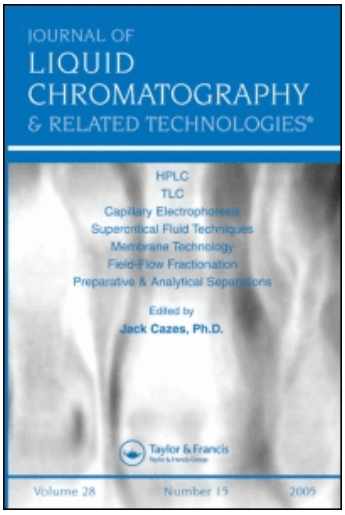

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STRATEGY OF PREPARATIVE SEPARATION OF ORGANIC COMPOUNDS BY THIN-LAYER CHROMATOGRAPHIC METHODS

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ABSTRACT

The preparative layer chromatography discussed in this review has been presented to facilitate the choice of the most effective, selective, cheap, and simplest method for separation and isolation of small quantities of some mixture components. Therefore, apart from very effective forced-flow development methods, the possibilities and applications of very simple sandwich chambers ES and DS are discussed. Both forced-flow and continuous planar chromatography development may be considered as quasi column preparative chromatography, carried out on smaller scale. Numerous application examples are also cited in this paper.

Key Words: Preparative layer chromatography; Basic planar techniques; Modes of development; Modes of sampling; Solvent characteristics; Adsorbent characteristics

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INTRODUCTION

The theoretical base is similar in principles both for thin-layer chromatography (TLC) for analytical purpose and preparative layer chromatography (PLC).

Generally, thin layer chromatography, which deals with samples above 1 mg on 1 g of adsorbent is called preparative, semipreparative, or micropreparative, depending on loading mode and layer thickness.

Preparative layer chromatography has already been described by Nyiredy in his chapters in "Handbook of Thin-Layer Chromatography",[1] in "Planar Chromatography",[2] and in numerous publications with co-workers which will be cited further in this paper. Numerous books[3–5] and review articles[6,7] also contain chapters regarding principles and applications of preparative layer chromatography.

The primary goal of this paper is to propose an author's viewpoint on strategy of optimisation of preparative layer chromatography separation using simple methods (used in our laboratory) and available in every laboratory.

The classic TLC was, among others, applied as a pilot method to optimisation of chromatographic systems for column chromatography.[8] According to the preparative layer chromatography, analytical TLC, as a cheap and simple technique, is indispensable for determination of mutual relation: substance–adsorbent–solvent, separability, scaling-up procedure and, thus, for anticipation of loadability and yield of separation. First, all essential differences between TLC and PLC should be taken into account (see Table 1).

GENERAL CONSIDERATIONS

Goals

Preparative layer chromatography is applied to: (a) isolation of one or a few compounds for further identification by physico-chemical methods; (b) obtaining some chromatographic standards; (c) investigations of biological activity.

The main purpose of PLC is isolation of pure components from a mixture with maximal yield. In separation of a few compounds with similar retentions (i.e., closely related compounds), the purity of the product is a compromise to loadability and process yield. In case of touching bands, components are isolated only from central parts of zones. The components of upper and lower edges of bands are often rechromatographed in a system of different selectivity.[9,10]

Table 1. Comparison of Main Differences Between Analytical Thin-Layer Chromatography (TLC) and Preparative Layer Chromatography (PLC)

TLC	PLC
A. Goals	
1. Quantitative determination and/or identification of all sample components	1. Isolation of one or a few sample components
B. Methods and modes of mobile phase flow	
2. Either straight-phase or reversed phase chromatography is applied	2. Straight-phase method of chromatography is prefered.
3. The mobile phase migrates by capillary action as well as by pressure or centrifugal force	3. As in TLC
C. Sampling	
4. Samples are introduced in simple way, using capillary or automated sampler. Sample volume about 2 μL	4. More complicated sampling of large volumes of solution
D. Adsorbents	
5. All known adsorbents and precoated plates for TLC	5. As in TLC. Possible is using more coarse adsorbents and special adsorbents for PLC
E. Sample solubility and solvents	
6. Solubility of sample is not important	6. Sample solubility is very important
7. Volatility and viscosity of mobile phase are not important	7. Volatility and viscosity of mobile phase are very important
F. Detection	
8. Directly on plate in day-light or UV-light or using all[a]	8. Possible off-line and on-line detection, but not all[b]

[a]Methods: biological, radiometric, and chemical derivatisation.
[b]Detection methods can be used.

Methods and Modes of Mobile Phase Flow

For preparative separations, the method of normal-phase chromatography is preferable because of its wide range of differentiated selectivity of various solvents and of greater velocity of mobile phase than on nonpolar adsorbents. On the other hand, reversed phase chromatography gives the possibility to separate homologous compounds, whereas polar adsorbents prove greater ability to group selectivity, which permits the separation of positional isomers.

Such differentiated behaviour of adsorbents is exploited for re-chromatography of touching or overlapping bands.[9]

In conventional planar or ascending methods of PLC, the mobile phase migrates by capillary action which leads to a longer time of development (i.e., time development of a plate to a distance 10–20 cm may take 20–60 min, respectively). However, longer time development produces band diffusion, although it enhances the mutual displacement effect. Moreover, in conventional methods, avoiding the contact of polar adsorbent with the surrounding atmosphere is impossible.

The attainment of an equilibrium state in triphasic systems (solid polar phase—mobile phase—gas phase) is very important in order to avoid additional undesirable effects, especially those of thick layer: solvent demixing, evaporation of the mobile phase, and preadsorption of the vapours from the chamber atmosphere. Separated substances migrate in the layer with different velocities, depending upon evaporation velocity of solvent: quickly on the surface of a layer; with a delay in locations adjacent to the plate. Such effects may be minimized, or avoided completely, by using saturated chambers or sandwich tanks equilibrated by prewetting of adsorbent layer with eluent on a calculated distance.[11] For preparative chromatography, all types of chambers for TLC described in Chapt. 4 of Ref. (2) can be used. A flat tank constructed by Soczewinski (see Fig. 1)[12,13] is very useful for preparative separation. The design of the chamber enables spotting the sample behind the solvent front (equilibrium conditions), bandwise sample application, dosage of large volume from the edge of the layer (frontal + elution chromatography),[14] bidimensional development of larger samples,[15] stepwise gradient elution,[16,17] simultaneous development of standards, and bandwise applied sample.[14] Sandwich chambers are especially convenient for the separation and on-line purification of crude plant extracts contaminated with lipids.[18] Sandwich chambers may be adapted for self-prepared plates, or for commercially precoated layers on glass or plastic (aluminium) foils.

The chamber constructed by Dzido[19] (see Fig. 2) is a modified version of both sandwich (ES) chamber and Brenner–Niederwieser chamber.[20] It has been modified[21,22] and adapted to different variants of development techniques, e.g. stepwise gradient elution,[24,25] for simultaneous development of six parallel chromatograms and micropreparative purposes.[22,23]

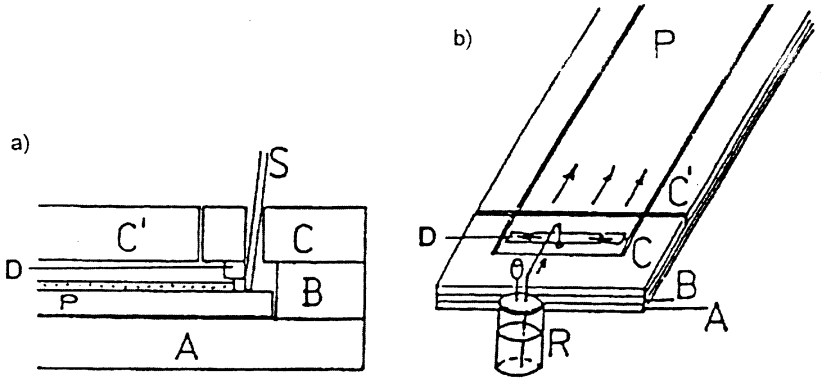

Figure 1. Horizontal glass chamber (ES); (a) cross-section of chamber fragment with the eluent distributor, (b) view of chamber; A—base, B—distance frame, C,C′—two cover plates, P—chromatographic plate with the adsorbent layer, D—distributor of eluent, S—end of the capillary siphon.

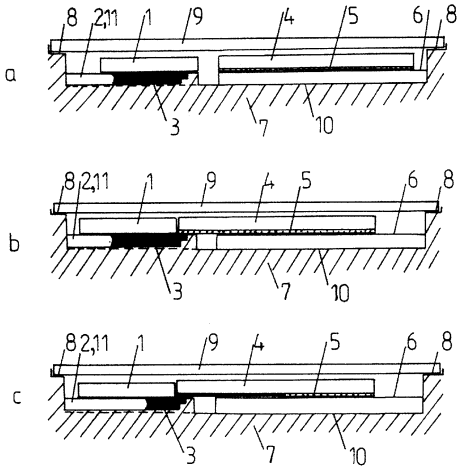

Figure 2. Principles of action of DS horizontal chamber with a horizontal bottom of eluent reservoire; 1—glass cover plate, 2—eluent reservoire, 3—solvent, 4—chromatographic plate, 5—thin layer of adsorbent, 6—ledge supporting the TLC plate, 7—body of the chamber, 8—rectangular depression, 9—glass cover-plate of the chamber, 10—bottom, 11—edges of the eluent reservoire. (a) original position, (b) beginning of development, (c) advanced development.[20]

The optimal distance for PLC should be no longer than 20 cm because of a decrease flow rate with increase of development distance. Simultaneously, diffusion of zones and losses of resolution increase. This disadvantageous phenomenon, as well as a demixing effect, are eliminated by modern forced-flow techniques, either for analytical planar chromatography or for preparative separations.

In the review paper of Tyihak, et.al.[26] three forced-flow planar liquid chromatographic methods were described with schematic drawings of instruments for rotational planar chromatography (RPC), high-speed TLC (HSTLC), overpressured layer chromatography (OPLC), and some theoretical considerations. More recently, Tyihak, et al., characterized the off-line OPLC method.[27] Forced-flow techniques relative to preparative applications were elaborated in a review publication by Nyiredy, et al.[28] Using medium pressure liquid chromatography (MPLC) and the Chrompress 25 apparatus, Zogg et al. carried out preparative and semipreparative separations of Furanocomarin isomers.[29] The same authors investigated the influence of operating parameters on separations in preparative OPLC. All the separations of *Heracleum sphondylium* extracts were performed with a Chrompress 10 apparatus. Using analytical TLC, the authors optimized the mobile phase with the "Prisma" model for following OPLC separation. They also determined the loading limit by a scale-up separation process from analytical to preparative OPLC.[30]

Considerable improvement of resolution was achieved using a new preparative circular layer chromatographic method.[31]

Several, very interesting, publications on a novel version of forced-flow planar chromatography should be mentioned. It is a long-distance overpressured layer chromatography. Such a modification was first elaborated by Tyihak, et al.[32] for simultaneous development of several plates. The principles of a new multilayer technique and its application were described by Botz, et al.[33,34] The authors emphasised that, owing to a significant increase of spot capacity, long distance OPLC may be applied to separation of complex natural mixtures and also adapted for preparative separations.

Sampling

The necessity for introducing a large sample onto a layer is a great problem, especially with reference to:

(a) which type of overloading mode (volume or concentration) is more advantageous;
(b) sampling mode.

For optimization of systems for preparative chromatography, determination of maximal volume and concentration of sample (sample amount) is of essential importance for the separation to achieve touching band separation. The sample amount depends on components' resolution which can be determined by analytical TLC data. It should be above 1.5. But, on the other hand, anticipation of possible resolution on the basis of the Kirkland equation:[35]

$$R_s = \underset{\text{selectivity}}{\frac{1}{4}(\alpha - 1)} \underset{\text{capacity}}{\left(\frac{k}{1+k}\right)} \underset{\text{efficiency}}{N^{1/2}} \tag{1}$$

in overloaded systems becomes impossible. Introduction of large samples influences all three parameters: selectivity, capacity and efficiency. The increase of solute weight above 1 mg/g of adsorbent results in a drastic decrease of k and α parameters and an increase of plate height (decreases efficiency). The overloading can be also defined as a sample amount where the k parameter decreases 10% in relation to analytical scale.

There are two ways in which a chromatographic system may be overloaded. First, when the injected sample volume is so large that the eluted band (or concentrating profile) is significantly broadened (volume overloading) or when the concentration of an injected solute is so large that the eluted bands (or concentration profiles) become asymmetric (sharp front and elongated tail) according to analytical scale sampling (mass overloading) (see Fig. 3).

It is evident that band broadening may lead to overlapping of bands. Concentration overloading is more advantageous if sample solubility allows. It should be taken into account that introduction of concentrated solutes may be disadvantageous, owing to crystalization of substances into the adsorbent pores. As the result of this, strong local overloading and disturbance of desorption–adsorption processes (substance is gradually dissolved) is possible. In the case of the necessity of large volume introduction (e.g., biologically active mixtures which, by their nature, are diluted and thermolabile) investigators have to use a multiple sampling. The design of a sandwich (ES) chamber enables preconcentration of sample directly on the layer by the evaporation of sample solvent beyond the narrow cover plate.[13] The use of plates with concentration zones is advantageous in such a case.

The second factor influencing separability is the manner of delivery of larger sample volumes. In a conventional method, the samples are applied by syringe or pipette, using multiple spotting or streaking by a special mechanical device (e.g., programmed applicators) which causes a complex starting band (compare Figs. 4 a,b). During the elution step, the starting band components are rearranged to a sequence according to their R_F values. This problem may be solved by introduction of sample from the edge of the chromatographically

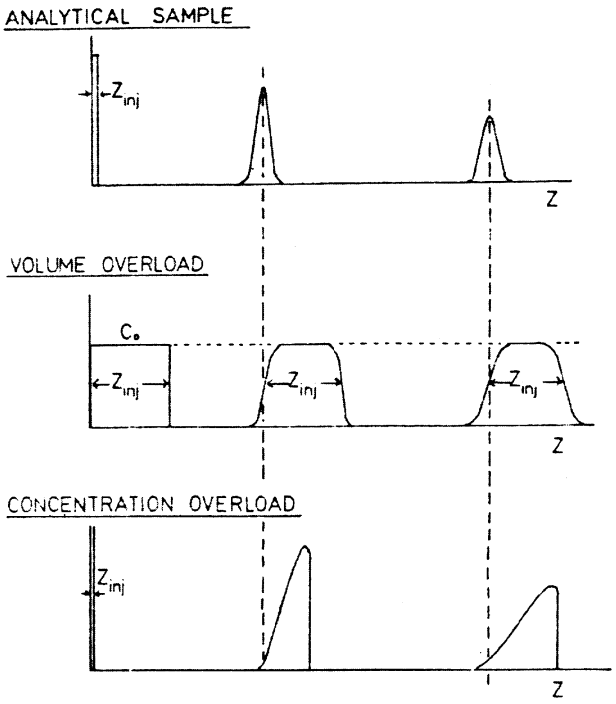

Figure 3. Development of peak profiles during migration along a column for analytical overloaded samples (with permission).[36]

equilibrated bed (behind the solvent front). Flat chambers for continuous development, proposed and elaborated by Soczewinski et al.[12,14,37,38] permit zonal sample application from the edge of the layer. The sample components of the starting band are already partly separated (the frontal chromatography stage—compare Fig. 4c).

During development (elution chromatography stage), components may be fully separated. The larger sample volumes can be introduced gradually from a syringe or from a container equipped with a capillary siphon. A glass (quartz) cover plate enables the observation and recording of zone movement. Thus, a dynamic picture of band migration and determination of loading limit[14] can be performed (see Fig. 5).

The DS chamber also enables zonal application. In an OPLC system, the sample is injected into the continuous stream of eluent across the plate. Similar on-line sample introduction is used in centrifugal RPC.[2] Botz et al. described a new solid phase sample application technique and application device. It also

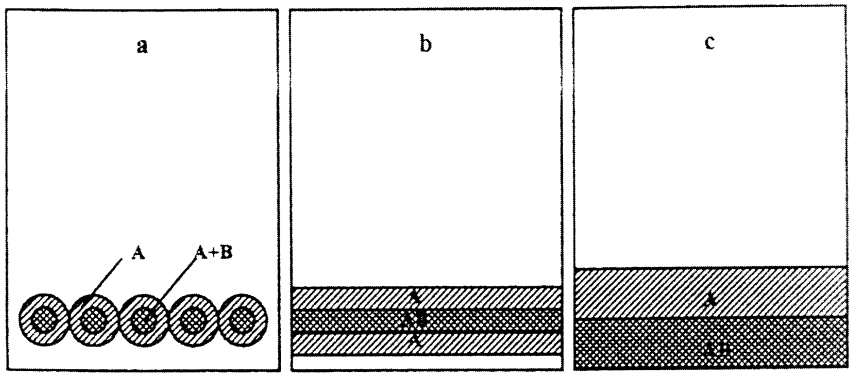

Figure 4. Application of large quantity of mixture (a)—as a series of spots (series of microcirculars chromatograms); (b)—as a narrow streak on the adsorbent layer; (c)—from the edge of the layer—partial separation occurs as a result of frontal chromatography.[13]

enables sample concentration and clean up and it may be used either for conventional chromatography (migration of mobile phase by capillary action) or for OPLC, RPC (forced-flow chromatography).[39]

Adsorbents

One of the requirements for satisfactory preparative separation is homogeneity of the layer of adsorbent. For the preparation of thicker layers (*ca*. 0.5–1.0 mm), all adsorbents applied in analytical TLC, such as: silica, alumina, Florisil, cellulose, and silanized silica may be used. For preparative chromatography, the more coarse particles of size range 5–40 μm may also be used but, for forced-flow chromatography, use of only fine-particle sorbents is recommended. Generally, preparation of preparative layers requires a lower amount of water (proportion to silica 1 : 2 or 1 : 1.7, for alumina 1 : 0.9), greater amount of adsorbent-adequately to layer thickness. In order to produce layers free from cracks, the plates should be dried in a horizontal position in a draft-free room for much longer time than is done for preparation of thin analytical layers (*ca* 0.25 mm), according to producers' instructions.

Besides self-made plates, commercially available precoated layers of 0.5–2 mm thick are used more frequently because of their higher resolution capacity and greater reproducibility. The plates with preconcentrating zones are recommended mainly for separation of natural mixtures containing extraneous substances. But, first of all, such plates improve the separability because the

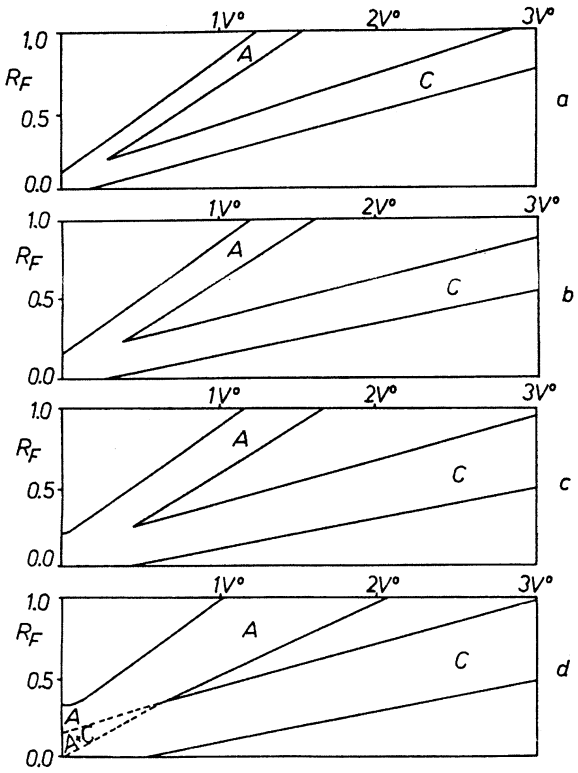

Figure 5. Effect of sample volume on the migration of zones of dyes A and C. Sample: 0.02 M solution of each dye in the eluent 4 : 1 methylene chloride—*n*-hexane. Sample volume: (a) 0.2 mL (b) 0.6 mL; (c) 1.0 mL; (d) 1.5 mL.[14]

starting diffuse band is compressed to a narrow band on the boundaries of two adsorbents (see also Refs. 1, 2, and 40).

Sample Solubility and Solvents

Solubility of samples in analytical TLC or HPLC is important only for quantitative investigations. For other cases it may be neglected. Because of the purpose of preparative chromatography, sample solubility is very important. It should be taken into account that sample solubility may affect the bands' resolution, owing to the fact that ratio of sample solvent volume to volume of

eluent is greater than in analytical TLC or in column chromatography, and may change the eluent strength significantly.

For the sampling stage, three cases may be considered:[40,41]

- the sample is dissolved in the eluent used as mobile phase;
- the sample is dissolved in diluted or more concentrated eluent;
- the sample is dissolved in a solvent of different qualitative composition.

In first case, only the starting band widening may be expected. Considering the second case, two possibilities should be taken into account—sample dilution (weaker solvent than mobile phase) which is advantageous because of formation of a narrow starting band and, thus, effecting an increase of separability. Formation of the starting band and subsequent zones' migration may be described by an equation for a two-step gradient. The equation enables computer simulation of all separation processes[41] and is illustrated by schematic representation of band behaviour during the sampling and elution process, assuming a two-step gradient (Fig. 6).

In Fig. 7, the effect of sample concentration is illustrated. Injection of a large volume of sample, diluted with a nonpolar diluent (*n*-heptane) results in greater overloading without loss of resolution. This is the effect of peak compression known in column chromatography.[42] In Fig. 7, the experimental bands are slightly narrower than theoretically predicted (under the assumption of a linear range of adsorption isotherm). In reality, for overloaded systems (in the

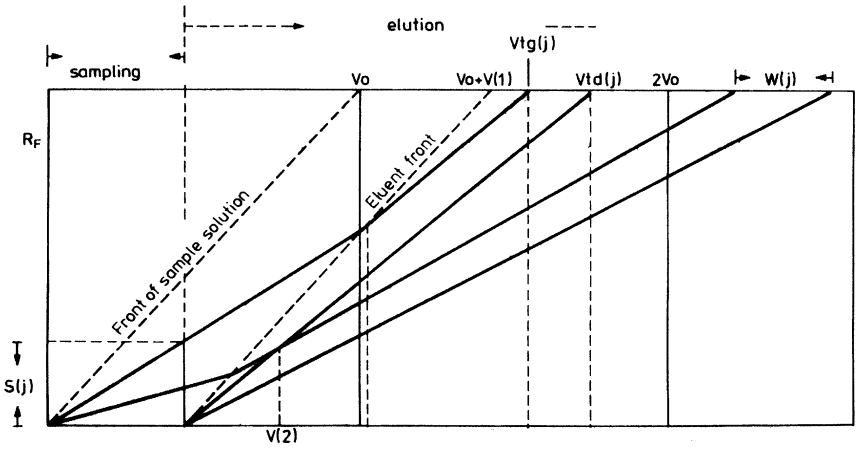

Figure 6. Theoretical model of band separation during introduction of sample and elution for two-step gradient in overloaded systems.[41]

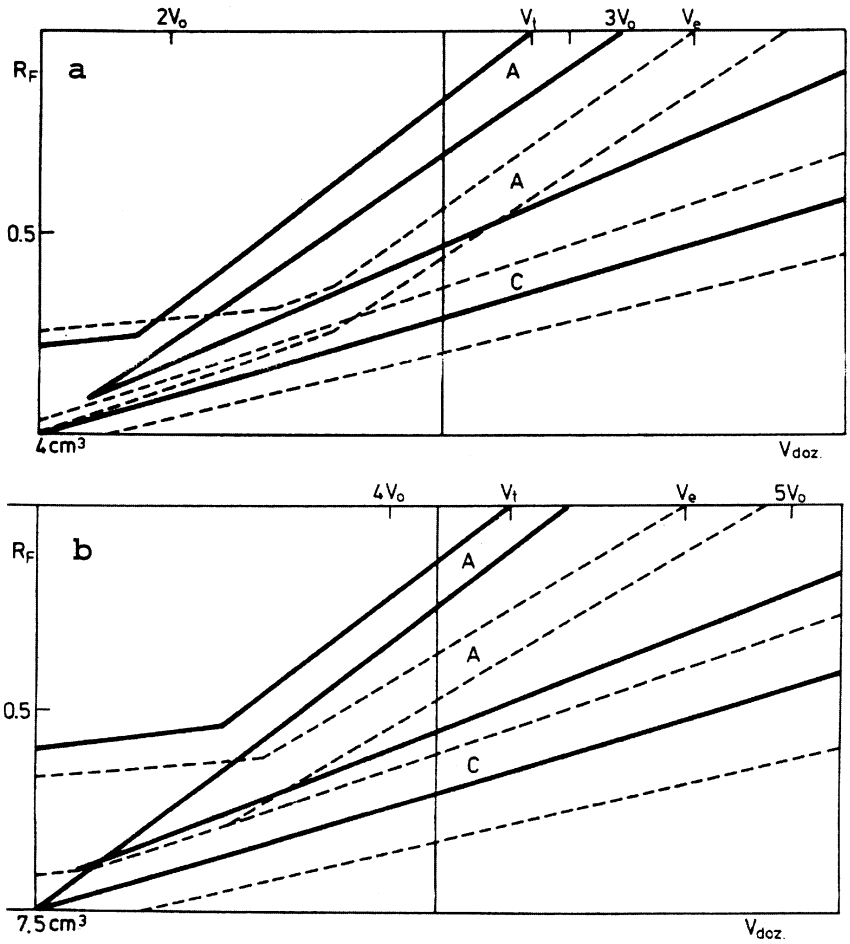

Figure 7. Dynamic representation of zones migration for sample A + C; (a) 4 mL of 0.04 mol L^{-1} solution of each dyes; (b) 7.5 mL of the solution. Eluent: dichloromethane + *n*-heptane (4 + 1). Solid lines—theoretical calculated, dashed lines—experimental data.[41]

nonlinear range of the isotherm), the deviation is a result of a mutual displacement effect and solute–solvent interaction.

Use of eluent of higher concentration of modifier as a sample solvent results in widening of the starting band adequately to R_F values. For a third case, where the sample is dissolved in solvent which is different from the eluent, it may disturb the separation process, owing to a change of eluent strength and the

precipitation of the solutes. The substances which are stagnant in the adsorbent's pores are gradually eluted, forming elongated zones from the start line. Therefore, on-line application of the sample[2] or from the edge of equilibrated plate and solid sample application[39] are advantageous.

The choice of mobile phase for optimization of the chromatographic system for PLC may be achieved using an eluotropic or isoeluotropic series "Prisma" model[43] and subsequent selection by preliminary TLC experiments with increasing sample amount for determination of sampling limit. More advantageous are eluents giving R_F values in the lower range (0.1–0.5) because the application of larger sample volumes leads to widening of the starting zone and increasing of R_F values. On the other hand, it influences mutual displacement effects. The choice of mobile phase or chromatographic system may be facilitated by considering correlation diagrams (e.g., log k_I vs. log k_{II} or R_{MI} vs. R_{MII}) for two different systems and different selectivities determined for TLC[44] (Fig. 8).

The second requirement referring to the mobile phase is the volatility of its components isolated from scraped adsorbent or eluted by on-line chromatography.[2] Such solvents as: ethyl acetate, isopropylether, diethylketone, chloroform, dichloromethane, and toluene as modifiers, and *n*-hexane as diluent, are usually recommended for normal phase chromatography. For reversed phase systems—methanol or acetonitrile as modifiers. Such components as acetic acid or acetate buffer, as well as ion association reagents, should be avoided.

Detection

For preparative chromatography, the main goal is the recovery of pure mixture components and, therefore, the localisation of separated bands is an important consideration. For forced-flow chromatography and on-line

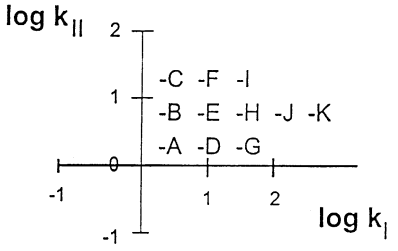

Figure 8. Schematic representation of log k_I vs. log k_{II} correlation. In the first stage the system I can be applied for group separation: (A + B + C), (D + E + F), (G + H + I). Then the individual groups of components can be separated in system II.[44]

systems (continuous development), the effluent is detected by an appropriate detector and collected automatically. This allows repeated use of plates several times.

For off-line planar chromatography, the localisation of bands directly on plates in daylight (for coloured substances), or mostly in UV light, is more convenient. The majority of adsorbents and commercially available precoated plates have a fluorescent indicator, e.g., silica gel 60 $F_{254+365}$. In several cases, separated bands may be localised in iodine vapours if substances form only unstable complexes. Brown or yellow zones, produced this way, should be immediately outlined.

For drastic derivatization methods in layer after development, 1–2 cm wide paths are marked along the two longer sides of the plate. The centre part of the layer should be covered and the two side segments sprayed with appropriate reagents. Two channels scraped in the adsorbent along the plate secure flow of reagent to the central part of the plate. The zones corresponding to products of derivatization are scraped and gathered into small tubes or small funnels for extraction of components.

Special Modes of Development

For the chromatography of complex mixtures (separation of isomers or closely related compounds with similar retention), the main problem is to improve the resolution. This might be achieved by increasing separation efficiency. Besides, for the analysis of such complex mixtures, it is advantageous to achieve (if possible) a large peak capacity. Improvement of separation efficiency and, thus, resolution, can be obtained by use of special modes of development, such as gradient elution, multiple development, two-dimensional development (2D), incremental multiple development (IMD), and gradient multiple development. All of these methods influence separation efficiency and peak capacity, owing to a spot reconcentration effect.

Gradient Elution

The problem of the separation of samples containing components of widely different polarities is difficult because of the "general elution problem".[45] This can be solved by using gradient elution. This technique can also be applied in TLC especially for separations carried out in sandwich chambers and stepwise development.[12] As has been observed, in TLC separation of plant extracts, gradient elution markedly improves the separation of spots owing to stronger displacement effects under conditions of numerous adsorption–desorption

processes.[25,46] In general, the increase of eluent strength of the mobile phase passing through the partly separated starting zone, causes consecutive sample components to reach the optimal range in order to increase R_F values. Because the lower edge of the zone is overtaken earlier than the upper edge by mobile phase front of increasing eluent strength—the zone becomes narrower than the starting band (see Fig. 9).[4]

For separation of less complex mixtures, the application of step-wise gradient elution enables the introduction of larger sample by comparison to the described method.[47]

Two Dimensional Micropreparative Separation

The ES chamber can be used for two-dimensional separation on a larger scale rather than a traditional 2D technique. The first technique requires the use of

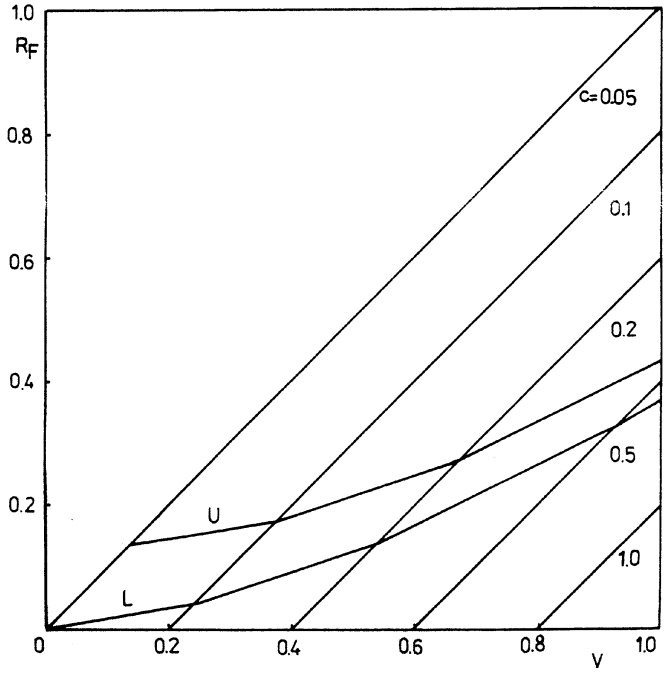

Figure 9. Compression of a zone under conditions of five-step gradient elution (5–100% modifier concentration): the upper edge U is overtaken by the fronts of increased modifier concentrations (ascending lines) with delay relative to the lower edge of the zone L.[47]

a cover plate with two distributors. A shorter (*ca*. 1 cm) distributor is used for introducing the sample, longer distributor for simultaneous delivery of pure eluent. Thus, radial sample spreading is avoided. For more details, see Ref. (2), Chapt. 5, pp. 13, 15. The possibility of using solvents of different selectivities improves the separation.

Multiple Development

Multiple development in TLC is a modern technique which enables high spot capacity and improves separation and resolution. Multiple development consists in repeated developments of the same plate with solvent for the same distance and in the same direction. Therefore, it is called a uni-dimensional multiple development (UMD). After each development, the plates are dried in air. Each consecutive development results in band reconcentration and, thus, increases the efficiency of separation. Compare Publication[48] and Review[49]. Perry et al.[50] derived an equation which can be used for prediction of R_F values after "n" developments:

$$R_{Fn} = 1 - (1 - R_F)^n \tag{2}$$

where n is a number of development, R_F is the retention factor after a single development.

It should be emphasised that multiple development is advantageous, especially for the separation of complex mixtures and overlapped bands, but, more frequently, for nonoverloaded systems. For overloaded systems, layer chromatography is used, rather, for separation of mixtures composed of a few components, and mainly for rechromatography of superimposed bands with lower R_F values. Therefore, the eluent strength of the solvent should be moderate. Experiments carried out with overloaded chromatography of compounds with similar retentions confirmed that a two-fold development of the plate was sufficient for complete separation[51] (see Fig. 10).

Incremental Multiple Development (IMD)

In this technique, the development distance is increased linearly in 10 or 20 mm steps with evaporation of the mobile phase from the plates after each step, by using of the same solvent, or a series of solvents, for modified IMD technique. In gradient incremental multiple development, the eluent strength is reduced stepwise.

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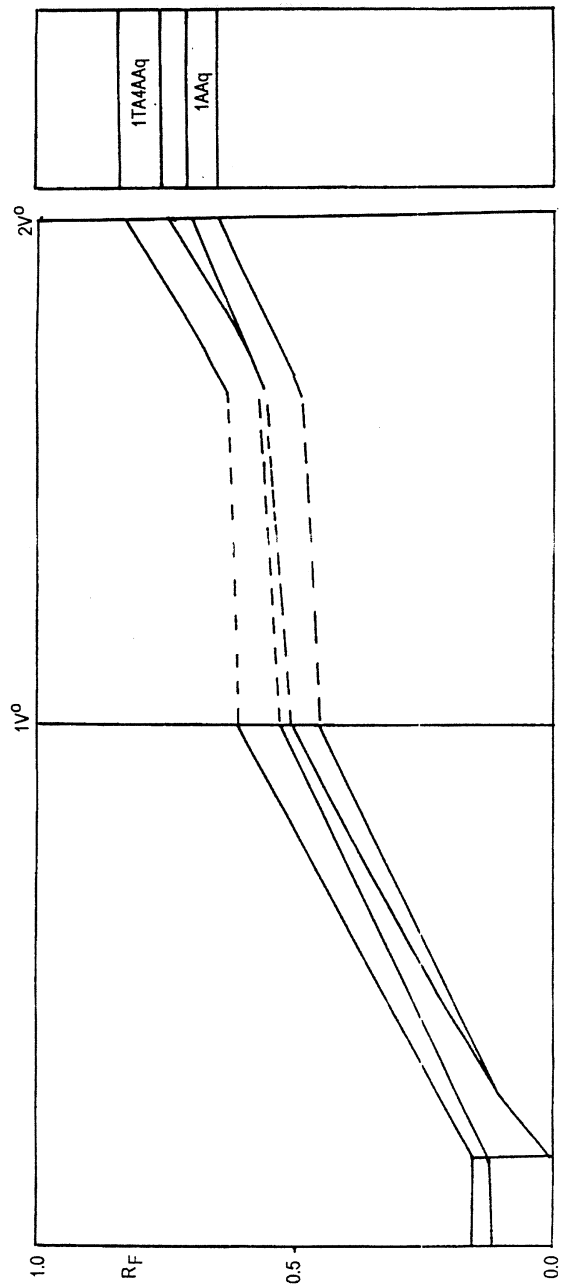

Figure 10. Two-fold development of mixture 1-N-(p-tolyl)-amino-4-aminoantraquinone and 1-aminoantraquinone on 100 × 100 × 0.5 mm silica layer. Sample: 1 mL of 0.78% solution of dyes. Eluent: 5% ethyl methyl ketone in toluene.[51]

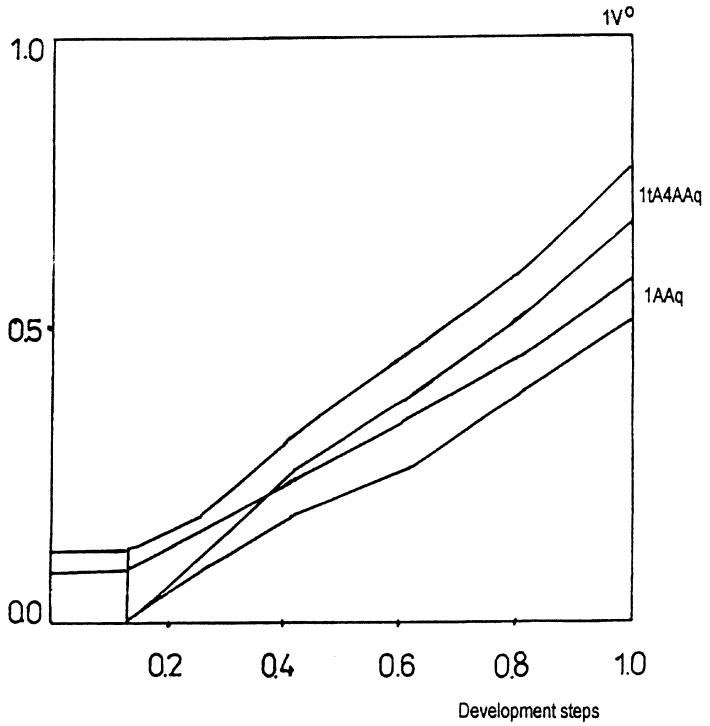

Figure 11. Incremental multiple development of 1-*N*-(*p*-tolyl)-amino-4-aminoantraquinone and 1-aminoantraquinone on $100 \times 100 \times 0.5$ mm silica layer. The development distance increased in 15 mm steps. Sample: 1 mL of 0.78% solution of dyes. Eluent: 5% ethyl methyl ketone in toluene.[51]

As is seen, from Figs. 10 and 11, multiple development and incremental multiple development are effective separation techniques applicable for preparative layer chromatography of closely related compounds, although other factors such as displacement effects and structural electronic interactions can also influence the separation. Better results were obtained using incremental multiple development. Difficult separations can be achieved, especially, by use of incremental multiple gradient development with stepwise reduction of eluent strength (see Fig. 12).[51]

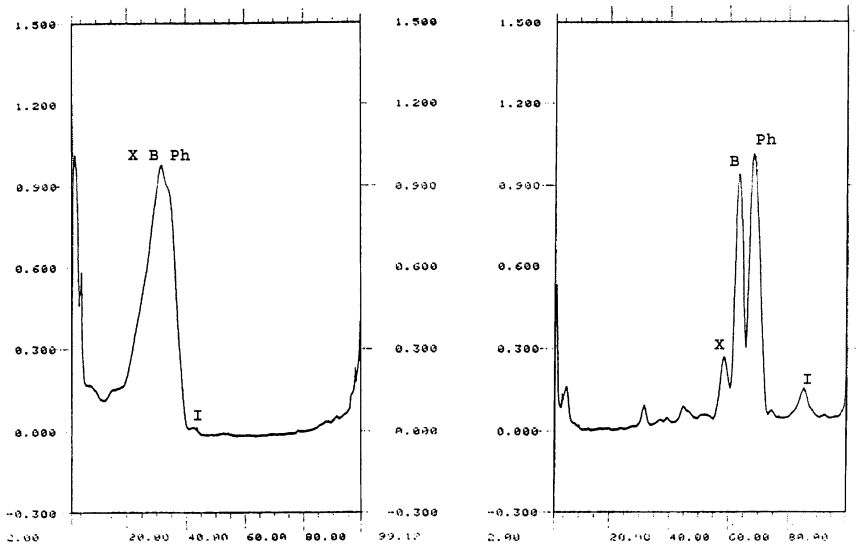

Figure 12. Densitograms illustrating the performance of incremental multiple development for 0.1 mL of 0.5% solution of extract of the *Heracleum sphondylium* fruits. (a) Isocratic development of the extract with ethyl acetate—*n*-heptane (1 + 9) as mobile phase, (b) IMD of the extract over distances increasing in 10 mm steps with acetate—*n*-heptane (1 + 9) as mobile phase. Adsorbent: silica.[51]

Applications of Preparative Layer Chromatography

Preparative layer chromatography is widely used for different purposes. PLC is usually applied for isolation of natural mixture components, e.g., plant extracts before identification of isolated compounds by various physicochemical methods such as ^{1}H-NMR, ^{13}C-NMR, IR (FTIR), and mass spectrometry (MS, TDMS, HR-EIMS). There are also numerous examples of use of thin-layer chromatography on a preparative scale as a method of sample preparation. Purification and/or fractionation of mixtures can be, therefore, used before GC or HPLC analysis (often connected with an MS detector), with reference to environmental samples as well as crude plant extracts or biological samples. Preparative layer chromatography is also used to obtain small quantities of

Table 2. Application of Preparative Layer Chromatography for the Separation of Alkaloids

Compounds	Sample	Plates	Eluent	Detection	Remarks	Ref.
Alkaloids and limonoids	*Tetradium trichotomum*	SiO_2	$CHCl_3$–MeOH	UV	Chemotaxonomy	52
Quaternary alkaloids	*Chelidonium maius*	SiO_2	Aqueous buffered MeOH	UV 355 nm	—	53
Tobacco alkaloid myosmine	*Arachus hypogea Corylus avellana*	SiO_2	$CHCl_3$–MeOH	UV	HPLC, GC/MS	54
Lycopodium alkaloids	*Lycopodiaceae* sp.	SiO_2	$CHCl_3$–MeOH–NH_3	Dragendorff's reagent	Identification	55
Protoberberine alkaloids	*Fissistigma balansae*	SiO_2	$CHCl_3$–MeOH–NH_3	Dragendorff's reagent	Identification	56
Anthranilate alkaloids	*Ticorea longiflora*	SiO_2	Hx–AcOEt	—	—	57
Aspidofractine-type alkaloids	*Kopsia teoi*	SiO_2	Et_2O–AcOEt; $CHCl_3$–MeOH	—	Centrifugal TLC	58
Diterpenoid alkaloids	*Aconitum leucostomum*	SiO_2	$CHCl_3$–MeOH–Me_2CO;	Dragendorff's reagent	IR, NMR, EI-MS	59

Benzylisoquinoline alkaloids	Leaves *Anisocycla jollyana*	SiO_2, Al_2O_3	Multicomp. eluent	—	—	60
Quinoline alkaloids	Stems *Orixa japonica*	RP18 SiO_2	MeOH–H_2O B–AcOEt	UV 254 nm	Isolation	61
Xanthine alkaloid	Leaves *Bosistoa floydii*	SiO_2	Petrol ether AcOEt	—	Centrifugal TLC	62
Quaternary alkaloids	Roots *Chelidonium majus L.*	SiO_2	Buff. aqueous eluents	Dragendorff's reagent, UV	Quantification	63
Cyclopeptide alkaloids	Root bark *Zizyphus lotus*	SiO_2	$CHCl_3$–MeOH	Dragendorff's reagent	Isolation	64
Polar alkaloids	*Schumanniophyton magnificum*	SiO_2 + paraffin	MeOH–H_2O buff.	—	—	65
Alkaloids	*Mitragyna speciosa*	SiO_2 RP18	$CHCl_3$–AcOEt-MeOH–H_2O	Dragendorff's reagent, UV	Identification	66
Tropane alkaloids	*Erythroxylum* sp.	SiO_2, Al_2O_3	Me_2CO–H_2O–NH_3; Et_2O–EtOH	—	Identification	67
Norditerpenoid alkaloid	*Delphinium tatsienense*	SiO_2	Et_2O–MeOH	—	Centrifugal TLC	68

Table 3. Application of Preparative Layer Chromatography for the Separation of Phenolic Compounds

Compounds	Sample	Plates	Eluent	Detection	Remarks	Ref.
2,4-Dihydroxy-benzoic acid derivat.	*Louchocarpus nicon* roots	SiO_2	$CHCl_3$–MeOH	UV	Centrifugal TLC	69
Isoflavone- and flavonol glycosides	*Trifolium striatum L.*	SiO_2	AcOEt–Me_2CO–H_2O	Anisalde-hyde	—	70
Flavonoids	*Cassia obtusifolia*	SiO_2	Multicomponent eluents	—	MS after elution	71
Isoflavonoids	*Milettia grif-foniana* root, bark	SiO_2	B + petroleun ether + AcOEt	—	Isolation, IR, NMR, MS	72
Methoxylated flavones	*Primula veris*	SiO_2	Hx + AcOEt	—	Isolation, HPLC	73
Flavonoid glycosides	*Astragalus* sp. roots	SiO_2 RP-2	$CHCl_3$ + MeOH + H_2O; MeOH + H_2O	UV	HPLC-MS	74
Furanocoumarins	Plants Apiaceae	Silanized silica	MeOH + H_2O	UV	Identification by HPLC	10
Coumarins	*Ticorea longiflora*	SiO_2	Hx–CH_2Cl_2–MeOH	UV	—	57
Hydroxycoumarins	*Cassia obtusifolia*	SiO_2	Multicomponent eluents	UV	MS after elution	71
Coumarins, furanocoumarins	*Harbouria trachypleura*	SiO_2	Hx + AcOEt, CH_2 Cl_2 + Me_2CO	—	Isolation, ^{1}H-NMR	75
Anthocyanidins	*Hypericum perforatum* L.	Cellulose	AcOH–HCl–H_2O	UV	Identification UV	76
Anthocyanins	Champagne vintage	—	—	—	Identification ^{1}H-NMR	77
Isoprenylated xanthones	*Cudrania tricuspidata*	SiO_2	Hx–Me_2CO	$FeCl_3$	Isolation	78

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Table 4. Application of Preparative Layer Chromatography for the Separation of Terpenes

Compounds	Sample	Plates	Eluent	Detection	Remarks	Ref.
Terpenoids: danshenol-A	*Salvia gluinosa*	SiO_2	B–AcOEt	—	Quality control	79
Diterpenes	*Euphorbia segetalis*	SiO_2	CH_2Cl_2–T–MTB	—	—	80
Furano-diterpenes	*Croton campestris*	SiO_2	Hx–AcOEt	UV 254	Quantitative analysis	81
Cytotoxic diterpenes	*Salvia miltiorrhiza*	SiO_2 RP18	Hx–AcOEt MeOH– H_2O	—	Isolation	82
Euphane triterpenes	*Schinus molle*	SiO_2	T–AcOEt–AcOH	Anisaldehyde reagent	Qualitative identification	83
Sesquiterpenes	*Chlorantus japonicus*	SiO_2	Et_2O–MeOH; $CHCl_3$– MeOH	UV	Isolation	84
Monoterpenes, sesquiterpenes	*Lippia ducis*	SiO_2	Hx–Me_2CO	UV 254	Identification	85
Neo-clerodane diterpenoids	*Teurcium yemense*	SiO_2	$CHCl_3$–MeCN	Anisaldehyde reagent	Centrifugal TLC	86
Sesquiterpene	*Eriostemon fitzgeraldi*	SiO_2	$CHCl_3$–AcOEt petr. ether–AcOEt	—	Centrifugal TLC	87
Triterpenoids	*Sanguisorbeae* species	SiO_2	T–AcOEt–HCOOH	Anisaldehyde reagent	Chemo-taxonomy	88
Sesquiterpene lactones	*Peniophora polygonia*	SiO_2	CH_2Cl_2 (T)–AcOEt–MeOH	UV	Identification	89
Norsesquiterpenes	*Pteridium aquilinum*	SiO_2	CH_2Cl_2–Me_2CO	UV	Isolation of pterosin B	90

(*continued*)

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Table 4. Continued

Compounds	Sample	Plates	Eluent	Detection	Remarks	Ref.
β-dihydro-agarofuran sesquiterpene	Root bark *Celastrus rosthornianus*	SiO_2	B–Me_2CO	—	—	91
Pentacyclic triterpenoids–lantadenes	*Lantana camara var. aculeata*	SiO_2 GF	Petroleum + AcOEt + AcOH	UV	Purification, HPLC	92
Lactone diterpenes	*Potamogeton natans*	SiO_2	B + AcOEt	—	Isolation, NMR, FAB-MS	93
Triterpene	*Eugenia sandwicensis*	SiO_2	$CHCl_3$ + MeOH	—	Isolation, IR NMR, MS	94
Tetraterpenoid–trianthenol	*Trianthema portulacas-trum*	SiO_2	Hx + Me_2CO + AcOEt	—	Isolation, NMR, HR-EIMS	95
Euphosalicin–diterpene polyester	*Euphorbia salicifolia*	SiO_2	CHx + AcOEt + EtOH	—	Isolation, IR, NMR, MS	96
Triterpene saponins	*Hedera helix*	SiO_2	$CHCl_3$–MeOH–AcOH–H_2O	Anisaldehyde	Isolation of hederacoside	97
Saponines	*Gynostema longipes*	SiO_2	$CHCl_3$–MeOH– H_2O–AcOH	Densitometry	Identification NMR, MS	98
Triterpene saponins	*Verbascum songaricum*	SiO_2	$CHCl_3$–MeOH–H_2O	—	Isolation	99
Ginseng saponin, ginsenosides	*Ginseng*	SiO_2	CH_2Cl_2–MeOH–sol. Na_2CO_3	—	Rotating-disk TLC	100

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Table 5. Application of Preparative Layer Chromatography for the Separation of Sterols, Taxoids, Guaianolids, and Phtalides

Compounds	Sample	Plates	Eluent	Detection	Remarks	Ref.
Cholesterol oxidation prod.	—	SiO_2	Hx + Et_2O + EtOH	Flourescein reagent	Analysis by GC/TDMS	101
Sterols	Jojoba oil	SiO_2	Hx + Et_2O	UV 365 nm	Identification	102
7-Hydroxy sterols	—	SiO_2; C18	Hx + AcOEt; MeOH + H_2O	—	QSAR	103
Cytotoxic sterols	*Pseudobersama mossam-bicensis*	SiO_2; C18	Hx + iPrOH; MeOH + H_2O	—	Identification	104
Stigmasterol β-Sitosterol	*Cardamon*	Argentation TLC	Hx + AcOEt	UV	—	105
Cholesterol, oxysterols	Poultry meat	SiO_2	Hx + Et_2O	—	Sample prep. CGC	106
Taxoids	*Taxus baccata*	SiO_2	B–$CHCl_3$–Me_2CO–MeOH	UV 254	Optimization of eluent	107
Taxoids	*Taxus* sp.	SiO_2	B–$CHCl_3$–Me_2CO–MeOH	UV 254	Fractionation, HPLC	108
Taxoids	*Taxus* sp.	SiO_2; RP-2	CH_2Cl_2–DX–Me_2CO– MeOH; MeOH–H_2O	UV 254 densito-metry	Optimization, fractionation, HPLC	109
Taxoids	*Taxus* sp.	SiO_2	CH_2Cl_2–DX–Me_2CO– MeOH;	UV 254 densito-metry	Isolation	110
Guaianolides	*Anthemis carpatica*	SiO_2	Multicomponent eluents	—	Identification	111
Phthalides	*Angelica sinensis* roots	SiO_2	Hx + Me_2CO	—	Isolation, LC-MS	112

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Table 6. Application of Preparative Layer Chromatography for the Separation of Lipids, Fatty Acids, and Gangliosides

Compounds	Sample	Plates	Eluent	Detection	Remarks	Ref.
Oils and FAME	*Saussurea spp.*	SiO_2	Hx–Et_2O	Phospho-molybdic acid	Identification GC/MS, IR	113
Fatty acids	Rat liver	Argentation SiO_2	Petr. ether–Et_2O–AcOH	UV	Identification GC/MS	114
Glycisphingolipids	—	SiO_2	$CHCl_3$– MeOH–H_2O	Reagents + densitometry	—	115
Glycosphingolipids phospholipids	—	Paper	—	Primuline reagent	TLC monitoring	116
Phospholipids, non-phospholipids	Marine sediments	SiO_2	$CHCl_3$ + MeOH + AcOH	—	Identification, GC-MS	117
Fatty acids, triglycerides	Pappaya seed oil	Argentation SiO_2	Petr. ether–Me_2CO	Bromine vapour, densitometry	Preo. TLC of lipid classes	118
Octadecenoic acids	Edible fats	Argentation SiO_2	Hx–Et_2O	Flourescein reagent	Quantification by GC	119
Vernolic, cyclo-propenoic fatty acids	*Piper nigrum*	SiO_2	Hx–Et_2O	Flourescein reagent	Preo. TLC of acids fractions	120
n–3 fatty acids	Fish oil	SiO_2	Hx–AcOH–H_2O	Iodine vapour	Fractionation	121
Oleic, eruic acid	Postrafination waters	SiO_2	B–Me_2CO	Iodine vapour	Identification	122
19 FAME	Soyabean oil	Argentation SiO_2	$CHCl_3$	Flourescein reagent, UV	Identification GC, FTIR	123

Triglycerides of fatty acids	*Thunnus alalunga*	$SiO_2 + 5\%$ boric acid	Hx–Et_2O–AcOH	—	Purification	124
Forbesin–glycolipid	*Asterias forbesi*	SiO_2; C-18	$CHCl_3$–MeOH–NH_3; MeOH–H_2O	—	Identification	125
Phospholipids	Rat liver	SiO_2	$CHCl_3$–MeOH–AcOH	—	Recovery of procedure	126
Polar lipids	Cotton var.	SiO_2	$CHCl_3$–Me_2CO–nPrOH–AcOH	Flourescein reagent, densitometry	—	127
Triacylglycerols	Plant oils	Argentation SiO_2 RP-TLC	—	H_2SO_4 temp. densitometry	Quantification	128
Monoacylglycerols	Butter oil	SiO_2	Hx + Et_2O + HCOOH	UV, I_2	Sample preparation GC-MS	129
Serum gangliosides	—	SiO_2	$CHCl_3$–MeOH–0.3% sol. $CaCl_2$	Orcinol reagent	Removal non-polar lipids	130
Gangliosides	—	SiO_2	$CHCl_3$–MeOH– H_2O	Nondestr. fluorochromes	—	131, 132
Gangliosides	Embrioic chicken brain	SiO_2	$CHCl_3$–MeOH– H_2O	—	AMD	133
Gangliosides	—	SiO_2	$CHCl_3$–MeOH–0.3% sol. KCl	Orcinol reagent	—	134

Table 7. Application of Preparative Layer Chromatography for the Separation of Various Compounds

Compounds	Sample	Plates	Eluent	Detection	Remarks	Ref.
Anthraquinones	*Cassia obtusifolia*	SiO_2	Multicomponent eluents	Methanolic KOH	MS after elution	71
Naphthoquinones	*Catalpa ovata*	SiO_2	Hx–AcOEt; B–AcOEt	UV 254, 366 nm	Identification	135
Podophyllotoxin derivatives	*Forythia intermediata*	SiO_2	Et_2O	UV	—	136
Steviol glucosides	*Stevia rebaudiana*	SiO_2	AcOEt–iPrOH–H_3O	Vanilin reagent	Identification	137
Acetogenin derivatives	*Annona cherimola*	SiO_2	$CHCl_3$–MeOH; AcOEt–Me_2CO	Kedde's reagent	Identification	138
Carotenoids	*Capsicum annuum*	SiO_2	Petroleum ether-Me_2CO–Et_2NH	UV 254, 366 nm	Identification	139
Carotenoids	Brown algae *Phaeophyceae*	SiO_2	Hx–Me_2CO	—	Quality control	140
Carotenoids	Red pepper	SiO_2	Petr. ether-B–Me_2CO–AcOH	—	OPLC	141
Polyacetylenes	*Bellis peremnis*	SiO_2	Pentane–Et_2O	UV 254 nm	Isolation	142
Natural polyamine–agmatine	—	SiO_2	CHx + AcOEt	UV	DANS–derivat., MS	143
Alkamides	*Echinacea purpurea* roots	C18	MeOH + H_2O	—	LC-MS	144

Pungent constituents	Ginger	SiO_2	Hx + Me_2CO	UV	Purification HPLC-MS	145
Benzofuranones, chromanes	*Coniothyrium minitans*	SiO_2	B + AcOEt	—	Isolation NMR, MS	146
Dibenzofurans	*Lecanora cinereocarnea*	SiO_2	T + Me_2CO	—	Isolation, IR, NMR, HR-EIMS,	147
Naphthopyranones	*Guanomyces polythrix*	SiO_2	CH_2Cl_2 + MeOH	—	Isolation, IR, NMR, MS	148
Riboflavin + derivatives	Food	SiO_2	BuOH + AcOH + H_2O	—	Sample preparation	149
Flavin derivatives	Baker's yeast	SiO_2	BuOH + AcOH + H_2O	—	Sample preparation	150
Aromatic, polar, non-eluted fractions	Coal-derived products	SiO_2	T; CH_2Cl_2–MeOH	UV	Identification	151
Aliphatic, aromatic, polar fractions	Bitumen, coal tars, host rock, lignite	SiO_2	Petroleum ether, CH_2Cl_2	Ethanolic sol. of rhodamine	Identification of various compounds	152
Azaarenes	Sewage sludges	SiO_2	CH_2Cl_2–MeOH	UV 254, 366 nm	GC-MS after elution	153
Herbicide–pendimethalin	Rat tissues	SiO_2	$EtCl_2$	Autoradiography	—	154
Imazapyr herbicide	Aqueous media	SiO_2	7 solvent systems	UV, autoradiography	—	155

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standards from natural mixtures. References cited have been chosen from various publications from the last 15 years. Examples of applications are divided according to chemical groups in Tables 2–7. The applied systems, detection, and goal of chromatography are also presented.

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