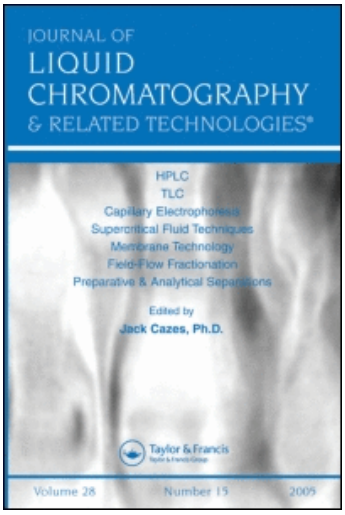

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DISPLACEMENT THIN-LAYER CHROMATOGRAPHY

J. Bariska[a]; T. Csermely[b]; S. Fürst[b]; H. Kalász[b]; M. Bathori[c]
[a] National Institute of Rheomatology and Physiotherapy, Budapest, Hungary [b] Department of Pharmacology and Pharmacotherapy, Semmelweis University of Medicine, Budapest, Hungary [c] Department of Pharmacognosy, Albert Szent Györgyi Medical University, Szeged, Hungary

Online publication date: 15 February 2000

To cite this Article Bariska, J. , Csermely, T. , Fürst, S. , Kalász, H. and Bathori, M.(2000) 'DISPLACEMENT THIN-LAYER CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 23: 4, 531 — 549
To link to this Article: DOI: 10.1081/JLC-100101470
URL: http://dx.doi.org/10.1081/JLC-100101470

J. LIQ. CHROM. & REL. TECHNOL., 23(4), 531–549 (2000)

DISPLACEMENT THIN-LAYER CHROMATOGRAPHY

J. Bariska,[1] T. Csermely,[2] S. Fürst,[2] H. Kalász,[2,*] M. Bathori[3]

[1] National Institute of Rheomatology and Physiotherapy
P.O.Box 54
H-1525 Budapest, Hungary

[2] Department of Pharmacology and Pharmacotherapy
Semmelweis University of Medicine
Nagyvárad tér 4, P.O.Box 370
H-1445 Budapest, Hungary

[3] Department of Pharmacognosy
Albert Szent Györgyi Medical University
Eötvös. u 6, P. O. Box 121
H-6701 Szeged, Hungary

ABSTRACT

Thin-layer chromatography has several advantages in comparison to column chromatography, e.g., simultaneous separation of several samples, easy realization of two-dimensional separation, direct observation of both the chromatographic development and the separation using colored compounds, sensitive and easy detection of the spots with specific spray reagents. The entire procedure, i.e., loading, development, and evaluation are relatively simple. An additional feature is the optimization possibilities of mobile phase flow velocity, based on the complexity of driving force where capillarity results in a concave flow profile that may be counterbalanced by convex profile of laminar flow. Displacement thin-layer chromatography has the advantages of planar arrangement of the stationary phase, and opens new possibilities for analytical separations.

This paper outlines basic features of planar displacement chromatography, including differences from elution chrom- atography, and that of column displacement chromatography, the possibility of using spacer displacement chromatography. Parameters limiting displacement chromatography, and their optimization to achieve better separation are discussed.

INTRODUCTION

Analytical and/or preparative separation methods are widely used in chemical, biochemical, biological etc., sciences and industries. One of the generally used separation methods is chromatography. The components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction. The solutes (sample components) are subjected to impelling and retarding forces. Impelling forces are hydrodynamic at liquid chromatography (operating with liquid mobile phase), and results in flow of the mobile phase through the particles of stationary phase. Although the major forcing actions of the mobile phase are done either by using pumps or generated by capillarity, other possibilities are also utilized such as gravity, centrifugal forces, the electroosmotic flow, etc. Retarding forces act as the consequences of interactions of the solute and the stationary phases, such as adsorption, partition, ion exchange, hydrophobic interaction, etc. Movement by the mobile phase and retardation by the stationary phase of a solute is demonstrated by the simplified expression, the ratio of average duration of sojourn, in each phase. Migration of individual sample components is determined by the outcome of impelling and retarding forces and separation is determined by relative migration of the solutes. Resolution of any two solutes also depends on the width of their bands.

Tiselius[1] classified the chromatographic procedures according to the modes of developments such as elution, frontal, and displacement chromatography. In the case of elution chromatography, the size of sample load is small; the equilibrium characterizing the distribution of sample components between the stationary and mobile phase follow a linear low. It means that their distribution coefficients remain constant over a wide range of concentration. This is the situation in most cases of separations when analytical procedures are carried out both in gas chromatography and liquid chromatography, and this situation is called linear elution chromatography. One of the sample components can be characterized as non-retarded, and it will migrate with the mobile phase, that is, it will be eluted at $\mathbf{t}_o$. Other components will be eluted after $\mathbf{t}_o$. The shape of concentration characteristics of the individual zones may be Gaussian or quasi-Gaussian (with either tailing or leading).

Looking at this situation from another point of view, the low load means that the stationary and mobile phases of the chromatographic system are poor-

ly utilized, and also the compounds are diluted in comparison to their original concentration in the sample. The yield in time unit is poor and also the yield divided by the stationary phase amount and the mobile phase used is also very low. When the amount of sample is increased, the system starts to be overloaded, and thereby the efficiency of separation, number of theoretical plates, resolution, yield, calculated to a definitely pure product, etc.) are decreasing. The parallel increase of the sample size and the column size can keep the separation characteristics optimal, however, the dilution of the separated sample, as well as an increase of the cost of separation will occur.

In some cases, the stationary phase has to be regenerated by the use of a regenerant (to remove any solute to be strongly adsorbed) and, also, the starting mobile phase has to remove the regenerant as well. Gradient elution is also frequently used when solutes of divergent characteristics are used. In the cases of both stepwise or continuous gradient elution, the separation cycle is completed when the starting eluent is applied to re-equilibrate the system.

Frontal chromatography operates with the continuous supply of the load, and the component of the mixture with least interaction with the stationary phase migrate with the highest rate of movement.

The third type of development is displacement chromatography,[1-3] that was mentioned by Tswett[2-4] and introduced into practice by Tiselius.[1] Displacement chromatography uses three different mobile phases, the carrier, the displacer, and the regenerant,[5] and three main steps: loading the sample, development of displacement train - collecting the fractions of the separated bands, and regeneration. The sample compounds are adsorbed on the stationary phase when the carrier is used; the displacer forces them to move. There is a competition between displacer and sample components. Displacer replaces sample compounds on the stationary phase. There is a series of competitions among the sample components; the stronger adsorbing force to move forward weakly adsorbed sample components. Finally, the regenerant or regenerants are supplied to remove both the remaining sample components, and the displacer itself from the mobile phase. The final step of displacement operation is to flash the regenerant by the carrier from the stationary phase[5-7] (Figure 1). Displacement chromatography operates with non-linear conditions that are through the non-linear interval of the Langmuirian isotherms. Through loading, the mobile phase is adequate for non-movement or very slow movement of the zones of the solutes.

High performance displacement chromatography has been used for preparative scale separation of biologically active organic compounds. In a fully developed displacement train, rectangular zones are generated instead of the Gaussian or near-Gaussian elution curves. The sample load of displacement chromatography may be several magnitudes higher than that of elution chro-

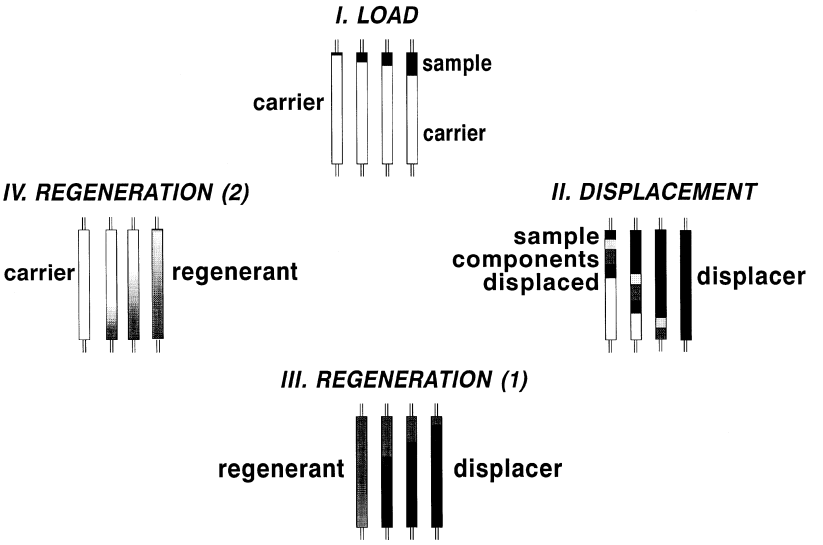

Figure 1. Operational steps of column displacement chromatography. The 1st step (load) is performed when the stationary phase has been equilibrated with the carrier, and the sample components are sorbed on the top of column. The 2nd step starts with the supply of displacer that displaces the sample compounds. The well separated zones can be collected using a fraction collector. The 3rd and the 4th steps are regeneration using regenerant that elutes the displacer as well as carrier, which elutes regenerant.

matography. Porath and Li[8] used DC for separation of biomolecules. Large-scale purification of proteins has been more and more important.

The carrier displacement chromatography used various aliphatic alcohols, and it has reached a renaissance in the work of Peterson et al.,[9,10] who purified monoclonal antibodies on cellulose ion-exchangers, and used carboxymethyldextrane, as spacing displacer.

Displacement chromatography has been used by Horváth et al.[5-7,11-14] to separate various classes of compounds, such as phenoloids, ethylene glycol oligomers, amino acids and their derivatives, peptides, serum proteins, antibiotics, enzymes, nucleic acid fragments, steroids. Separation of geometrical and structural isomers, enantiomers, and even isotopes was also published by various authors.

The majority of HPDC separations used the regular set-up of the HPLC, but frequently special techniques were used. Hodges et al. used sample dis-

placement HPLC (SDC) of peptides[15] and discussed various multi-column SDC cases, when a single peptide component or a multiple component peptide mixture were applied. They investigated the effect of flow rate, sample load, and running time on the yield. Sample displacement HPLC was also used by Veeraragavan et al.[16] to purify proteins with loads of several hundred mg.

Kim and Cramer[17] used metal affinity displacement chromatography for the separation of cytochrom c, chymotrypsinogen A, lysozyme and lactoferrin. Behavior in displacement train means the migration speed and the order of the components. The order of the components can be derived from the statement of Tswett,[3] who reported that the more strongly adsorbed pigments displace the more weakly adsorbed ones. The migration speed of the ith component is characterized by the equation from Helfferich and Klein[18] according to equation 1.:

$$u_1 = \frac{u_0}{1 + \frac{q_i}{c_i}} \tag{1}$$

As in a fully developed displacement train the migration speed of all bands, that is the band also is equal to that of the displacer (equation 2.),

$$u_1 = u_2 = u_3 = \ldots = u_i = u_n = u_D \tag{2}$$

therefore,

$$\frac{q_1}{c_1} = \frac{q_2}{c_2} = \frac{q_3}{c_3} = \frac{q_i}{q_i} = \frac{q_n}{c_n} = \frac{q_D}{c_D} \tag{3}$$

It means that as the migration speed of any member in the fully developed displacement train is the same (it is the speed of the displacer front), and as u_o and u_i are the same for any member of the displacement train, the q_i/c_i ratios should also be the same (equation 3.), as q_i and c_i are the only component dependent parameters in the equation 1. At the same time, these quotients are on the operating line of the system that is on the line between the actual concentration of the displacer and origo. Therefore, the concentration of the displacement zone of each component is determined by the crossing point of the operating line with the individual adsorption isotherm.

Horváth et al.[5] wrote the basic equations and considerations on HPDC. They outlined the essential conditions for the bona fide displacement chromatography, and also gave formulas for calculations of efficacy, sharpness of the boundary between the two zones, purity, and the effect of the operational parameter, such as flow rate, feed amount, feed volume, column length, as well as selection of column, carrier, and displacer, etc. Veress et al.[19] modeled the efficiency of DC and that of the recycling mode of operation. Frey[20] presented

general treatment of theory and calculation of free energy consumption at self-sharpening concentration front for isotherm arbitrary shape and application in HPDC. He detailed how to calculate the solute bands in DC when isotherms of the individual components are not crossing each other, and how to handle isotherms that do cross each other. Yu and Do[21] performed computer simulation of displacement chromatography on the basis of Langmuir isotherm, species-dependent saturation capacities. Phillips et al.[22] suggested a mathematical model for the simulation of non-ideal HPDC. Katti et al.[23] used semi-ideal models to compare separations of binary mixture by both elution and DC; they also modeled the behavior of trace components when one main and two trace components were present in the displacement train.[24] Gu et al.[25] made a theoretical model to check the effects of maximum adsorption capacity of the displacer, that of the displacer concentration, and that of the adsorption equilibrium constant of displacer.

Frenz et al.[26] published one of the most interesting application of high-performance displacement chromatography by its direct connection with mass spectrometry.

Planar chromatography is originated in the early work of Runge et al. (for overview, see Ettre[2]) who separated dyes and salts on filter paper. The method was utilized for practical purposes by Izmailov and Schraiber,[27] Brown,[28] Kirchner et al.,[29] and Stahl[30] on powdered adsorbents placed on glass (and sometimes sandwiched also); paper strips and sheets were also widely employed. Excellent books summarize the essence of thin-layer chromatography.[31-33]

Thin-layer chromatography operates with dry stationary phase; and the impelling force to generate the mobile phase flow is the capillarity.[34,35] TLC may be performed in a simple glass chamber; the mobile phase is placed in it, and the bottom edge of TLC plate is dipped into the solvent system of the mobile phase. Although the chromatographic characteristics of the solutes depend on several factors (including the saturation degree of the vapor phase, temperature), use of standards makes reliable identification and quantitative evaluation possible. The advantages of TLC are:

1. Either several spots can be separated at the same time, or two-dimensional developments can be easily done,

2. Progress of the mobile phase front can be visually observed,

3. chromatographic behavior of colored solutes may be followed,

4. specific and sensitive reagents can be used for detection of the majority of compounds,

5. stationary phases with UV active ingredients are widely available and/or color reagents help the location of spots of the solutes,

6. contact detection methods such as bioautography or x-ray sensitive films facilitate identification of antibiotics and radiolabeled compounds, respectively.

Pre-coated thin-layer plates become available with a wide choice of stationary phases, and sophisticated instrumentation for TLC has been wide spread since the '70s, including spotting devices and scanners for improved quantitative evaluation. In spite of these instrumental supplements, the basis of TLC separations have remained unchanged.

The development of TLC starts with a dry stationary phase, i.e., the stationary phase is neither prewetted nor preequilibrated with the mobile liquid phase. At the same time, both the mobile phase (located at the bottom of the chamber), and the dry plate are arranged to be open, that is, the mobile phase components can evaporate from the bottom of the chamber, and can condense on the stationary phase. During the development, the capillarity impels the mobile phase running; thereby the wet part of the TLC plate may also be the source of both evaporation and condensation. In general, the mobile phase is a multi-component mixture that runs with multiple solvent front, where the most apolar solvent component runs first in silica or alumina stationary phase, and the fronts of the other liquid constituents follow it in the sequence of their decreasing adsorption ability to the stationary phase. The apolar components are generally volatile and the progress of their solvent front is mostly influenced by the saturation degree and temperature of TLC chamber. The time versus front distance curves during the process are declining, and generally can be characterized with the equation 4:

$$t = k \times s^2 \qquad (4)$$

The value of the constant depends on the degree of saturation in the chamber, the temperature, and the stationary phase. Another determining factor is the mobile phase, where the composition of the solvent front, including their specific mass, viscosity, and interaction (capillarity), with the stationary phase is important.

Remarkable progress has occurred in regulation of the mobile phase migration by the use of centrifugal thin-layer chromatography, forced-flow thin-layer chromatography (FF-TLC; overpressure layer chromatography, OPLC), and automated multiple development (AMD).

Forced flow displacement chromatography operates with a covered stationary phase and the solvent delivery pump generates mobile phase flow with

a constant velocity. The stationary phase is tightly covered in FF-TLC, and therefore, the vapor phase as the major uncertainty factor is eliminated. In FF-TLC, the efficiency of TLC is highly increased by generating optimal conditions (for review, see Kalász et al.[34,35] In case of forced-flow thin-layer chromatography, the time versus front distance curve is linear as given in equation 5:

$$t = k \times s \tag{5}$$

The value of the constant (**k**) depends on the flow rate generated by the pump. This paper deals with a special type of chromatography where the impelling and retarding forces differentiate the sample components to be separated, however, each band of the separation train interact with the neighboring ones - displacing the less sorbed band and being displaced by the sample component that has stronger interaction with the stationary phase.

PLANAR DISPLACEMENT CHROMATOGRAPHY

Planar displacement chromatography can be realized using the same arrangement as is done for the elution mode of development. The dry stationary phase is dipped into the mixture of carrier and displacer, and the displacing system (the proper arrangement of carrier and the displacer) is formed in situ on the planar sorbent. There is an essential difference of migration ability of the carrier and the displacer, therefore, the carrier front runs first, wetting the previously dry stationary phase. Behind the carrier front, another front moves forward, it is the displacer, if there is such a constituent present in the solvent system used for development (Figure 2). Displacer front migrates depending on the stationary phase, the carrier, the displacer, and the concentration of displacer in the carrier. Although equation 3 defines the speed of any component in the displacement train, the equality of the migration velocity is not valid for the carrier, that is the carrier front moves faster than the displacer train. In thin-layer chromatography the relative migrations are designated by a parameter called R_F. The value of the displacer front is called R_{DI}, and characterizes the migration of displaced front at a given displacement train that includes the stationary phase, the carrier, the displacer, and its original concentration in the carrier. R_{DI} values can be determined by the usual way of planar chromatography. The lower values are the displacer concentrations, the smaller ones are the R_{DI} values (Figure 3) in a given system.

The migration types of the solute spots can be divided into three main classes:

1. the solute is displaced by the displacer front,

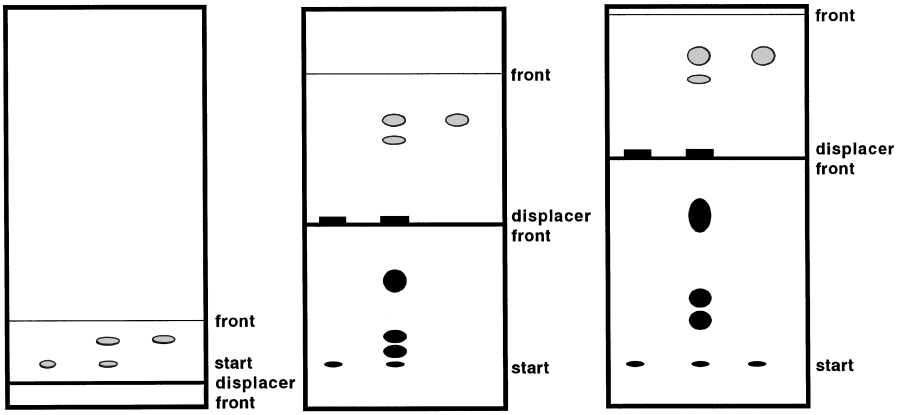

Figure 2. Operational steps of planar displacement chromatography. There is one single step of operation, development of the plate using the proper concentration of displacer in the carrier.

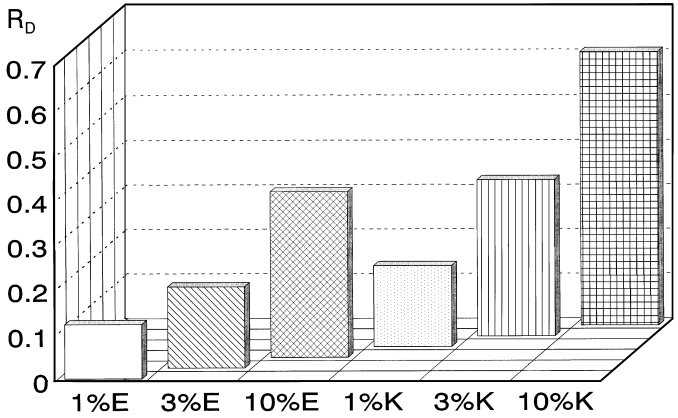

Figure 3. R_{DI} versus displacer concentration. 1%E, 3%E and 10%E mean 1%, 3% and 10% triethanolamine in dichloroethane, while 1%K, 3%K and 10%K mean 1%, 3% and 10% triethanolamine in chloroform. The stationary phase was TLC plate 20 x 20 cm, silica gel 60 of Merck (Darmstadt, Germany).

2. the solute migrates faster than the displacer front,

3. the solute migrates slower than the displacer front, but it is eluted by the displacer.

As the displacement procedure is based on the interaction of the displacer (front) and the solute, a necessary condition of the displacement procedure is that the individual solute and the displacer has to be in physical contact.

Similar migration velocity of the solute spot and the displacer front characterize the displacing equilibria (case No. 1). Otherwise (case of No. 2) if the solute migrates faster (that is the displacer front moves slower), either the slower migration of the solute can be generated by the adequate change of the carrier composition, or the faster movement of the displacer front can be arranged by the increase of displacer concentration in the carrier. In case No. 3, the composition of the displacer system has to be changed, i.e., either the stationary phase, or the carrier, or the displacer, or the whole system has to be replaced.

Thin-layer chromatography has made possible visual observation of the displacement train. Bona-fide displacement is generated when the displacer front and the solute spot are close to each other. The displacer concentration is high enough to generate migration of displacer front faster than that of the spot (Figure 4, left side), therefore, there is no minimum in the scan of the displacement train. Quasi displacement train is generated when migration velocity of displacement front is very similar, or slower than that of sample spot (Figure 4, right side). Therefore, a minimum is observable between the two maxima, one maximum belonging to the displacer front, the other one to the solute spot.

There are some particular phenomena of the displacement chromatography that have never been detected at high performance displacement chromatography. One of them is the displacer front distortion, the other one is the displacer front multiplication.

Displacer front distortion may be observed at the planar stationary phase where the displaced solutes do not occupy the entire length of the displacer front. The displacer front shape is deformed; that is, where the displacer front-displaced spot interaction takes place.

Carrier and displacer front multiplications are specific phenomena characteristic to the displacement thin-layer chromatography with forced-flow supply of the mobile phase. Front multiplication can be observed when the mobile phase pumps generate slow flow rate whereby capillarity of dry stationary phase acts as a sucking pump. When the mobile phase supply is ad libitum (that is the case when the TLC plate is dipped into the mobile phase) the supplement

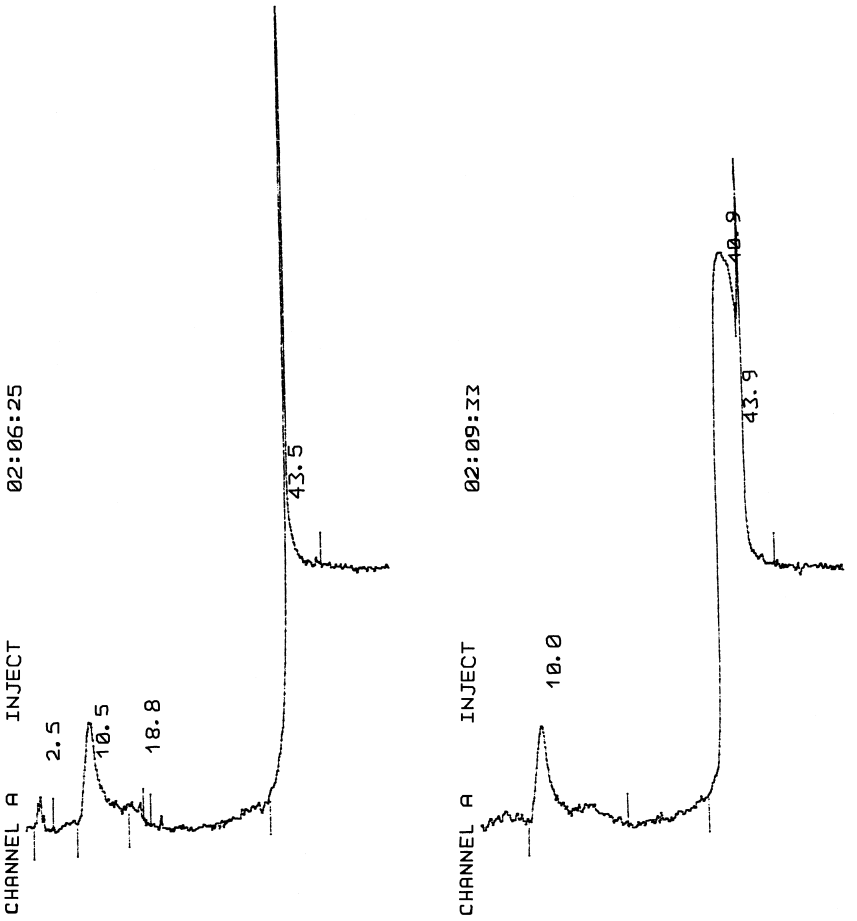

Figure 4. Bona fide- (left side) and quasi-displacement (right side) chromatograms.

of both the carrier and that of the displacer is enough to replace the forward moving mobile phase. The strongly adsorbed mobile phase components (both the carrier, and the displacer) indicate one front, the mobile phase components reaching the saturation indicate the second front.

Capillary flow development has shown another interesting phenomenon. The presence of displacer modifies the time versus solvent front distance curves when silica stationary phase and chlorinated hydrocarbon carriers are used (Figure 5.). The larger the displacer concentration, the slower is the

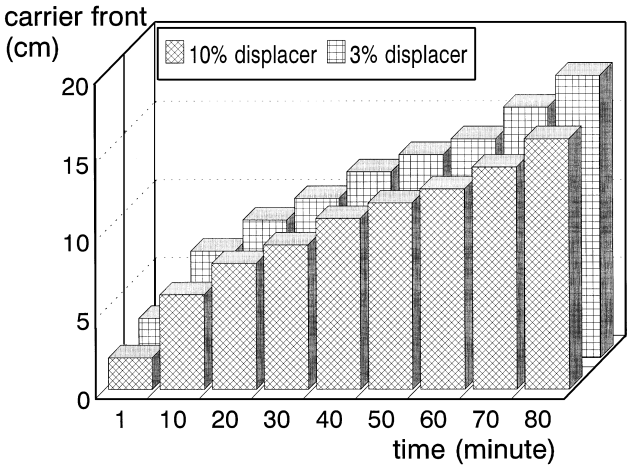

Figure 5. The presence of displacer modifies the time versus solvent front distance curves when silica stationary phase and chloroform carrier was used. 10% and 3% triethanolamine. The stationary phase was TLC plate 20 x 20 cm, silica gel 60 of Merck (Darmstadt, Germany).

progress of the carrier front, the faster the displacer front, and the higher the R_{DI} value of the displacer.

Carrier displacement (column) chromatography was used to improve purification of substances to be isolated. Partridge and Brimley[36] used a series of unsaturated fatty acids to improve separation of saturated fatty acids. In the case of displacement thin-layer chromatography, a simplified version of carrier displacement chromatography, spacer displacement chromatography, was used. Both carrier and spacer displacement chromatography use an additive multicomponent mixture; the constituent of this mixture improve the separation. While carrier displacement chromatography includes the extra compounds dissolved in the displacer part of the mobile phase, spacer displacement chromatography adds the additives to the samples.

In the case of separation of colorless compounds, a mixture of colored substances improve both the separation and the detection. The multicomponent Test Substance II (of Camag, Muttenz, Switzerland) is commercially available, and especially its lipophilic constituents, a large number of similarly behaving Sudan Black compounds, were used to improve separation of various phenylalkyl amines.

Planar displacement chromatography, or more exactly, displacement thin-layer chromatography (D-TLC) was used to scout the optimal conditions for HPDC,[7] and the optimization for carrier selection, displacer, and displacer concentration was confirmed by HPDC. We have used the same stationary phase for both HPLC and TLC. Later on, the possibility to follow visually the chromatographic procedure was utilized when individual operation steps were analyzed, and either colored compounds were monitored or the separation of the colorless spots were made visible by the help of the multicomponent dye Sudan Black. The D-TLC method made the deformation of the displacement front around the components to be displaced easily detectable. It was also shown that the order and arrangement of the sample is independent from the loading order of the sample components, and that the length of the displaced zone depends on the amount of the sample in the displacement train, but not on the sample size.[37-40]

Displacement thin-layer chromatography (D-TLC) was used to scout the optimal conditions for high performance displacement chromatography.[7] The aim was to perform preparative scale separation of corticosterone, deoxycorticosterone, and Reichstein's substance S with high performance liquid chromatographic columns and instrumentation. Thin-layer chromatography in the displacement mode was used to find an adequate carrier as well as suitable displacer and its appropriate concentration. The same stationary phase for both HPLC and TLC were used. Displacement TLC indicated the optimum composition of the stationary and mobile phases, and subsequent displacement HPLC had good results. It was direct proof that a good preparative scale separation can be modeled by planar chromatography.

As displacement chromatography is based on the similar interactions between the stationary phase and the solute, displacement thin-layer chromatography provides an excellent tool to establish the sequence of lipophilicity for a series of compounds, e.g., metabolites.[41] It was utilized when the lipophilicity of deprenyl metabolites – deprenyl-nordeprenyl-methamphetamine-amphetamine-norephedrine – were investigated. The fast and easy displacement TLC gave the same order of lipophilicity for these metabolites as the more expensive and rather time consuming reversed phase thin-layer chromatography did.

The feasibility to follow visually the chromatographic procedure was utilized when the individual operation steps were analyzed, and either colored compounds were monitored or the separation of the colorless spots were made visible by the help of the multicomponent dye Sudan Black. The D-TLC method made the deformation of the displacement front around the components to be displaced easily detectable. It was also shown that the order and arrangement of the sample is independent from the loading order of the sample com-

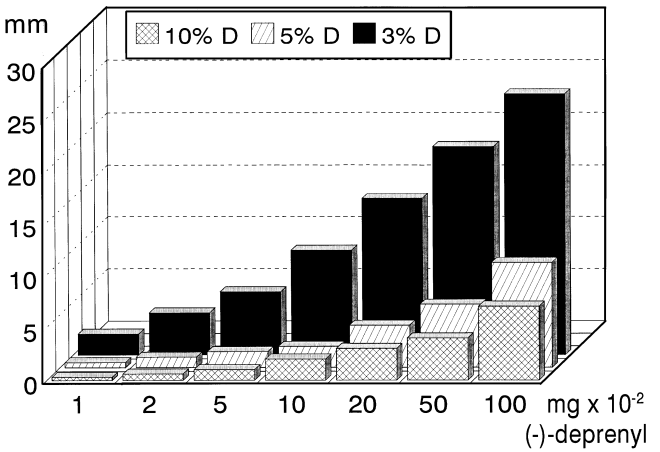

Figure 6. Both the displacer concentration and the load influence the length of the displaced zones. 10%D, 5%D and 3%D means the displacer concentration in the carrier, the length of zones of (-)-deprenyl were determined. The stationary phase was TLC plate 20 x 20 cm, silica gel 60 F_{254} of Merck (Darmstadt, Germany).

ponents and that the length of the displaced zone depends on the amount of the sample in the displacement train, but not on the sample size.[36]

Displacer concentration defines the length of the displacer zones, as given in Figure 6. The other factor defining the length of displaced zones is the amount of compounds spotted.[37] Tiny amounts (even if they were spotted in a large fields) of components are effectively concentrated by the displacement train. This procedure is based on the observation of Horváth et al.,[42] who purified antibiotics from fermentation broth; several liters of solution was concentrated to microliters using displacement HPLC.

At the same time the displaced compounds may deform the displacer front. This deformation may be easily observed by planar arrangement of the displacement system (Figure 7.). The position that relates to the arrangement of the solute and displacer is taken in a very short distance of displacement run, and it does not change through further development.

Arrangement of the solutes to form a displacement train is performed within several millimeters of displacement run. The requirement for the generation of this situation (fully developed displacement train), however, depends on the concentration of the displacer. The larger the displacer concentration is, the shorter distance of displacement run is required.

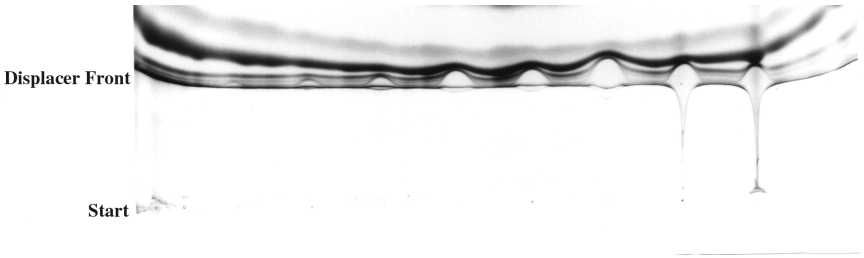

Figure 7. Displaced compounds may deform the displacer front, this phenomenon may be easily observed by planar arrangement of the displacement chromatography system. 0.01, 0.02, 0.05, 0.1 (twice), 0.2, 0.5, and 1 mg of L-amphetamine and 0.01, 0.02, 0.05, 0.1 (twice), 0.2, 0.5, and 1 mg L-deprenyl were co-loaded from left to right. The spots and the displaced zones were visualized by the use of Sudan Black components of Test Mixture II of Camag (Muttenz, Switzerland) that was loaded through the front line.

A method with regulated propagation of mobile phase is when either a pressure at the inlet[43-46] or a vacuum[47] at the outlet of the mobile phase is applied. Such a later case was published by Berezkin,[47] who used a vacuum-operated system. The basic characteristic of the forced-flow systems is that the planar stationary phase is covered by a membrane, and this membrane prevents the mobile phase from evaporation. This covers the mobile phase consequences that the behavior of the spots of compounds follows the rule of saturated-chamber system at R_F values under about 0.6, and follows the rule of super-unsaturated system when they migrate near the solvent front. Therefore, displacer front keeps an adequate and beneficial distance from the front, leaving thereby space enough for spreading the displaced compounds. Forced-flow thin-layer displacement chromatography (FF-D-TLC) means displacement type of development when propagation of the mobile phase is done by a pump. Over the practical significance of FF-D-TLC, the well-concentrated spots were able to indicate deformation of their shape. Therefore, both the displacement front bifurcation and severance in the displacement train may be observed. The probable cause of this effect is the insufficient supply of the mobile phase.

Two Dimensional Planar Displacement Chromatography

One of the basic advantages of planar chromatography is the possibility of simple and easy arrangement using two-dimensional separation. Even in the case of the two-directional separations, the peak capacity of the procedure is multiplied with the square root of 2. In case of real two-dimensional planar separation, either chromatography-electrophoresis, or straight-phase-reversed phase chromatography, or elution-displacement separations are usually

employed. We have used elution and displacement chromatography in the 1st and 2nd dimensional development, respectively.

Xenobiotics, metabolites, degradation products, and natural products can be easily analyzed using two-dimensional thin-layer chromatography.[39] The 1st dimensional run is usually done with elution type of development; displacement mode of development is preferred in the 2nd dimension. Metabolites extracted from urine or blood, and natural products isolated from plant extract were beneficially monitored using two-dimensional elution-displacement thin-layer chromatography.

PLANAR DISPLACEMENT CHROMATOGRAPHY, WHAT FOR?

Thin-layer displacement chromatography has been used for almost two decades.[7] It was successfully used for scouting and optimization of stationary and mobile phases for displacement development using HPLC. On the basis of TLC optimization, displacement development of HPLC generated highly concentrated bands, and also the overlapping parts of bands of the preparative scale separation was diminished.

Spacer-displacement (thin-layer) chromatography perfectly eliminated the overlapping parts. Thin-layer chromatography with displacement mode of development has also brought its own independent role into practice. D-TLC of radiolabeled drugs and metabolites has proven to be an easy and inexpensive way to find metabolites, identify and quantitatively measure the known metabolites, and find the new, earlier unknown metabolic products of drugs. The results are based on the effective separation by D-TLC and also by the application possibility of contact detection method by the use of an X-ray film.

Two-dimensional planar chromatography offers an unusual way of detection. The 2nd dimensional run with displacement mode of development concentrates the band instead of the usual band-spreading. In addition, the separation mechanism of the D-TLC basically differs from the elution mode, therefore, transforming the two-directional run into a bona-fide two-dimensional separation.

ACKNOWLEDGMENTS

This project was financially supported by the grant Nos. T023370 and T025412 of Otka, Budapest, Hungary. The authors thank Prof. J. M. Varga (University of Innsbruck, Austria) for his advice. Present affiliation for T. Csermely: Clinics of Anesthesiology and Intensive Therapy, Army Hospital, Budapest, Hungary.

REFERENCES

1. A. Tiselius, Ark. Kemi. Mineral. Geol., **16A,** 1-8 (1943).

2. L. S. Ettre, "Evolution of Liquid Chromatography: A Historical Overview," in **High Performance Liquid Chromatography**, Vol. 1, Cs. Horváth, ed., Academic Press, New York, 1980, pp. 1-74.

3. M. S. Tswett, Ber. Dtsch. Bot. Ges., **24**, 235-244 (1906).

4. M. S. Tswett, Ber. Dtsch. Bot. Ges., **24**, 384-393 (1906).

5. Cs. Horváth, A. Nahum, J. Frenz, J. Chromatogr., **218**, 365-393 (1981).

6. H. Kalász, Cs. Horváth, J. Chromatogr., **215**, 295-302 (1981).

7. H. Kalász, Cs. Horváth, J. Chromatogr., **239**, 423-438 (1982).

8. J. Porath, C. H. Li, Biochim. Biophys. Acta, **13**, 268-281 (1954).

9. E. A. Peterson, Anal. Biochem., **90**, 767-775 (1978).

10. A. R. Torres, E. A. Peterson, J. Biochem. Biophys. Methods, **1**, 349-360 (1979).

11. Cs. Horváth, J. Frenz, Z. El Rassi, J. Chromatogr., **255**, 273-293 (1983).

12. S. M. Cramer, Z. El Rassi, Cs. Horváth, J. Chromatogr., **394**, 305-314 (1987).

13. S. M. Cramer, Cs. Horváth, Preparative Chromatography, **1**, 29-42 (1988).

14. K. F. Kalghatgi, I. Fellegvári, Cs. Horváth, J. Chromatogr., **604**, 47-53 (1992).

15. R. S. Hodges, T. W. L. Burke, C. T. Mant, J. Chromatogr., **444**, 349-362 (1988).

16. K. Veeraragavan, A. Bernier, E. Braendli, J. Chromatogr., **541**, 207-220 (1991).

17. Y. J. Kim, S. M. Cramer, J. Chromatogr., **549**, 89-99 (1991).

18. F. Helfferich, G. Klein, **Multicomponent Chromatography**, Marcel Dekker, New York, 1970, p. 37.

19. G. Veress, Cs. Horváth, E. Pungor, "Efficiency of Separation Processes as Applied to Displacement Chromatography," in **, New Approaches in Liquid Chromatography**, H. Kalász, ed**.,** Elsevier, Amsterdam, 1994, pp. 45-56.

20. D. D. Frey, J. Chromatogr., **409**, 1-13 (1987).

21. Q. Yu, D. D. Do, J. Chromatogr., **538**, 285-292 (1991).

22. M. W. Phillips, G. Subramanian, S. M. Cramer, J. Chromatogr., **454**, 121 (1988).

23. A. M. Katti, E. V. Dose, G. Guiochon, J. Chromatogr., **540**, 1-20 (1991).

24. R. Ramsey, A. M. Katti, G. Guiochon, Anal. Chem., **62**, 2557-2565 (1990).

25. T. Gu, G.-J. Tsai, G. T. Tao, Biotechn. Bioengin., **37**, 65-70 (1991).

26. J. Frenz, C. P. Quan, W. S. Hancock, J. Bourell, J. Chromatogr., **557**, 289-305 (1991).

27. N. A. Izmailov, M. S. Shraiber, Farmatsia, **3**, 1-12 (1938).

28. W. G. Brown, Nature, **143**, 377-379 (1939).

29. J. G. Kirchner, J. M. Miller, G. J. Keller, Anal. Chem., **23**, 420-425 (1951).

30. E. Stahl, Pharmazie, **11**, 633-637 (1956).

31. **Thin-Layer Chromatography. A Laboratory Handbook**, English Edition, E. Stahl, ed., Academic Press, New York, 1965.

32. **Handbook of Thin-Layer Chromatography**, 2nd Edition, J. Sherma, B. Fried, eds., Marcel Dekker, New York, 1996.

33. **Modern Thin-Layer Chromatography**, N. Grinberg, ed., Marcel Dekker, New York, 1990.

34. H. Kalász, M. Báthori, LC∗GC International, **10**, 440-445 (1997).

35. H. Kalász, L. S. Ettre, M. Báthori, LC∗GC Magazine, **15**, 1044-1050 (1997).

36. S. M. Partridge, R. C. Brimley, Biochem. J., **44**, 313-318 (1951).

37. H. Kalász, Cs. Horváth, "Effect of Operating Conditions in Displacement Thin-Layer Chromatography," in **New Approaches in Liquid Chromatography**, H. Kalász, ed**.,** Elsevier, Amsterdam, 1994, p. 57.

38. H. Kalász, L. Kerecsen, J. Nagy, J. Chromatogr., **316**, 95-104 (1984).

39. H. Kalász, J. High Resol. Chromatogr., Chromatogr. Commun., **6**, 49-50 (1983).

40. H. Kalász, M. Báthori, B. Matkovics, J. Chromatogr., **520**, 287-293 (1990).

41. J. Lengyel, K. Magyar, I. Hollósi, T. Bartók, M. Báthori, H. Kalász, S. Fürst,J. Chromatogr., **762**, 321-326 (1997).

42. Cs. Horváth, J. Frenz, Z. El Rassi, J. Chromatogr., **255**, 273-291 (1983).

43. E. Tyihák, E. Mincsovics, H. Kalász, J. Chromatogr., **174**, 75-81 (1979).

44. H. Kalász, J. Nagy, E. Mincsovics, E. Tyihák, J. Liquid Chromatogr., **3**, 845-855 (1980).

45. H. Kalász, J. Nagy, J. Liquid Chromatogr., **4**, 985-1005 (1981).

46. H. Kalász, M. Báthori, J. Nagy, Chromatographia, **27**, 253-256 (1989).

47. V. G. Berezkin, V. V. Buzaev, J. Chromatogr., **758**, 125-134 (1997).

Received October 30, 1998 Author's Revisions October 14, 1999
Accepted June 3, 1999 Manuscript 4933