

Milestones in Chromatography

**Leslie S. Ettre and
Huba Kalász**

This installment of “Milestones in Chromatography” surveys the evolution of thin-layer chromatography and compares the technique with high performance liquid chromatography.

Leslie S. Ettre
Milestones in Chromatography
Editor

The Story of Thin-Layer Chromatography

The evolution of chromatography in the past 100 years has been characterized by a number of important milestones, each heralding the start of an important branch. Partition chromatography started in 1941 and modern gas chromatography (GC) in 1951–1952. Of the more recent techniques, high-performance liquid chromatography (HPLC) was initiated in the mid-1960s, capillary electrophoresis (CE) around 1980, and capillary electrochromatography (CEC) in the past decade. In all these techniques, separation takes place in a column. It is, however, also possible to carry out the separation on a planar surface. In fact, the first widespread application of partition chromatography was paper chromatography introduced in 1944; then, toward the end of the 1950s, thin-layer chromatography (TLC) practically replaced paper chromatography as one of the most popular, routine chromatographic techniques.

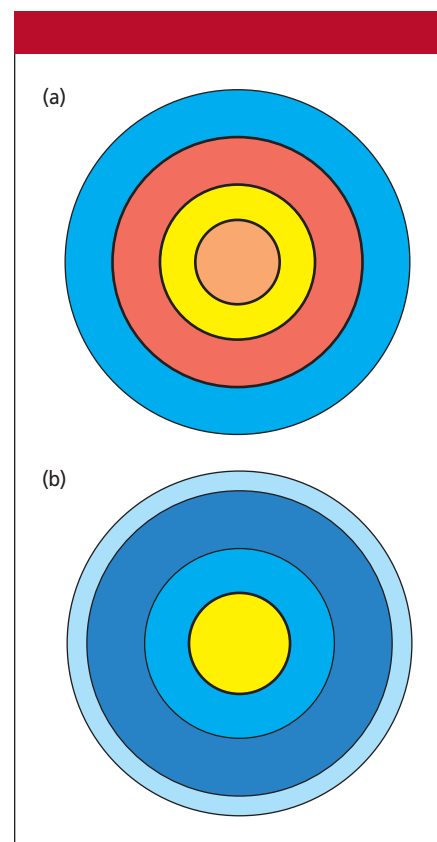
The subject of this “Milestones in Chromatography” column is TLC, with particular emphasis on the technique’s evolution, which is an excellent illustration how new scientific improvements directly follow from the achievement of the previous contributor. It is not our aim to discuss theoretical aspects or go into detail on the many variations of the operational parameters of the technique or the wide range of its applications. Readers interested in these questions can find a number of excellent summaries in the literature (1–6) which, in turn, provide hundreds of references for further details.

It should be noted that when dealing with the activities of the pioneers we shall consistently use the term *thin-layer chromatography* although — as explained below — this term is of more recent origin.

The Beginnings

The technique of TLC was first used in 1937–1938 at the Institute of Experimental Pharmacy of the State University of

Kharkov, Ukraine, by Nikolai A. Izmailov (1907–1961), the young head of this institute, and Maria S. Shraiber (1904–1992), his graduate student. As described in her recollections (7), they were searching for appropriate methods for the rapid analysis of galenic pharmaceutical preparations (plant extracts). They were aware of classical column chromatography, but such an analysis would have required too much time. Therefore, they felt that if the adsorbent would be prepared in the form of a thin layer on a glass plate, it would behave like in a column, but the time needed to characterize the sample would be much shorter. Accordingly they coated micro-



Idealized *ultrachromatograms* of extracts of (a) belladonna (*Atropa belladonna*) and (b) digitalis (*Digitalis purpurea*) according to Izmailov and Shraiber (8).

scope slides with a suspension of various adsorbents (calcium, magnesium, and aluminum oxide), deposited one drop of the sample solution on this layer, and added one drop of the same solvent one would use in a column to develop separation. The test was successful: the separated sample components appeared as concentric rings that fluoresced in various colors under a UV lamp. In the paper summarizing their results (8), Izmailov and Shraiber demonstrated that the sequence of the concentric multicolored rings on the plate was identical to the sequence of the colored rings one would have obtained on a regular chromatographic column containing the same adsorbent, however, the time needed for an analysis was much shorter. They called the new variant *spot chromatography* and the result on the microscope slides *ultrachromatograms*. In their paper, Izmailov and Shraiber showed a number of idealized drawings of these ultrachromatograms and tabulated the colors of the rings for a few plant extracts used as medications. It was also their intention to compile a manual presenting color drawings of the ultrachromatograms of a large number of galenic preparations; however, the war interfered with these plans (7).

The paper of Izmailov and Shraiber was published in a Russian pharmaceutical journal that was practically unknown outside the Soviet Union. However, its abstract was included in a Russian review journal and through it in *Chemical Abstracts* (9). This was read by M.O'L. Crowe of the New York State Department of Health, who then adapted the technique for his use. Crowe prepared the adsorbent layer in a Petri dish, added a drop of the sample solution in the center and then dropwise the developing solvent until sufficient separa-

tion was obtained. According to his brief note published toward the end of 1941, he had used this technique for the previous two years to scout for the best developing solvent in column chromatography (10).

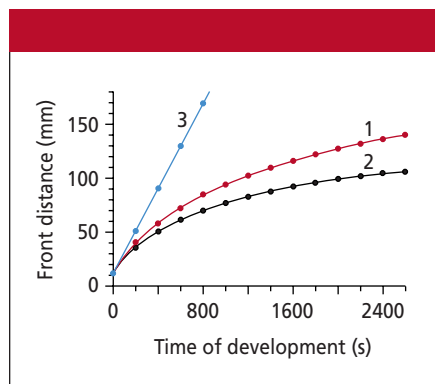
In his 1947 textbook on chromatography, T.I. Williams described a further improvement of the method of Izmailov and Shraiber (11). He now prepared the adsorbent-coated glass plates in the form of a sandwich: the adsorbent layer was covered by a second glass plate with a small hole through which the sample (and solvent) drops could be applied.

The next step in the development of TLC was the work of Meinhard and Hall at the University of Wisconsin. They now used a binder (corn starch) to hold the coating on the glass plate and added a small amount of Celite powder to the adsorbent particles to improve the consistency of the layer. Meinhard and Hall called the technique *surface chromatography* and used it for the separation of inorganic ions (12).

As mentioned, in these early investigations development of the sample spot was carried out by adding one or a few of drops of a solvent. This type of chromatography strongly resembled the spot-test analysis technique (Tüpfelanalyse) developed in the 1920s and 1930s by Fritz Feigl, an Austrian scientist. Almost completely forgotten today, it was a quite popular analytical method at that time. In spot-test analysis, a drop of the sample solution is placed on a filter paper strip and drops of a specific reagent solution are added, observing the various colored reaction products. If the sample represents a mixture, some separation is also taking place. These spot-tests were mainly used for the qualitative analysis of inorganic ions.

TLC Matures

Modern TLC started 50 years ago with the work of Justus G. Kirchner (1911–1987) at the U.S. Department of Agriculture Fruit and Vegetable Laboratory in Southern California. Kirchner investigated the flavor components of the juices of citrus fruits. According to his personal recollections (13), very large volumes of the fresh juices had to be processed (3000 gal of orange and 2760 gal of grapefruit juice) because the amount of the flavor material was exceedingly small. The next problem was to find an analytical method for the investigation of the composition of the juice concentrates. Column chromatography would have been adequate for this purpose, except that the compounds to be separated were



Front distance versus time of development. Conventional TLC using (1) saturated and (2) unsaturated development chamber; (3) forced-flow TLC.

colorless (or did not have a distinct color), thus their identification would have been quite difficult. Paper chromatography (by then well established) seemed to be suitable, particularly because of the convenience of visualizing the separated spot by spraying the paper with selective reagents. However, they soon found that paper was too mild an adsorbent for the separation of terpenoid compounds present in the juices. Paper impregnated with silica gel held some promise, but its sample capacity was too small. Then one day, Kirchner remembered the abstract of the paper by Meinhard and Hall (12) he had read in *Chemical Abstracts*, and decided to follow it. He coated a layer of silicic acid (using starch as the binder) on strips of glass, but instead of adding just a drop of the developing solvent (as it was done by the earlier investigators), he developed the plates in the so-called ascending mode used in paper chromatography. (In this technique, the spotted plates are placed into a closed chamber, dipping one side of the plate into the solvent [the mobile phase], which would then ascend through capillary action, carrying with it the sample components that are separated during their passage on the plate.) The experiments proved to be successful and their publication can be considered the start of modern TLC (14). Soon after, the possibility of quantitative analysis was also demonstrated, using absorbance measurement of the separated spots (15).

Kirchner introduced the term *chromatostrips* for the adsorbent-coated glass plates. It should be noted that his group used not only narrow glass strips but also square coated plates, permitting them to run multiple samples on a single plate simultaneously. They also demonstrated the possibility of two-dimensional chromatography on such plates, a technique well known at that time in paper chromatography. In addition they introduced the use of reactions carried out on the plate, for identification purposes.

Kirchner also developed another variation of TLC. He coated silicic acid (bound with gypsum) on a glass rod. In this way a silica column was created without the containing envelope (that is, the tube); we may also consider these *chromatobars* (Kirchner's name for them) as a thin adsorbent layer wrapped around a glass rod. Development of the chromatograms was carried out similar to the chromatostrips, in the ascending manner, dipping the end of the rod into the solvent and the separated zones could also be identified in a similar manner as in paper chromatography or TLC, by spraying with various reagents (16). It is interesting to note that Kirchner's chromatobars recently were reinvented and are now even produced under the name *chromarod* by Iatron Laboratories (Tokyo, Japan). The only difference is that while Kirchner used standard glass rods and prepared a fairly thick adsorbent layer around them, present-day chroma-

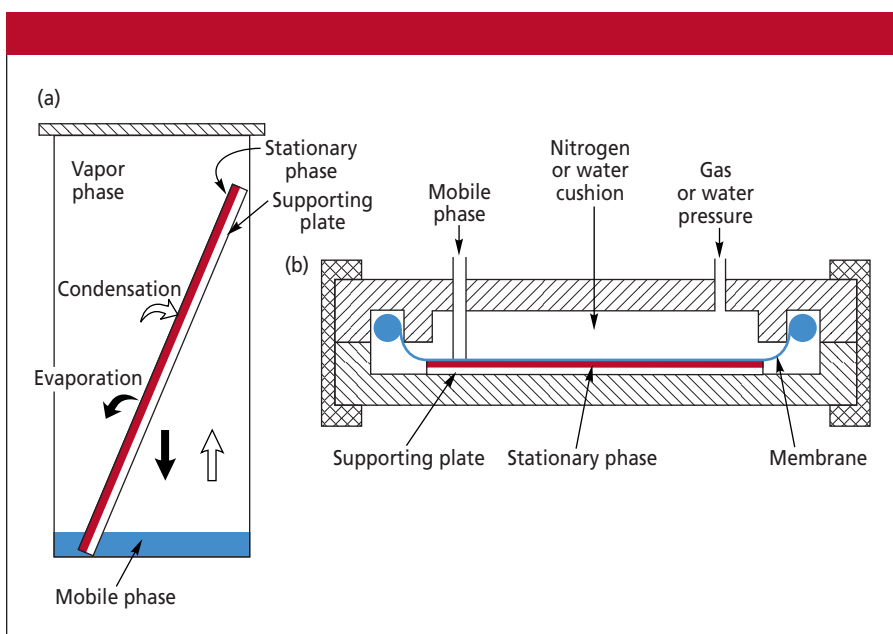
rods utilize a thin (0.9 mm) quartz wire as their core and a thin (75 μm) coating around it (17,18).

After Kirchner's publications, a limited number of laboratories started to use his technique. Noteworthy among these investigators was Reitsem (19), who utilized such *chromatoplates* (his term) for the analysis of a wide variety of essential oils. In spite of this, however, it was more than a decade before TLC became a generally accepted, major variant of chromatography.

The Activities of Egon Stahl

TLC became a universally accepted analytical technique, a full-fledged variant of chromatography, mainly due to the activities of Egon Stahl (1924–1986). Stahl was associated first with the University of Mainz and, from 1958 on, with the University of Saarbrücken in Germany (20). In fact, he was the first who consistently used the term thin-layer chromatography (Dünnschicht-Chromatographie) to characterize the technique, and his choice of this name was almost immediately universally accepted.

Stahl was involved in the investigation of various essential oils and was able to obtain good results using adsorbent-coated glass plates, following the recommendations of Kirchner. However, he soon found out that neither the method nor the adsorbent to be used had been optimized. He also learned that the commercially available adsorbents had to be modified and treated in various ways before they could be used for coating the plates. Therefore, Stahl started systematic investigations of the operational parameters and the preparation of the proper adsorbents. He first reported his preliminary findings in 1956, in a German pharmaceutical journal (21); however, this publication was largely ignored by the scientific public. Meanwhile, Stahl continued his efforts to standardize the method, construct simple equipment that enabled the application of a uniform thin layer, and have standard adsorbents commercially available that could be directly coated on the plates without further preparation. His efforts were finally fulfilled by the spring of 1958, when the necessary basic instrumentation made by Desaga and "silica gel G according to Stahl for TLC" by E. Merck were both introduced at the international Achema exhibition of chemical equipment in Frankfurt. Simultaneously, Stahl also published an informative article (22) dealing with the use of this system and show-



Functional schematics of (a) ascending development chamber for conventional TLC and of (b) forced-flow TLC chamber.

ing a wide range of applications. Now the situation changed. The standardized method aroused a wide interest and, within a few years, TLC became a widely used laboratory technique. Stahl significantly contributed to the meteoric rise of its application, by further improving the technique and expanding its fields of application. His activities culminated in 1962 with the publication of a very useful and highly popular handbook of TLC that was translated into a number of languages (23).

High-Performance TLC

Although TLC soon enjoyed a wide application, it was essentially considered as a qualitative technique for the analysis of relatively simple mixtures. Further advances were directed toward instrumentation of TLC and on improvements in the technique itself. Instrumentation was developed to permit more precise spotting of the sample onto the plates and the quantitative evaluation of the separated spots. Improvements in the technique itself resulted in higher separation power and faster analysis. Just as the name change of liquid chromatography to "high-performance liquid chromatography" (HPLC) characterized the significant change in performance capabilities, this improved TLC was also named high-performance TLC (HPTLC) by R.E. Kaiser, who was instrumental in its development (24).

The main difference between conventional and high-performance TLC was in the particle size and range of the adsorbent. The original "silica gel for TLC according to Stahl" had a fairly broad particle size range (10–60 μm), with an average of about 20 μm , but the material for HPTLC had a narrower range and an average particle size of only about 5 μm . The plates were also smaller, 10 \times 10 cm against the conventional 20 \times 20 cm, and the sample volume was reduced by an order of magnitude. The method of sample application was also improved with the design of mechanical applicators (dosimeters) permitting a reduction in the diameter of the starting spots. These improvements significantly reduced the time needed for an analysis, with a simultaneous increase of the separation efficiency.

The use of very fine particles, however, results in some additional problems. For example, the movement of the mobile phase on the plate will significantly slow down after a relatively short distance. On the other hand, as emphasized by Guiochon and co-workers (25–29), a fast and constant flow velocity of the mobile phase is needed to obtain an optimum efficiency. To overcome this problem, Kaiser started to apply pressure to the TLC plate. This then led to the development of the so-called forced-flow TLC technique.

Forced-Flow TLC

Developing the TLC plates in the conventional developing chambers has a shortcoming. In addition to the stationary phase (on the plate) and the liquid mobile phase (ascending by capillary action), the vapor of the mobile phase is also present, forming a third phase. During development, molecules of the mobile phase will condense from the vapor phase onto the plate, both above the ascending mobile phase front (where the plate is dry) and below the front (where it is wet). At the same time, molecules of the mobile phase will evaporate from the wet part of the plate. Due to this constant condensation–evaporation process, the speed of the movement of the mobile phase front will depend on the degree of saturation of the vapor phase. Also, with the increase of the front distance, the upward movement of the mobile phase on the plate will slow down; this is a direct consequence of the increasing weight of the developing solvent on the plate. In the case of mixed mobile phases, there will also be a solvent composition gradient on the plate due to differ-

ences in the ascending speed of the mobile phase components and in their vapor pressures.

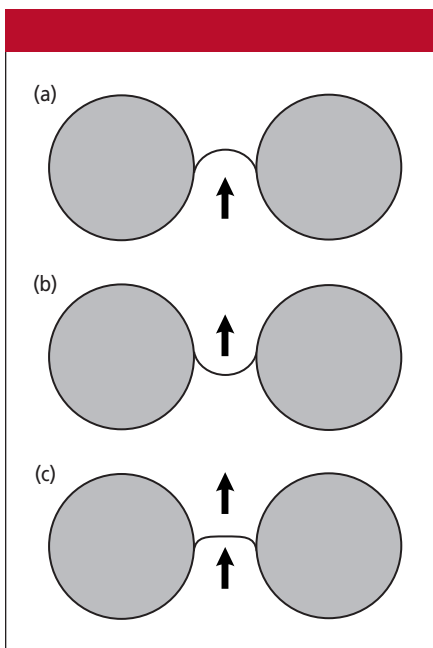
To overcome the problems caused by the changing velocity of the mobile phase in the plate and also to eliminate the presence of the vapor phase in the TLC system, Tyihak, Mincovics, and Kalász developed the so-called over-pressured TLC (OPTLC) or forced-flow TLC (FFTLTLC) (30–31).

In the FFTLC system, the samples are spotted on the dry plate, which then is placed into the pressurized development chamber. There the stationary-phase layer is tightly covered and sealed on its sides by an elastic membrane (a plastic sheet), pressurized by an inert gas or water filling up the cushion above the layer. The mobile phase is delivered with help of a pump, at a constant velocity, through a slit in the membrane directly to the stationary phase layer. Depending on the construction and location of the solvent inlet, various configurations can be handled. For example, systems can have a linear or circular front, or the entry point may be either at the lower edge or the middle of the plate.

It is interesting to compare the mobile-phase flow profile in liquid column chromatography with that of both conventional and forced-flow TLC. In column LC, the stationary-phase particles are wet, because the mobile phase is continuously flowing through the column, and the flow is pressure-driven; here, the profile of the microflow among the particles is convex. In TLC, the stationary phase is dry before the advancing front of the developing solvent. In conventional TLC, the solvent flow is propagated in the dry plate by capillary action and thus, has a meniscus representing a concave profile. In FFTLC, the flow is again pressure driven as in column chromatography, but now, moving on a dry plate; here the two effects — the convex front and the meniscus — compensate one another, resulting in a straight flow profile.

Instrumentation

Basically, TLC is a simple technique and for its conventional version, very little instrumentation is needed. Samples can be applied to the stationary-phase layer with a micropipette or syringe, and the developing chambers have simple glass construction. For detection, the separated spots can be checked by visual inspection or made visible by spraying the plate with a reagent. The only equipment needed is for the reproducible coating of the glass plates but



The shape of the microflow profiles between the stationary phase particles. (a) HPLC; (b) conventional TLC; (c) forced-flow TLC.

today, a wide variety of precoated plates are commercially available.

For more advanced systems a number of instruments have been developed. The samples may be spotted by automated loading devices (dosimeters) permitting the application of small and uniform sample spots. Developing chambers with more sophisticated design are also available: the FFTLC system represents such an improved construction. For the quantitative evaluation of the separated sample components (spots), the plates can be scanned by densitometers, using absorbance or fluorescence measurements. Such an evaluation actually can produce chromatograms with peaks of the individual separated spots recorded against the length of the plate, and their area is proportional to the amount present. It is also possible to combine separation by TLC with optional methods for structure elucidation such as mass spectrometry (MS) or Fourier transform infrared (FTIR) spec-

troscopy, creating complex hyphenated systems.

Newer Developments

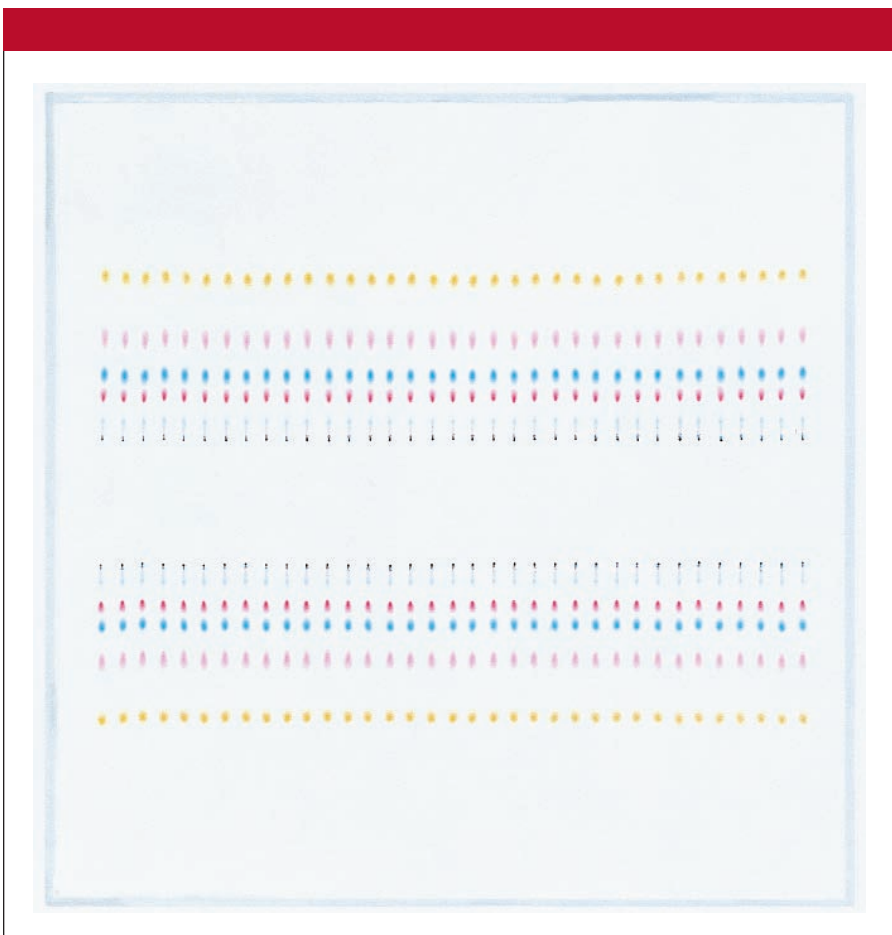
We have discussed in more detail the steps leading to the development of TLC as one of the important variants of chromatography. In recent decades, a number of further improvements have been introduced. However, their discussion is beyond the aim of such a historical retrospection; we only outline briefly some of the latest developments. Readers interested in more details may consult the general chromatography literature and the chromatography journals of which particularly two should be mentioned here: the *Journal of Planar Chromatography* (32) and the bibliography service of CAMAG (33).

In the first developmental period, silica gel or alumina was used most frequently as the stationary phase. However, the range of suitable phases is much wider today, including cellulose, ion-exchange resins,

polyamides, particles with chemically bonded groups, and various chiral phases. Although the plates can be prepared by the user, many different types of coated glass plates or plastic sheets are now commercially available.

With respect to the technique itself, displacement or electrophoresis can also be used in addition to the usual elution mode, and electrochromatography can also be carried out on a plate. The plates can be developed in three different ways: (a) in one direction (linear development); (b) as a circular chromatogram, introducing the mobile phase at the center, flowing toward the periphery of the plate and spotting the samples as a cluster of spots around the solvent entry position; and (c) as anticircular chromatogram applying the sample(s) on an outer circle and developing toward the center of the plate. The unique features of the latter technique are its high speed and higher sample capacity. In the case of linear development, one can also carry out two-dimensional TLC (similar to two-dimensional paper chromatography). In this technique, the spotted sample is first developed in one direction; then, after drying, the plate is turned 90° and developed again, but now with a different mobile phase.

In TLC, the development of the plate is usually finished before the solvent front — which is moving faster than the spots corresponding to the individual sample components — would reach the upper edge of the plate. Thus, at the end of a determination, the chromatogram is static: the separated spots are “frozen” on the plate and qualitative and quantitative evaluation is carried out with this plate. It has also been proposed to carry out TLC similar to the column chromatographic techniques, allowing a continuous flow of the mobile phase (the developing solvent). In this way the individual spots would eventually also reach the upper edge of the plate, just as the separated bands traverse the chromatographic column, eluting from its end. According to these suggestions, in such a continuous-flow TLC, a detector at the edge of the plate would sense the individual sample components, analogous to the detector in column chromatography located at the column's outlet. However, these suggestions were not followed in the practice.



TLC of a dye mixture using FFTLC. Plate: 20 × 20 cm silica, particle diameter: 5 μm. Mobile phase: toluene. Sample: toluene solution of CAMAG Test Substance II and Ceres Violet. Sample loading: manual, with a 1-μL syringe, in the center of the plate; 2 × 35 spots, loading time: 15 min. Cushion pressure (water): 50 atm. Running time: 3 min. (Courtesy of E. Mincsovic.)

TLC vs. HPLC

Although the basis of HPLC and TLC is the same, a number of major differences can be pointed out.

Naturally, the first difference is that HPLC is carried out in a column while TLC performs separation on an open arrangement of the stationary phase. In HPLC, one single sample is measured at one time in the chromatographic system (column, instrument); however, in TLC one can spot a large number of different samples on a single plate and develop them simultaneously.

In HPLC, the column is used for a long time, for the analysis of many samples. Because of this, excessively retained sample components accumulate in the column and may alter its characteristics or elute

much later when other samples are being analyzed, creating artifact peaks. On the other hand, the TLC plates are disposed of at the conclusion of each separation and the individual samples are analyzed parallel on the same plate, without interfering with one another.

In TLC, the detection process is static while in HPLC it is dynamic. As mentioned above, TLC produces a "frozen" chromatogram and (qualitative and quantitative) detection is carried out separately, independently of the separation process. On the other hand, in HPLC detection is continuous: the column effluent is continuously monitored. In HPLC, sample introduction, separation, and detection make up a continuous process in which these steps follow each other without interrup-

tion. However, TLC is a segmented process: it can be interrupted between sample application, development, and detection.

In HPLC, the time required to analyze n samples is n times the time required to separate one sample. However, in TLC, the time required to analyze n samples is the same as the time required for a single sample, where n is restricted only by the possible sample lanes on a single plate. It follows from this difference that TLC is a more effective technique for the routine analysis of a large number of samples.

Another advantage of TLC is that it enables visual observation of the separated spots on the plate and the use of using spraying reagents selective to certain compound groups. This permits the rapid screening of a large number of samples for the presence or absence of a certain substance, or for selecting the suitable mobile phase for optimum separation.

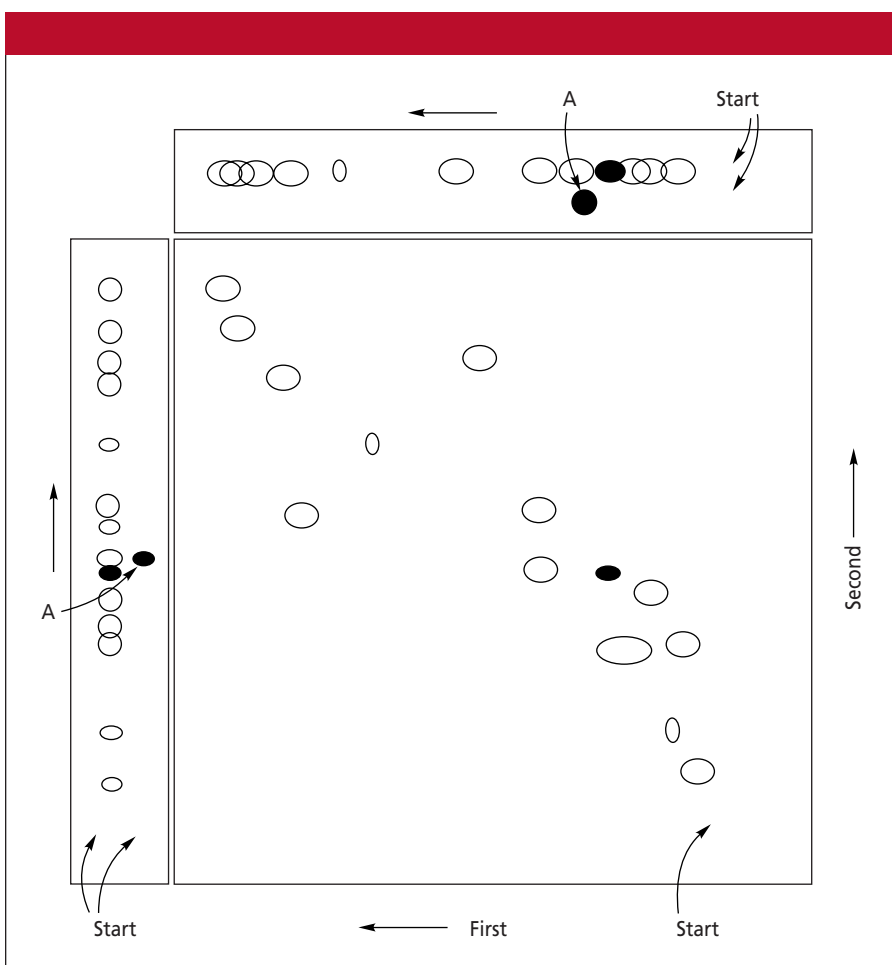
It is true that HPLC can generally provide a higher separation power (more theoretical plates) than TLC. However, in most separations carried out by HPLC, a high efficiency is really not necessary. In such applications the two techniques are quite comparable. The performance of TLC can be improved by automation and in the past decades, fully automated and computer-controlled systems have also become available, approaching the sophistication of the instrumentation in HPLC. However, one should not forget that in such highly sophisticated systems, TLC is losing its versatility and simplicity, the two basic characteristics that contributed to its high popularity and which continue to make it a universally used, routine analytical method.

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References

- (1) C.F. Poole and S.A. Schuette, *Contemporary Practice of Chromatography* (Elsevier, Amsterdam, 1984), pp. 619–699.
- (2) C.F. Poole and S.K. Poole, *Chromatography Today* (Elsevier, Amsterdam, 1991), pp. 649–734.
- (3) B. Fried and J. Sherma, *Thin-Layer Chromatography* (Marcel Dekker, Inc. New York, 4th ed., 1999).
- (4) Ch. Weins and H.E. Hauck, *LCGC* **14**(6), 456–469 (1996).
- (5) H. Kalász, L.S. Ettre, and M. Báthori, *LCGC* **15**(11), 1044–1050 (1997).



Two-dimensional TLC of a partially purified extract of the brown algae *Fucus serratus*, checking it for the presence of ecdysteroids. Silica plate, ascending development. After the first development, the plate was dried at room temperature, then turned 90° and redeveloped. Mobile phases: (1) 25:5:3 (v/v) chloroform–methanol–benzene; (2) 80:15:5 (v/v) ethyl acetate–96% ethanol–water. The spots were initially detected by UV illumination at 254 nm, then sprayed with vanillin–sulfuric acid reagent: spots in black (original color: turquoise blue) are indicative of ecdysteroids. The two side-tracks represent one-dimensional development of the same sample plus 20-hydroxyecdysone (spot A), with mobile phase 1 (top) and 2 (left). As seen, only one ecdysteroid was present in the sample, but it was not identical to 20-hydroxyecdysone. (Reproduced with permission from reference 34.)

- (6) H. Kalász and M. Báthori, in *Separation Methods in Drug Synthesis and Purification*, K. Valkó Ed. (Elsevier, Amsterdam, 2000), pp. 439–501.
- (7) M.S. Shraiber, in *75 Years of Chromatography — A Historical Dialogue*, L.S. Ettre and A. Zlatkis, Eds. (Elsevier, Amsterdam, 1979), pp. 413–417.
- (8) N.A. Izmailov and M.S. Shraiber, *Farmatsiya (Moscow)* **3**, 1–7 (1938). (For English translation, see N. Pelick, H.R. Bollinger, and H.K. Mangold, in *Advances in Chromatography*, J.C. Giddings and R.A. Keller, Eds. [Marcel Dekker, Inc., New York, vol. 3, 1966], pp. 85–118.)
- (9) *Khim. Referat. Zhur.* **2**(2) 90 (1939); *Chem. Abstr.* **34**, 855:9 (1940).
- (10) M.O.L. Crowe, *Ind. Eng. Chem. Anal. Ed.* **13**, 845–846 (1941).
- (11) T.I. Williams, *Introduction to Chromatography* (Blackie & Sons, Glasgow, 1947), p. 36.
- (12) J.E. Meinhard and N.F. Hall, *Anal. Chem.* **21**, 185–188 (1949).
- (13) J.G. Kirchner, in *75 Years of Chromatography — A Historical Dialogue*, L.S. Ettre and A. Zlatkis, Eds. (Elsevier, Amsterdam, 1979), pp. 201–208.
- (14) J.G. Kirchner, J.M. Miller, and G.J. Keller, *Anal. Chem.* **23**, 420–425 (1951).
- (15) J.G. Kirchner, J.M. Miller, and R.G. Rice, *J. Agr. Food. Chem.* **2**, 1031–1033 (1954).
- (16) J.M. Miller and J.G. Kirchner, *Anal. Chem.* **23**, 428–430 (1951).
- (17) R.G. Ackman, C.A. McLeod, and A.K. Banerjee, *J. Planar Chromatogr.* **3**, 450–462 (1990).
- (18) E.D. Hudson, R.J. Helleur, and Ch.C. Parrish, *J. Chromatogr. Sci.* **39**, 146–152 (2001).
- (19) R.H. Reitsema, *Anal. Chem.* **26**, 960–963 (1954).
- (20) E. Stahl, in *75 Years of Chromatography — A Historical Dialogue*, L.S. Ettre and A. Zlatkis, Eds. (Elsevier, Amsterdam, 1979), pp. 425–435.
- (21) E. Stahl, *Pharmazie* **11**, 633–637 (1956).
- (22) E. Stahl, *Chemiker Ztg.* **82**, 323–329 (1958).
- (23) *Dünnschicht-Chromatographie, ein Laboratoriumshandbuch*, E. Stahl, Ed. (Springer, Berlin). (Original German edition: 1962; second German edition: 1967; English edition: 1969.)
- (24) *HPTLC: High-Performance Thin-Layer Chromatography*, A. Zlatkis and R.E. Kaiser, Eds. (Elsevier, Amsterdam, 1977).
- (25) G. Guiochon, A. Siouffi, H. Engelhardt, and I. Halász, *J. Chromatogr. Sci.* **16**, 152–157 (1978).
- (26) G. Guiochon and A. Siouffi, *J. Chromatogr. Sci.* **16**, 470–481 (1978).
- (27) G. Guiochon and A. Siouffi, *J. Chromatogr. Sci.* **16**, 598–609 (1978).
- (28) G. Guiochon, F. Bressole, and A. Siouffi, *J. Chromatogr. Sci.* **17**, 368–386 (1979).
- (29) G. Guiochon, G. Körösi, and A. Siouffi, *J. Chromatogr. Sci.* **18**, 324–329 (1980).
- (30) E. Tyihák, E. Mincsovics, and H. Kalász, *J. Chromatogr.* **174**, 75–81 (1979).
- (31) H. Kalász, *Chromatographia* **18**, 628–632 (1984).
- (32) *Journal of Planar Chromatography*. Published jointly by the Research Institute for Medicinal Chemistry and Springer Verlag Hungarica, Budapest. The last volume published in 2000 was vol. 13.
- (33) “Bibliography Service on Thin-Layer Chromatography,” CAMAG Co. (Muttens, Switzerland). (The last volume published in 2000 was vol. 81.)
- (34) M. Báthori, G. Blunden, and H. Kalász, *Chromatographia* **52**, 815–817 (2000).

Huba Kalász is a research professor with the Department of Pharmacology of Semmelweis University of Budapest. He is a member of the Editorial Advisory Board of LCGC Europe.

Leslie S. Ettre
 “Milestones in Chromatography” editor
 Leslie S. Ettre is a research affiliate of the Chemical Engineering department of Yale University and a member of LCGC’s editorial advisory board. Direct correspondence about this column to “Milestones in Chromatography,” LCGC, 859 Willamette Street, Eugene, OR 97401, e-mail lcgcedit@lccmag.com.

