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Review

# Progress in forced-flow planar chromatography

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**Abstract**

This paper summarizes progress in forced-flow planar chromatography (FFPC) and demonstrates the importance of the different techniques like rotation planar chromatography (RPC), overpressured-layer chromatography (OPLC), and electro-planar chromatography (EPC). Special attention is paid to a novel analytical FFPC method in which continuous development and continuous evaporation of the mobile phase from the end of the chromatographic plate ensure forced-flow development. A simple, but powerful preparative forced-flow technique is also reported; in this technique hydrostatic pressure is used to increase mobile-phase velocity. Parallel- and serially coupled layers open up new vistas for the analysis of a large number of samples (up to 216) for high throughput screening and for the analysis of very complex matrices. The special features of fully off-line and fully on-line RPC, OPLC, and EPC are compared in a table. New detection methods—on-line coupling of OPLC with radiodetection and on-line OPLC–MS—are also discussed. The role of a new spraying device for post-chromatographic chemical detection and for biological detection is also discussed. Some applications, relating to different classes of substances, are given to demonstrate the versatility of the various FFPC techniques.


*Keywords:* Reviews; Forced-flow planar chromatography; Overpressured-layer chromatography; Electro-planar chromatography; Detection, FFPC; Rotation planar separation

## Contents



## 1. Introduction

In the last few years the state of the art of planar chromatography (PC) and thin-layer chromatography (TLC) has been summarized by several authors. The book ''Planar Chromatography–A Retrospective View for the Third Millennium'' was published at the end of 2001 [1]. An overview of TLC at the turn of the century was written by Poole [2] in 1999, and

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essential guides to method development in TLC/PC have been published by Poole [3] and by Nyiredy [4,5]. Recent developments in overpressured-layer chromatography (OPLC) [6,7], rotation planar chromatography (RPC) [8], electro-planar chromatography (EPC) [9], and both OPLC and EPC [10] have been summarized. A contemporary picture of the state of the art of forced-flow planar chromatography (FFPC) will be published in a book by Tyihák and Nyiredy [11] and in several chapters of a book by Sherma and Fried [12].

After listing all these abbreviations for the different methods we can point out that PC consists of two main categories: TLC and FFPC. FFPC can be divided into further categories like OPLC, RPC, and EPC. Theoretically all include analytical, micro-preparative, and preparative separation methods in which the mobile phase moves through a planar stationary phase (porous adsorbent), however, EPC has been applied for analytical purposes only because the use of this method is at an early stage.

In TLC, as an example of the conventional technique, the force driving solvent migration is the decrease in the free energy of the liquid as it enters the porous structure of the layer, the mechanism of transport is the action of capillary effects [13,14]. Movement of the compounds to be separated by TLC is the result of two opposing forces, the force driving the mobile phase and the retarding action of the stationary phase. The mobile-phase velocity—established by the system variables and otherwise beyond experimental control—varies as a function of time and migration distance, therefore capillary forces are inadequate for achieving the optimum velocity desired.

In FFPC, as an example of a modern technique, in addition to capillary action the solvent system migrates through the stationary phase under the additional influence of forced flow. Forced flow can be achieved either by the application of external pressure (vacuum, hydrostatic pressure or by application of a pump) for OPLC, centrifugal force for RPC, or an electric field for EPC [8].

In the following discussion the possibilities of approaching optimum mobile-phase velocity are summarized, progress in the three basic forced-flow techniques—RPC, OPLC and EPC—is given, and the state of the art of off-line and on-line chemical detection is reviewed. Sample application, stationary and mobile phases, development modes and detection methods in the different FFPC techniques are also compared with those of high-performance liquid chromatography.

## 2. Approaching the optimum mobile-phase velocity

The main problem of TLC is that capillary forces are inadequate for achieving the optimum mobile-phase velocity, and mobile-phase velocity declines as the solvent-front migration distance increases. Mobile-phase velocity is a complex function of the system conditions [13], and is mainly determined by the applied vapour phase conditions [14]. As shown in Fig. 1, the lowest linear development speed is achieved in a non-saturated chromatographic chamber whereas in a sandwich configuration chamber [15] the velocity is higher; the highest mobile-phase velocity—using capillary action—can be achieved in a saturated chromatographic chamber. When forced-flow is used—in linear development mode—this correlation is linear and constant over the entire separation distance.

Recently the flow TLC of Worontsov et al. [16] resulted in the idea of using continuous development [17,18] to achieve constant solvent velocity and,

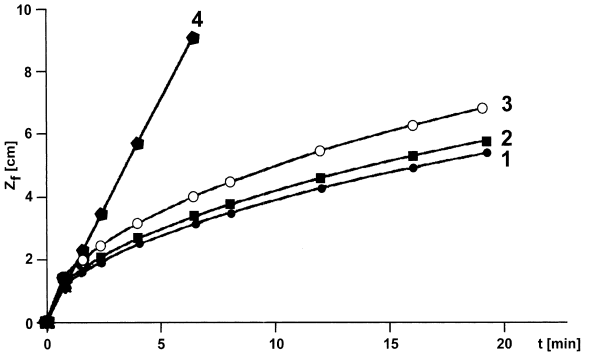


Fig. 1. Plot of solvent-front migration distance for dichloromethane on a HPTLC silica layer as a function of time, under different conditions. (1) Capillary-controlled flow in a non-saturated chromatographic chamber; (2) capillary-controlled flow in an ultramicro chromatographic chamber; (3) capillary-controlled flow in a saturated chromatographic chamber; (4) OPLC development at optimum velocity.

afterwards, for injection of the sample with a syringe. For realization of the technique no instructions were given in the paper. This was also true for the cover plate of the chamber and the detector system used. The novel scanner of the J&M company enables detection in diffuse light also, by use of optical fibres and a special fibre interface with a diode-array detector [19–21]. Because J&M could disconnect movement of the chromatographic plate in both directions ($X$ and $Y$), it was possible to perform on-line dynamic detection with the stationary chromatographic plate. A sandwich type chamber—with a distance of 1 mm between the stationary phase and the cover plate—was therefore constructed from Teflon by Nyiredy [22]; in this the chromatographic plate was inserted face-upwards and covered by a quartz glass cover plate, as illustrated in Fig. 2. The TLC/HPTLC plate was partly covered with a quartz glass plate 2 mm thick. The head of the optical fibres was placed directly on the quartz glass, near the end of the development distance [23]. At the end of the quartz glass cover plate the mobile phase was evaporated by means of nitrogen steam to ensure continuous development. After equilibration of the stationary phase with the mobile phase a small amount (0.5 μl) of the sample could be injected on-line through a septum-closed injection block by means of a Hamilton syringe.

Although no forces other than capillary action are used, evaporation of the mobile phase results in a linear mobile-phase velocity. The method can therefore be regarded as a type of forced-flow chromatography. The method integrates the idea of continuous development, flow TLC, and diode-array detection [23].

Another means of increasing the velocity of the mobile phase is the application of hydraulic pressure, as proposed by Botz et al. [24]. Fig. 3 illustrates the modified device for preparative circular separations in which the solvent reservoir, made of steel, and a Teflon sealing ring are placed on the layer and fixed by a magnet located below the chromatographic plate. To start the separation, the stationary phase is scraped off from the centre of the plate and the recess produced is filled with mobile phase. The device can be used with different types (ultramicro, normal) of chamber. Entry of the sample and mobile phase is regular over the whole cross-section of the preparative layer, irrespective of whether the sample is applied as a liquid or a solid. The device ensures rapid, efficient separation with all the advantages of circular development. Resolution is significantly higher in the lower $R_F$ range than that obtained from linear development, thus the resolution is of course lower in the higher $R_F$ range.

In an ultramicro chamber the glass cover plate is placed directly on the surface of the chromatographic plate. In a normal chamber the cover plate is placed on a 19-cm-diameter aluminium ring, the height of which can be varied between 0.5 and 2 cm, depending on the type of chamber used. To start development, the solvent reservoir is filled with the appropriate mobile phase and the level of this is kept constant by applying a constant hydrostatic pressure [24]. Needless to say, the same principle can be applied not only for off-line preparative chromatography, but also for analytical separations. Experi-

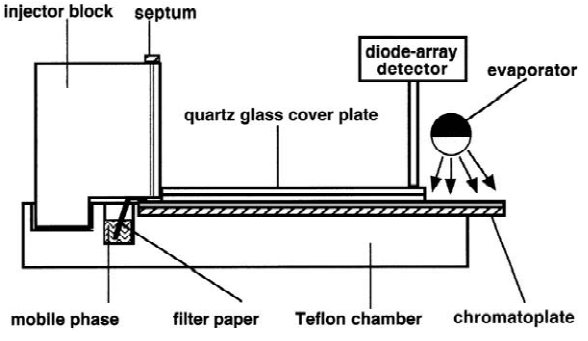


Fig. 2. The construction of the chamber for fully on-line HPTLC with diode-array detection using continuous development as the forced-flow technique.

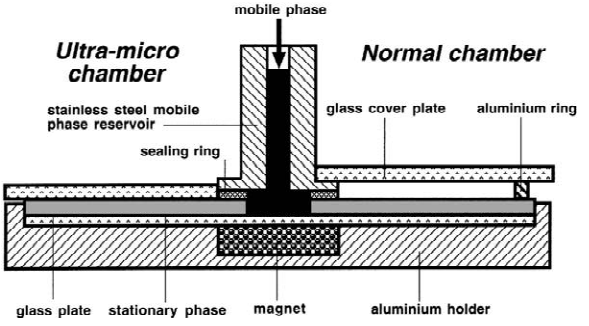


Fig. 3. Forced-flow circular preparative planar chromatographic device working with hydraulic pressure.

ments show that if a solvent reservoir with a diameter of 3 cm is used with 15 cm, hydrostatic pressure separations are quicker by 14%.

A paper published very recently by Berezkin et al. [25] described the applicability of hydrostatic pressure to TLC/HPTLC separations. In their experiments the authors covered silica gel layers with a polymeric membrane and applied hydrostatic pressure of heights of 10 and 70 cm. The paper shows how the presence of the vapour phase over the adsorbent layer affects migration velocity, the behaviour of the mobile phase, and the samples chromatographed. They found that elimination of the vapour phase over the stationary phase increased the distance of migration of the chromatographed compounds (higher $R_F$ values), furthermore that increasing the mobile-phase pressure resulted in a further increase in the distance of migration of the chromatographed substances compared with that observed for the isolated adsorbent layer at normal pressure. Eliminating the vapour phase and increasing the mobile-phase pressure also reduced the HETP value [25].

With modern RPC instruments (Rotachrom®, ExtraChrom®) the optimum mobile-phase velocity can be influenced by increasing the centrifugal force that means increasing the rotation speed. Increasing the speed of rotation threefold more than doubles the mobile-phase velocity, as is apparent from Fig. 4a. In general, the higher the speed of rotation the faster is the migration of the mobile phase [26]. Another means of increasing the mobile-phase velocity—to approach the optimum value—is to increase the diameter of the hole in the centre of the stationary phase at constant rotation speed. If this diameter is increased approximately fourfold, the mobile-phase velocity increases twofold as can be seen in Fig. 4b. The optimum rotation speed depends on the separation problem and also on the mobile phase used. The flow-rate is limited by the amount of solvent which can be accommodated by the layer without flooding over the surface. The greater the amount of solvent applied, the higher the rotation speed must be to keep the mobile phase within the layer [8].

Another possibility to increase the mobile phase velocity is to close the chromatoplate with sealing polymer on all four sides, to apply overpressure and to force the mobile phase through the stationary phase by means of a pump as it was first proposed for OPLC by Tyihák et al. [27]. Although using vacuum instead of a pump, the mobile phase can be also forced through the stationary phase—as it was

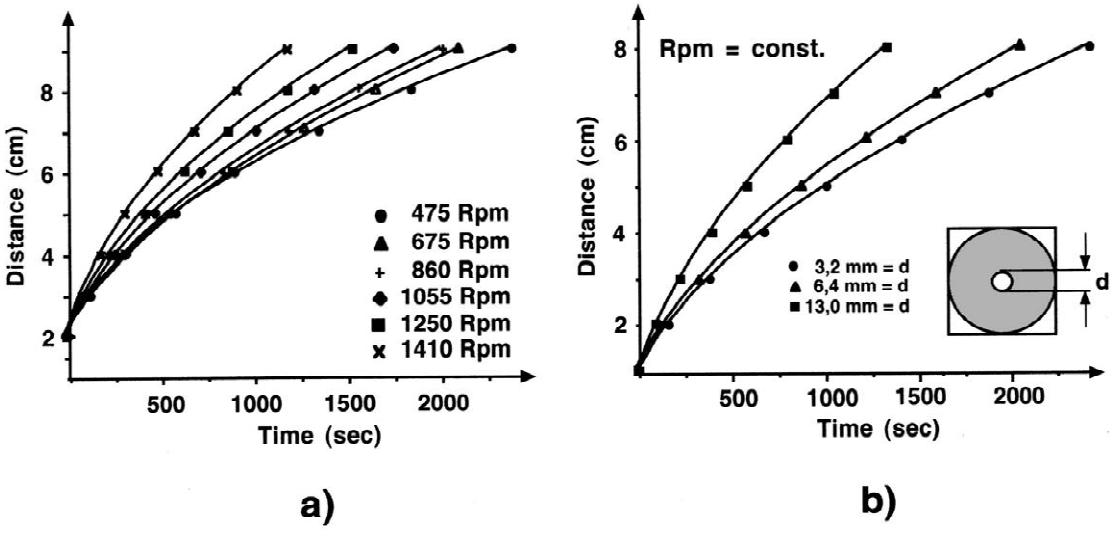


Fig. 4. Possibilities of increasing mobile-phase velocity by use of RPC. (a) Dependence of the distance travelled by the α front on migration time at different rotation speeds. (b) Dependence of the distance travelled by the α front on migration time for different perimeters of the hole in the centre of the stationary phase, at constant rotation speed.

later proposed by Delvordre et al. [28]—the mobile phase composition can be changed due to the vacuum; therefore this idea was not followed.

Increasing the external pressure in OPLC results in increased optimum mobile phase front velocity with an increased optimum velocity range. Increasing the external pressure in off-line OPLC reduces HETP. Despite this the same increase in efficiency was not observed for on-line OPLC [29], although application of a high external pressure resulted in the potential of reaching the optimum linear mobile-phase velocity, which was previously limited by the relatively low external pressure (10 bar). The results were similar when a 3-μm spherical particle size stationary phase was applied [30].

## 3. Progress in rotation planar chromatography (RPC)

The oldest FFPC method is the application of centrifugal force; this was first mentioned in 1947 by Hopf [31]. There have been different modifications of centrifugally accelerated paper and layer chromatographic techniques and equipment as has recently been summarized [8]. The term RPC [32], irrespective of the quality and type of stationary phase, embraces analytical, micro-preparative and preparative PC separations in which—besides capillary action—centrifugal force drives the mobile phase through the stationary phase from the centre to the periphery of the plate.

Depending on the size of the vapour space—an essential criterion in RPC—rotation planar separations can be classified as normal-chamber RPC, micro-chamber RPC, ultra-micro chamber RPC, and column RPC. All RPC techniques are single operating methods, whereas sequential RPC is a special combination of ''$n$'' steps of circular development and ''$n-1$'' anticircular zone concentration steps [8].

RPC can also be classified as an off-line or on-line separation technique. Analytical RPC methods are typical fully off-line processes [26], where the principal steps of sample application, development, evaporation of the solvent system, and densitometric evaluation are performed as separate operations. In fully off-line micropreparative or preparative RPC the zones are scraped off from the plate and the separated compounds are extracted from the stationary phase, by use of a solvent of high strength. In the fully on-line mode the principal steps are not performed as separate operations, the separated compounds are drained from the stationary phase by the centrifugal force and collected by means of a fraction collector [8].

In analytical separations up to 72 samples/chromatographic plates can be applied for qualitative assay [26] or quantitative determination of the separated substances, whereas for isolation and/or purification of compounds in micropreparative and preparative separation only one sample can be applied as a circle near the centre of the rotating stationary phase [8,33].

Although most of the progress in RPC is used as a preparative technique, as a qualitative method RPC is an excellent means for chromatographing a large number of samples [33] as is shown in Fig. 5 for the enantiomeric separation of 72 amino acid samples. Analytical RPC methods are rarely used for off-line quantitative determination because densitometric

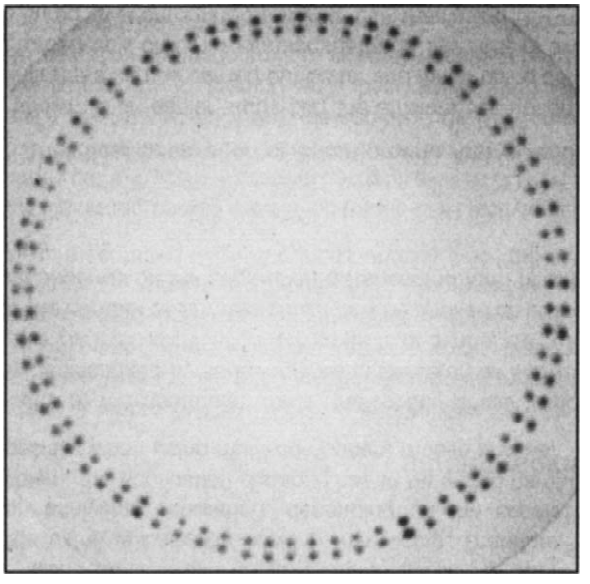

Fig. 5. Fully off-line analytical separation of 72 enantiomeric samples of phenyl-alanine on a chiral chromatographic plate. Conditions: mobile phase, methanol–water–acetonitrile, 5+5+3; flow-rate, 0.1 ml $min^{-1}$; rotation speed, 860 rev./min; temperature, 23 °C.

evaluation is rather complicated, and because of the lack of suitable software [8]. For as long as fully on-line TLC/HPTLC has been known [22,23], the use of optical fibres and a special fibre interface with a diode-array detector has enabled on-line detection of analytical RPC separations [34] through the quartz glass cover plate as is demonstrated schematically in Fig. 6. Thus, in addition to on-line preparative RPC separations using TLC or HPTLC plates, a single sample can also be analysed on-line.

Vuorela's research group [35] reported rapid analytical screening of indole-3-acetic acid and other indole derivatives in bacterial culture broth by TLC and RPC. Correlation was very good for TLC and RPC results obtained by use of the same mobile phase. Compounds which co-migrated in TLC were partially separated by RPC. To some extent this was due to the fact that in RPC higher $R_F$ values were obtained for the less strongly retained substances than in TLC. The higher $R_F$ values obtained by RPC meant that shorter separation distances could be used. Development over a distance of 5 cm took 9 min by RPC; development in TLC with a migration distance of 8 cm took 20 min.

Although RPC can be used for analytical separations, it is usually used preparatively, in particular for the isolation of compounds from complex biological samples. Hostettmann et al. [36] summarized the possibilities and applicability of RPC techniques and, in tabular form, gave an excellent compilation of different naturally occurring substance classes and the chromatographic conditions used, for example type of adsorbent, layer thickness, sample size, and mobile phase composition. Rodrigo et al. [37] recently reported the advantages of preparative RPC in the separation of a benzothiazinone from other compounds. It was found that although HPTLC could not be used to mimic RPC development, direct transfer of the mobile phase from HPTLC to RPC gave good results with low solvent usage and less time consumption in the separation of a mixture of organic sulphur derivatives.

Another promising innovation in rotation planar separation is the use of a rotation planar column, not only for RPC [8], but also for rotation planar extraction [38]. For both separation techniques the same planar column must be used. Because of the geometric design of the planar column, the amount of stationary phase or mass of the solid-phase to be extracted is constant over the cross-section. This

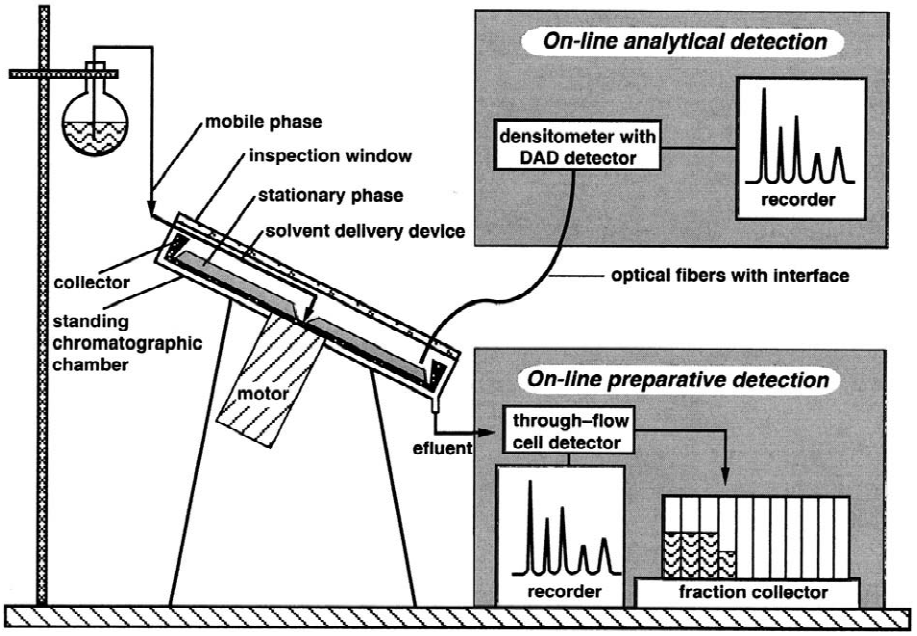


Fig. 6. Flow scheme for on-line analytical and preparative RPC separations.

special geometric design can be described by the function [8,38]:

$$h = \frac{K}{a + br + cr^2} \quad (1)$$

where $h$ is the actual height of the planar column at radius $r$, $r$ is the radius of the planar column, and $a$, $b$, $c$, and $K$ are constants.

Although column RPC seems to be a circular development mode, due to the fact that the volume of the stationary phase is constant along the radius, it is, in effect, a linear development mode, because the mobile-phase velocity will be linear over the entire separation distance. Since a planar column is a closed system, any commercially available fine-particle-size (3–5 μm) stationary phase (silica, RP-18, RP-8, amino, cyano) can be used with or without binder, which improves the separation significantly. The selected stationary phase must be carefully and continuously dry-filled into the centre of the planar column at a rotation speed of 2000 rev./min. Because of the high centrifugal force the stationary phase will be compressed. Sample is subsequently applied with a syringe to an equilibrated or non-equilibrated column, at a rotation speed lower than that used to fill the planar column. An impressive example has recently shown the application of an analytical high-performance liquid chromatographic mobile phase as the mobile phase for equilibrated preparative column RPC separation of a 350-mg flavanolignane-containing extract of *Silybum marianum* as is reproduced in Fig. 7 [8].

If finely powdered solid material to be extracted is placed in the planar column instead of the stationary phase, rotation planar extraction can be performed using a linear extraction solvent flow, accelerated by centrifugal force. In the course of rotation planar

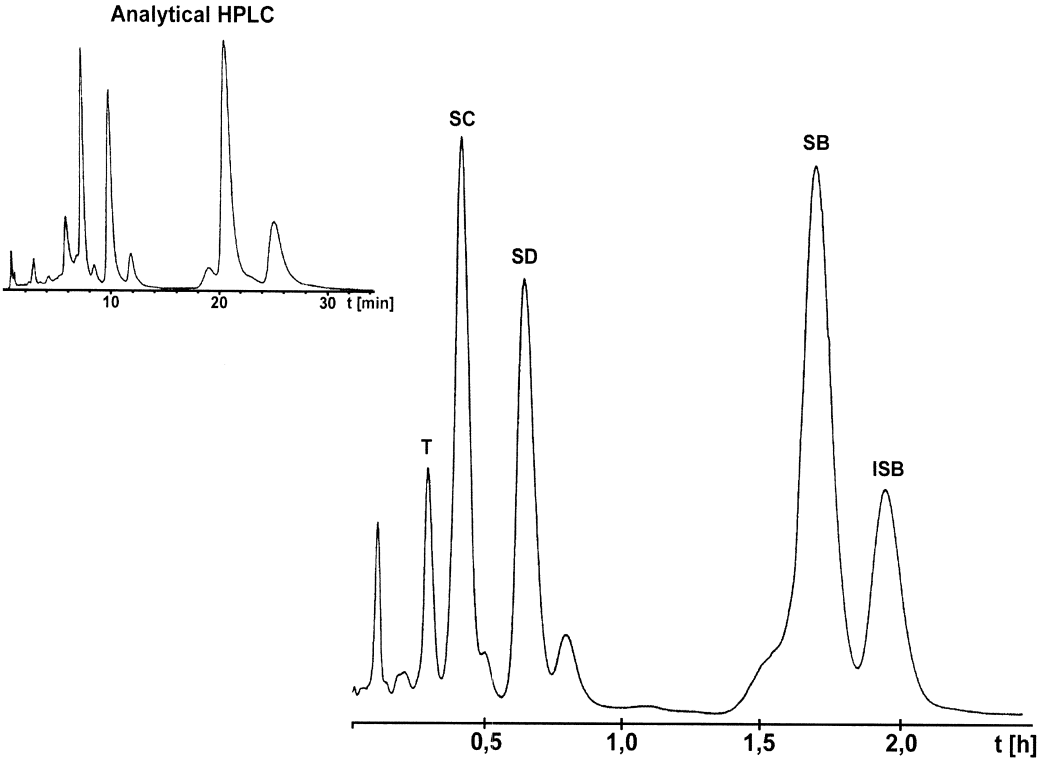


Fig. 7. Column RPC separation of flavanolignans from purified *Silybum marianum* extract by use of the mobile phase for analytical high-performance liquid chromatography. Conditions: stationary phase, Sepralyte $C_{18}$ (15 μm); mobile phase, water–tetrahydrofuran–methanol–acetonitrile, 75.24 + 17.78 + 3.85 + 3.13; flow-rate, 3 ml $min^{-1}$; rotation speed, 1200 rev./min; temperature, 23.1 °C.

extraction, exhaustive, discontinuously operated extraction is realized, working on the principle of the relative counter-current mechanism. The process is based on the observation that repetition of the dissolution and diffusion parts of extraction by use of an appropriate programme can have a beneficial effect on the extraction process [38]. Vovk et al. [39] recently used exhaustive rotation planar extraction for solid–liquid extraction of the constituents of *Allium cepa* L. Using the ExtraChrom® instrument it was possible to extract the main components, oligofructans, with a degree of polymerization up to 12. Experiments showed that the operation was simple, filling of the planar column was fast, extraction times were short, and solvent consumption was low. Vovk et al. [40] also reported the combined use of rotation planar extraction and different types of RPC for investigation of the constituents of *Quercus robur* L.

## 4. Progress in overpressured-layer chromatography (OPLC)

In recent years progress has been made in OPLC in the connection of chromatographic plates, either in parallel for the analysis of several samples (multilayer OPLC) [41,42] or serially for the analysis of complex samples (long-distance OPLC) [43,44].

Working with fully off-line OPLC—similarly to RPC—on a single chromatographic plate, 72 samples can be separated by circular development. OPLC in parallel connection mode (multilayer OPLC) is also suitable for the development of several chromatographic plates simultaneously if the plates are specially prepared [41]. All the chromatographic plates—except for that at the bottom—are furnished with a small hole, and mobile phase is delivered to all plates simultaneously, through a channel formed by drilling, to this small hole, by pressing the plates together. Fig. 8a shows a circular multilayer OPLC on three chromatographic plates for the separation of several samples. The aluminium backing of the plates is sufficiently flexible to conform to the surface of the chromatographic plates under conditions of OPLC. Needless to say, linear or bidirectional linear OPLC can also be applied in multilayer operating mode. The efficiency of multilayer OPLC separation of complex samples can be increased by use of different types of stationary phase for the development of several chromatographic plates.

Szücs et al. [42] recently demonstrated the use of multilayer OPLC for a high-throughput analytical strategy, with combined planar and column liquid chromatography, for the improvement of poppy (*Papaver somniferum* L.) to produce a variety with high alkaloid content. They reported the use of multilayer OPLC to screen the morphine content of 216 samples in a single run. The separation took 3 min, so the separation time per sample was less than 1 s. The method is used to screen 10 000 samples/year/genotype.

Another trend in OPLC is the serial connection of chromatographic plates (long-distance OPLC) for the analysis of complex samples [43,44]. This idea is based on the theory that if the bandwidth of the deposited spot is very narrow in OPLC, HETP is almost constant along the plate [46]. This means that the theoretical plate number increases linearly with

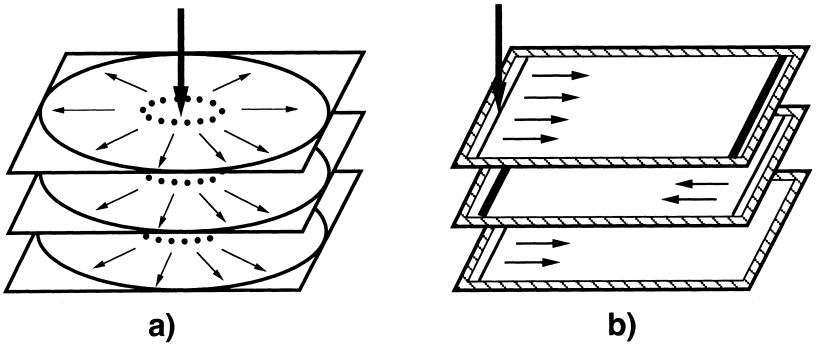


Fig. 8. Multilayer OPLC separation possibilities. (a) Principle of circular multilayer OPLC. (b) Principle of long-distance OPLC.

the development distance, in contrast to capillary-controlled TLC/HPTLC. On this basis Botz et al. [43,44] introduced the long-distance OPLC technique to increase the theoretical plate number and the spot and/or peak capacity. Long-distance OPLC is a multi-layer development technique with specially prepared flexible backing plates. Similarly to the preparation of layers for linear OPLC all four edges of the chromatographic plates must be impregnated with polymer suspension. Movement of the mobile phase with a linear front can be ensured by placing a narrow plastic sheet on the layer or by scraping a narrow channel in the adsorbent for the solvent inlet. Several plates are placed on top of each other to ensure a long development distance. A narrow slit (width ~0.1 mm) is cut at the end of the topmost chromatographic plate to enable the mobile phase to travel to a second layer where migration continues to the opposite end of the plate; there solvent flow can continue on the next subjacent (bottom) chromatographic plate or, if migration is complete, the mobile phase is led away. On this basis a 72-cm separation distance can be achieved by connecting four plates together [43]. In the arrangement illustrated in Fig. 8b, the upper plate has a mobile-phase inlet channel on one side and a slit on the other side for conducting the mobile phase to the next plate. The slit enables ready passage of the mobile phase and individual samples without mixing [44]. The pressure of the OPLC instrument is applied to the uppermost layer only, and each plate presses onto the layer below.

Fully off-line separation is complete when the mobile phase reaches the end of the lowest plate. The eluate can, furthermore, be led from the lower plate in a manner similar to that in which it was led in. In the latter case (fully on-line operating mode) all plates placed between the highest and lowest layers must have 1 cm cut from the length of the plate, to leave space for the mobile phase outlet. Two long-distance OPLC separations on different separation distances are depicted in Fig. 9. Using the idea of long-distance OPLC, Mincsovics et al. [45] introduced a special double-layer cassette system for OPLC separation over a 36-cm separation distance, to increase spot capacity and resolution for the separation of different, complex, biological samples.

A third trend in OPLC is the prediction of a variety of pressurized preparative column liquid chromatographic separations by use of results from nonequilibrated (off-line) or equilibrated (on-line) analytical OPLC separations [6,47]. By use of silica (TLC quality with average particle size of 15 μm) as stationary phase, the method usually used is to

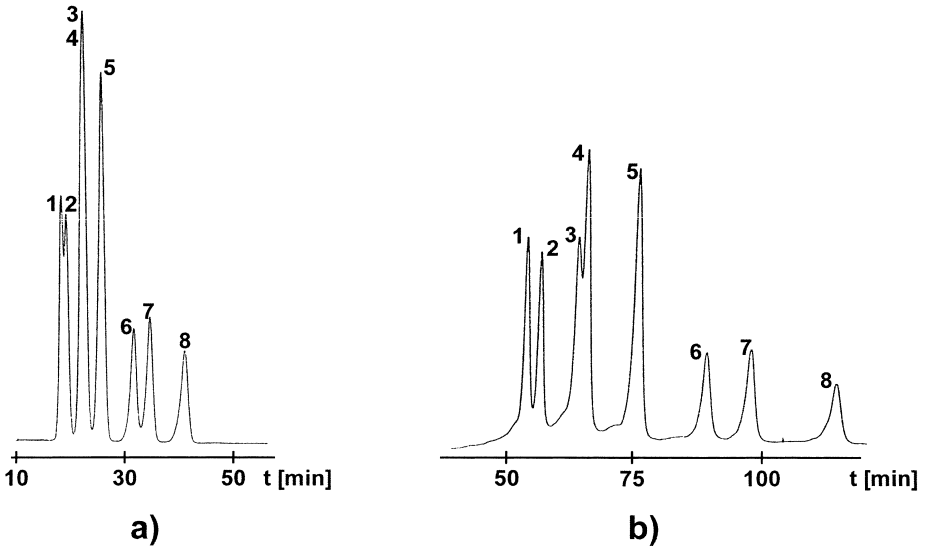


Fig. 9. Long-distance OPLC separation of furocoumarin isomers. (a) Separation over 18-cm separation distance. (b) Separation over 54-cm separation distance.

equilibrate the dry-filled pressurized column liquid chromatographic column with solvent in which the substances to be separated do not migrate and which was used for the prerun in analytical OPLC. Medium pressure liquid chromatography separation is then started with a nonsaturated chromatographic chamber and an optimized TLC mobile phase in which the substances are distributed over the whole $R_F$ range. Correlation of retention data from fully off-line OPLC with those from fully on-line OPLC and HPLC are given elsewhere [6]. Because of these linear relationships, in fully on-line OPLC or HPLC the separation times can be predicted for all compounds (after elution of the first three peaks) from the off-line OPLC $R_F$ values. Prediction of preparative medium pressure liquid chromatography is always possible if retention data from analytical fully on-line OPLC are known [18]. Analytical retention data of the first two peaks and the zero point enable calculation of the retention times and resolution of compounds eluted later in the preparative procedure. Transfer of the optimized TLC mobile phase via OPLC to medium pressure liquid chromatography was demonstrated by the separation of furocoumarin isomers from the roots of *Heracleum sphondylium*, ginsenosides from *Panax ginseng* C.A. Meyer, and anthraquinone aglycones from *Rhamnus frangula* [47].

## 5. Progress in electro-planar chromatography (EPC)

In EPC, in addition to capillary action, the force driving solvent migration is an electric field with a voltage gradient bigger than 1 kV $cm^{-1}$ [9]. During EPC the components of the sample are separated simultaneously by two processes, electrophoresis and adsorption. The use of an electric field results in the reduction of the analysis time and in a higher theoretical plate number; better separation can therefore be achieved [48]. TLC with electroosmotic flow was first described by Pretorius et al. in 1974 [49]. These authors used a pre-wetted chromatographic plate supported in a vertical chamber, and reported that separation by EPC was 15 times faster than the equivalent separation by conventional TLC. After an interval of nearly 25 years several papers have described the FFPC method in which the mobile phase is driven by electroosmotic flow. EPC can be performed on pre-wetted [49–52] or unwetted stationary phases [53–57].

By comparing results obtained by EPC on wetted layers and by conventional TLC, Nurok and coworkers [48,51] proved that analysis time was reduced by use of EPC, a better separation of the components of the sample was obtained, and the number of theoretical plates was increased. One result of application of an electric field is that Joule heat is evolved. In EPC the Joule heat generated results in increased evaporation of solvent/solvents from the surface of the chromatographic plate, which induced additional flow of the mobile phase on the plate. This effect was described for the first time by Kowalczyk et al. [58]. However, surprisingly, Shaflik et al. [59] stated that such flow had no effect on spot shape. When the evaporation effect occurs in conventional (vertical) chromatographic chambers, the evaporative flow can exceed the electroosmotic flow. The effect is not this dramatic when horizontal chromatographic chambers are used. Nurok et al. [52] found that a migration distance of 7 cm resulted in efficiencies up to ~5500 theoretical plates for compounds of high $R_F$. Although there is no theoretical limit to the maximum TLC plate length that can be used for EPC, obtaining reproducible separations is difficult if separation distances exceed 4 cm. This result depends on the amount of power that can be applied to the chromatographic plate—excessive mobile phase evaporation and even drying of the chromatographic plate must be avoided [52]. Nurok et al. recently discussed the reproducibility and quality of EPC separations obtained on 1 cm×5 cm, 2 cm×5 cm, and 4 cm×10 cm layers [55]. They found that separation quality and reproducibility were slightly lower for 1-cm and 4-cm plates. The effect of changing the electrode polarity when separating a four-component mixture is depicted in Fig. 10. This result suggests that electrode polarity is an additional parameter that has to be considered during an electro-planar chromatographic optimization process.

EPC can be also performed on dry layers, e.g. layers not wetted with the mobile phase. In horizontal chambers the samples can be developed from two—anode and cathode—sides simultaneously.

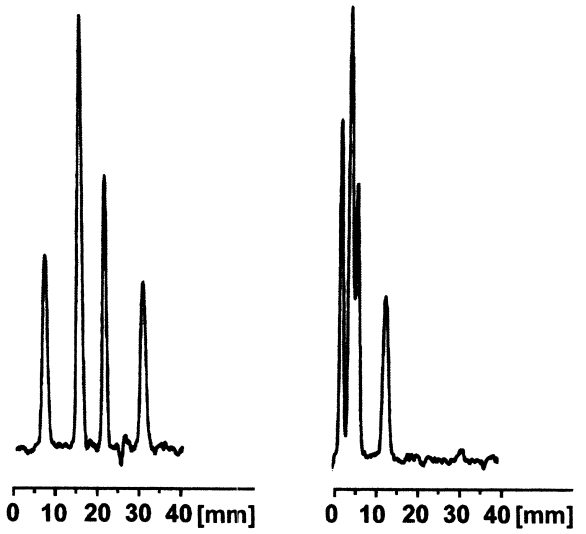


Fig. 10. Effect of changing electrode polarity when separating a four-component mixture on 2 cm×10 cm RP18 TLC chromatographic plates.

Pukl et al. [53] were the first to describe the application of EPC on unwetted layers. The use of this technique has the advantage that on unwetted layers there is no electric current, so the Joule heating effect is reduced. Malinowska reported [54–57] the use of EPC on unwetted silica gel and aluminium oxide. According to their results the electric field influences the migration velocity of the mobile phase, the migration of the chromatographed compounds and the shape of the chromatographic peaks. These effects were shown for a nonpolar binary mobile phase and nonpolar compounds, for example polycyclic aromatic hydrocarbons, and for a polar mobile phase and polar compounds (phenol and quinoline derivatives) [57].

Kreibik and co-workers [60–62] reported the enhancement of mobile-phase velocity in TLC by means of an external alternating electric field. They called this displacement effect on liquids, dielectroosmotic flow because of its similarity with electrokinetic phenomena. When electroosmotic flow and dielectroosmotic flow are compared, several differences are apparent.

- For electroosmotic flow it is necessary to connect two electrodes of opposite polarities to the ends of the tubes of the porous medium. In dielectroosmotic flow a continuous electric field cannot be used, an electric field is applied from outside.
- The solvents used in electroosmotic flow are conductive, those used in dielectro-osmotic flow are dielectric.
- Electroosmotic flow is the movement of liquid observed when an electric potential is applied to a conducting fluid trapped in a porous polar medium. When an electric field is applied outside a tube filled with liquid (in dielectroosmotic flow), Maxwell–Wagner polarization will appear between the tube material and the liquid, which affects the whole process—creation of an electric field in the layers and appearance of electric force at the meniscus. This polarization does not appear in electroosmotic flow [56].
- In electroosmotic flow a large amount of heat is generated when electric current is applied. This phenomenon is absent in dielectroosmotic flow.
- Electroosmotic flow cannot employ the audio-frequency range, dielectroosmotic flow can.

The preliminary results obtained by Kreibik et al. [60–62] suggest that application of an alternating electric field to porous media offers the possibility of using the dielectroosmotic flow effect to enhance the separating power of a TLC separation.

Most recent papers in the field of EPC describe the electrochromatographic process, yet only a few research groups have performed experiments and published results on the technique. Although routine methods for EPC analysis have not yet been developed, modern requirements already performed suggest that the time has come to develop this technique and to use it widely for the analysis of complex mixtures.

## 6. Progress in detection methods

Detection of separated compounds in FFPC can be performed by physical, chemical, and biological methods. Physical detection methods are based on substance-specific properties and are generally non-destructive; spectroscopy and radiochemistry can both be used. PC with UV–visible densitometry has recently been summarized by Dammertz and Reich [63]. Diode-array scanners [64,65] and image analysis [66–69] are also used for the evaluation of planar

chromatograms. Achievements in the coupling of TLC with Fourier-transform infrared and mass spectrometry have been outlined by Rager and Kovar [70] and by Busch [71], respectively. Wilson et al. reported the use of solid-state NMR spectroscopy (high resolution magic-angle spinning) for compound identification [72] and Klebovich has reviewed the applications of digital autoradiography in PC [73]. A short review of this method of detection is available elsewhere [74], and pre- and post-chromatographic derivatization have been summarized by Cimpan [75]. It can generally be stated that all detection methods used in PC can also be applied in FFPC.

Significant progress has been achieved as a result of a combination of OPLC and on-line radioactivity detection [76]. Sample preparation for purification of urinary metabolites of a $^{14}$C-labelled drug candidate was performed on octadecyl-modified silica solid-phase extraction columns and OPLC was conducted on silica gel layers with butanol–acetic acid–water as mobile phase. Eluate radioactivity was detected by use of a flow-cell radioactivity detector equipped with a solid scintillator; eluate fractions containing radioactivity were collected. Strongly retained radioactive compounds were detected off-line, after development, by use of digital autoradiography. Detection limits are slightly higher for radioactivity detection equipped with a flow cell than for off-line radioactivity detection methods [76]. On-line OPLC—with radioactivity detection combined with OPLC and digital autoradiography—is an effective tool which can be applied with advantage to metabolite research [77].

Chai et al. [78] recently reported the first on-line OPLC–ESI-MS separation and detection of glycolipids by direct linking of an OPLC 50 instrument and a Q-TOF mass spectrometer. Because precleaning is a prerequisite for achieving high-sensitivity detection in TLC–ESI-MS, a solvent wash of at least 100 bed volumes was necessary to reduce the background substantially. They found that the sequential use of methanol and a mixture of chloroform, methanol, and water might be a better means of reducing background, because different solvents were found to remove different contaminants [72]. Under optimized conditions, sensitivity of 5 pmol glycosphingolipid was readily demonstrated for TLC–ESI-MS, and 20 pmol for TLC–ESI-MS–MS product-ion scanning to derive the saccharide sequence and long-chain base/fatty acid composition of the ceramide. It was observed that chromatographic plates could be used repeatedly (at least 10 times) without adverse effects. On-line OPLC–ESI-MS might hold considerable promise for the analysis of different types of substance for which molecular mass and structural information are required [78].

FFPC is well suited to the demands of different screening methods, for example screening for a particular substance group, e.g. flavonoids, anthraquinones, alkaloids. The coupling of planar chromatography with in-situ bioassay for activity testing and for efficient biological activity-guided isolation of natural products is a significant trend [79]. OPLC and RPC are always suitable for in-situ determination of biological activity; different types of plate can be used for determination of the activity of isolated pure compounds, the activity of isolated fractions, and the activity of total extracts. The ChromaJet DS 20 automatic spray apparatus was recently developed on the basis of the idea of a ‘‘Compuspray’’ system [80]. This instrument enables computer-controlled post-chromatographic chemical derivatization, with documentation, in conformity with good laboratory practice and spraying of different microorganisms [81] onto chromatographic plates either in the direction of, or perpendicular to, mobile phase migration. The equipment sprays the tracks accurately, with high precision and reproducibility and minimal reagent consumption, and is environmentally sound and safe—no aerosols are produced in the working environment.

By use of FFPC methods, separating power without loss of resolution can be ensured over long separation distances. Shorter separation times, high resolution, high sample throughput, and reduced spot or band broadening are specific advantages of FFPC techniques which help to ensure optimum conditions for physical, chemical [75], and microbiological detection [81].

## 7. Comparison of the methods and conclusions

The most important characteristics of fully off-line and fully on-line FFPC are compared in Table 1.

Table 1
Comparison of fully off-line and fully on-line FFPC methods

| | Fully off-line RPC | Fully on-line RPC | Fully off-line OPLC | Fully on-line OPLC | EPC (fully off-line) |
|---|---|---|---|---|---|
| Type of forced-flow | Centrifugal force | Centrifugal force | Pressure using pump[a] | Pressure using pump[a] | Electric field |
| Separation | Development | Elution | Development | Elution | Development |
| Stationary phase | All commercially available | All commercially available | All commercially available | All commercially available | All commercially available |
| | Dry | Equilibrated | Dry or wetted | Equilibrated | Wetted or non-wetted |
| | Used once | Used several times | Used once | Used several times | Used once |
| for anal. separations | Precoated HPTLC | Precoated HPTLC | Precoated HPTLC | Precoated HPTLC | Precoated HPTLC |
| for prep. separations | Self-prepared | Self-prepared | Precoated | Precoated | Presently not possible |
| | Increasing volume | Increasing volume | Constant volume | Constant volume | |
| | $x = 15$ μm | $x = 15$ μm | 5 μm$< x <$40 μm | 5 μm$< x <$40 μm | |
| | – | Filled (C-RPC) | – | – | – |
| | | Constant volume | | | |
| | | 3 μm$< x <$15 μm | | | |
| Mobile phase | Cut-off value not important | Cut-off value important in UV detection | Cut-off value not important | Cut-off value important in UV detection | Cut-off value not important |
| Vapour phase | Variable | Variable | No, after prerun | No | Saturated or non-saturated |
| Sample application | Static | Dynamic | Static | Dynamic | Static |
| | Prepurification not important | Prepurification necessary | Prepurification not important | Prepurification necessary | Prepurification not important |
| | Solvent less important | Solvent important | Solvent less important | Solvent important | Solvent less important |
| Number of samples | Several samples (1–72) | Only one sample | Several samples (1–216) | Only one sample | Only one sample[b] |
| Development mode | Circular | Circular | Linear | Linear | Linear[c] |
| | Linear[d] | | Bidirectional linear | | |
| | Anticircular[d] | | Circular | | |
| | Multiple | | Anticircular | | |
| | | | Two-dimensional | | |
| | | | Multiple | | |
| Separation distance | 10 cm | 10 cm | ≤54 cm | 18 cm | 5 cm[e] |
| | theoretically unlimited[f] | | | | |
| Detection | Static | Dynamic | Static | Dynamic | Static |
| | Derivatization simple | Derivatization complicated | Derivatization simple | Derivatization complicated | No experiences |
| | UV–Vis (raw spectra) | UV–Vis (fine spectra) | UV–Vis (raw spectra) | UV–Vis (fine spectra) | UV–Vis (raw spectra) |
| | Fluorescence | Fluorescence | Fluorescence | Fluorescence | |
| | – | NMR | – | NMR | |
| | FTIR, in situ | – | FTIR, in situ | – | |
| | MS, in situ | MS | MS, in situ | MS | |
| Evaluation | Densitogram | Chromatogram | Densitogram | Chromatogram | Densitogram |
| | Repeatable | Unrepeatable | Repeatable | Unrepeatable | Repeatable |
| Analytical separation | Only for screening purposes | Acceptable method | Excellent for many samples | Comparable with HPLC | In progress |
| Isolation | Good, rapid method | Excellent, rapid method | Acceptable method | Acceptable method | At the moment not possible |
| amount of sample | 50–400 mg | 50–500 mg | 50–200 mg | 50–300 mg | |
| number of compounds | 2–7 | 2–10 | 2–5 | 2–7 | |

[a] Use of hydrostatic pressure is also possible.
[b] No papers exist about the separation of more than one sample.
[c] No papers exist about anything other than linear development.
[d] With special preparation of the plate for analytical separations.
[e] No papers exist about the separation of more than 5 cm separation distance.
[f] In the case of sequence technique.

In FFPC separations the plate height is independent of the mobile phase migration distance. A minimum value of ~20–25 μm is achieved for HPTLC chromatographic plates if the mobile-phase velocity range is 1.8–3.0 cm $min^{-1}$. With FFPC a zone capacity of 30–40 can be achieved; this is 2–3 times higher than for capillary-flow-controlled TLC.

This overview of the current state of the different

forced-flow planar chromatographic methods covers a special range of analytical and preparative methods. Its analytical characteristics lie between those of conventional off-line TLC and/or high-performance TLC and modern, on-line high-performance liquid chromatography, while its preparative features lie between those of classical preparative layer chromatography and modern on-line medium-pressure liquid chromatography. It can be stated that recent progress in FFPC, especially the flexibility to select the operating and development mode, the extent of saturation of the vapour phase (in RPC), and parallel or serial connection of plates (in OPLC) covers a special range of modern instrumental separations.

The different FFPC techniques do not compete with any other analytical or preparative liquid chromatographic method. Instead, the different approaches are complementary and together enable successful and rapid separation. In my opinion OPLC has, and probably will always have a role in the qualitative and quantitative analysis of pharmaceuticals and foods, and in toxicological and environmental analyses, whereas the different RPC methods afford excellent possibilities for the isolation of naturally occurring compounds or synthetic products.

Preliminary results obtained from different types of EPC as well as the achievements in capillary electrochromatography suggest that EPC has the potential to become a useful forced-flow separation technique. Further progress in the future could be the combination of RPC and/or OPLC with electric fields.

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