the eluted sample showed higher purity in comparison with the original substance (Fig. 2). Similar results were obtained when 5% or 15% methyl ethyl ketone was used as eluent.

In a second series of experiments, azobenzene and Sudan I were used as a test mixture. It was found, in preliminary experiments, that good separations were obtained when mixtures of chloroform and heptane were used as eluent. In Fig. 3a-c, the migration of the bands is illustrated for 1, 2 and 3 ml samples containing 2,4 and 6 mg of each component, respectively. The spectra of the eluted components were practically identical with those of the original solutes (Fig. 4).

The technique is very economical and the capacity of the adsorbent is optimized for instance, a single 5 x 20 cm plate contains about 2g of silica and, under favourable conditions, can be used to separate a 10 - 20 mg sample. This is more than sufficient for determination of infrared spectra and corresponds to the loading ratio 1:100 - 1:200. The expenditure of eluent for a single run, including predevelopment, is also very low. The advantages of the technique are probably due to relatively slow development which is favourable for equilibration of the system and partial separation of the compounds during the formation of the starting band. The performance of the system depends, needless to say, on the selectivity of separation and would be lower for components of similar adsorption affinity.



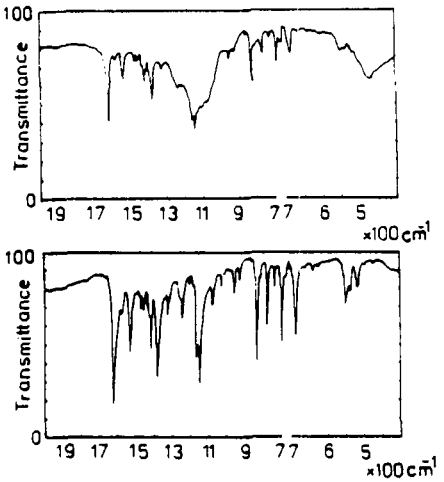


FIGURE 2

IR spectra of 4-dimethylaminoazobenzene (KBr pellets, UR-20 spectrophotometer, Zeiss, Jena): a, original solute; b, eluted solute.

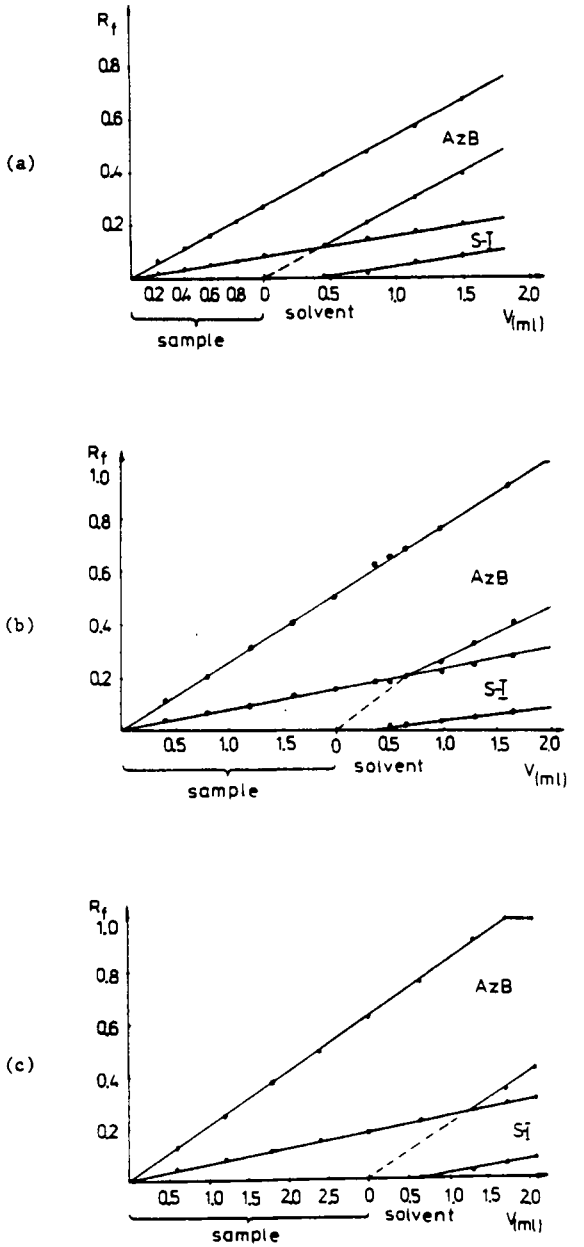


FIGURE 3

Separation of azobenzene and Sudan I on 5 x 20 cm layers of silica (0.5 mm) eluted with chloroform-heptane (4:6). Sample volume: a, 1 ml; b, 2 ml; c, 3 ml.

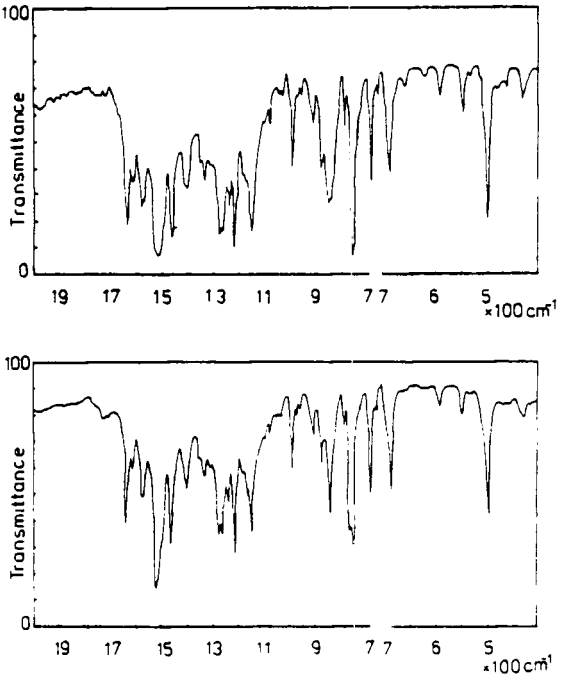


FIGURE 4

IR spectra of Sudan I: a, original solute; b, eluted solute.



ACKNOWLEDGEMENT

These investigations were supported financially by a grant from the Polish Academy of Sciences (03.10).

REFERENCES

1. Soczewinski, E., Maciejewicz, W., J. Chrom., 176, 247 (1979).
2. Soczewinski, E., J. Chrom., 138, 443 (1977).
3. Soczewinski, E., Wawrzynowica, T., Chromatographia, 11, 466 (1978).
4. Soczewinski, E., Czapinska, K., J. Chrom., 168, 230 (1979).
5. Soczewinski, E., Psionka, B., Chem. Analit., (Warsaw) in press.
6. Wotschokowsky, M., Kontakte (E. Merck, Darmstadt, F.R.G.), 1, 14 (1975).