Review

Planar chromatography at the turn of the century

Colin F. Poole*

Department of Chemistry, Wayne State University, Detroit, MI 48202, USA

Abstract

An overview of the state-of-the-art of modern thin-layer chromatography (planar chromatography) is presented with emphasis on the complementary features of thin-layer and column liquid chromatographic separations. The reasons for selecting thin-layer chromatography for a particular analysis are identified by its attributes: a disposable stationary phase; simultaneous parallel separations; static detection free of time constraints; storage device for chromatographic information; all sample components are observed in the chromatogram. Future prospects for improved separation performance in TLC using zone refocusing, forced flow and electroosmotic flow methods are discussed as well as increasing zone capacity by using two-dimensional development and coupling to column chromatographic methods. Advances in coupling thin-layer chromatography with spectroscopic methods for structural elucidation are also considered. Finally, some predictions are made for how thin-layer chromatography will be practiced in the future.   © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Planar chromatography; Thin-layer chromatography

Contents

*Tel.: +1-313-577-2881; fax: +1-313-577-1377.

E-mail address: cfp@chem.wayne.edu (C.F. Poole)

1. Introduction

Thin-layer chromatography (TLC) can trace its origins to the introduction of drop chromatography by Izmailov and Shraiber in the late 1930s as a fast, convenient and more powerful separation tool for analytical applications than conventional column liquid chromatography (see [1]). Thin-layer chromatography as we know it today was established in the 1950s, due in part to the efforts of Stahl [2] and Kirchner [3], who devised standardized procedures to improve separation performance and reproducibility and contributed to the development of many new applications. At about the same time commercialization of materials and devices commenced making the technique accessible to all laboratories. This heralded in a golden age in the evolution of TLC, Fig. 1, which quickly displaced paper chromatography as the main liquid chromatographic separation method in laboratory investigations. The 1970s saw a declining interest in TLC as modern column liquid chromatography was developed into an automated and fully instrumentalized separation method. Further optimized conditions for TLC, more generally known as high-performance TLC or modern TLC, failed to sustain the previous interest in the technique. By the 1980s modern TLC had become fully instrumentalized but at the same time detached from the main stream of chromatographic research. In its conventional form TLC continued to provide a quick, inexpensive, flexible, and portable method for monitoring synthetic reaction mixtures and similar applications. On the other hand, modern TLC was becoming increasingly marginalized and virtually invisible at major symposia on chromatography. At the turn of the century modern TLC faces an uncertain future, while conventional TLC is likely to survive as a general laboratory tool in the same mold as precipitation, crystallization and distillation, having survived as indispensable, low cost and low technology operations. The general invisibility of modern TLC, more so on the American continent than Europe, is its main enemy today. Training in modern TLC is virtually nonexistent within industry and academia, and the loss of institutional knowledge means that many contemporary scientists are unaware of when TLC could be the method of choice for solving certain problems. Modern TLC survives
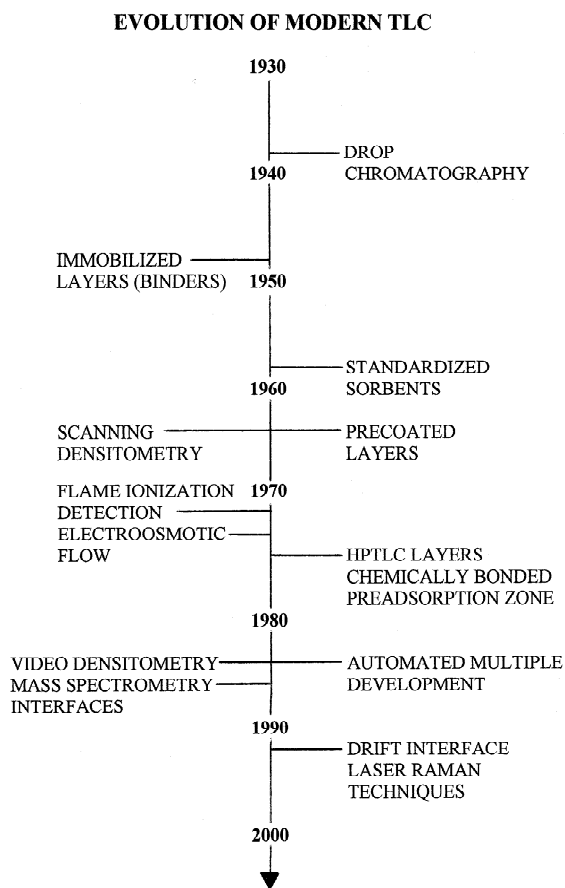
EVOLUTION OF MODERN TLC

Fig. 1. Time line depicting the evolution of modern thin-layer chromatography.

because of strong support from a few enthusiastic individuals who continue to develop and nurture the technique, and because its world wide user base is still large enough to support its commercialization. The last decade has seen strong growth in the use of TLC in the technically less advanced countries where the latest technology for column chromatography is often not cost efficient for solving local problems.

If modern TLC had no role to play in the future of separation sciences, then further comment would not be required. The disappearance of paper chromatography as a common laboratory tool was compensated for by the evolution of other techniques, ironically, including TLC. In my opinion modern TLC still has a role to play in separation science as a complement to other, column-based liquid chromatographic tech-

niques. It is this case that will be presented in this article, together with an appraisal of the current state-of-the-art in TLC, and identification of those areas in need of further development.

The main characteristic features of modern TLC are the use of fine particle layers for fast and efficient separations; sorbents with a wide range of sorption properties to optimize selectivity; the use of instrumentation for convenient (automated) sample application, development and detection; and the accurate and precise in situ recording and quantitation of chromatograms. These features are the exact opposite of conventional TLC, which thrives because it does not require instrumentation and has low oper-

ating costs. Expectations in terms of performance, ease of use, and quantitative information from the two approaches to TLC are truly opposite. As an example of expectations for a separation by modern TLC we show the chromatogram in Fig. 2. The ethynyl steroids are the main components of the birth control pill. Since they have a similar biological function, their structures are similar, and high selectivity is required for their separation. Typical tablet formulations contain two steroids only but the method illustrated was designed for universal application requiring the baseline separation of all possible active ingredients. Baseline separation is obtained with a short migration distance typical of fine
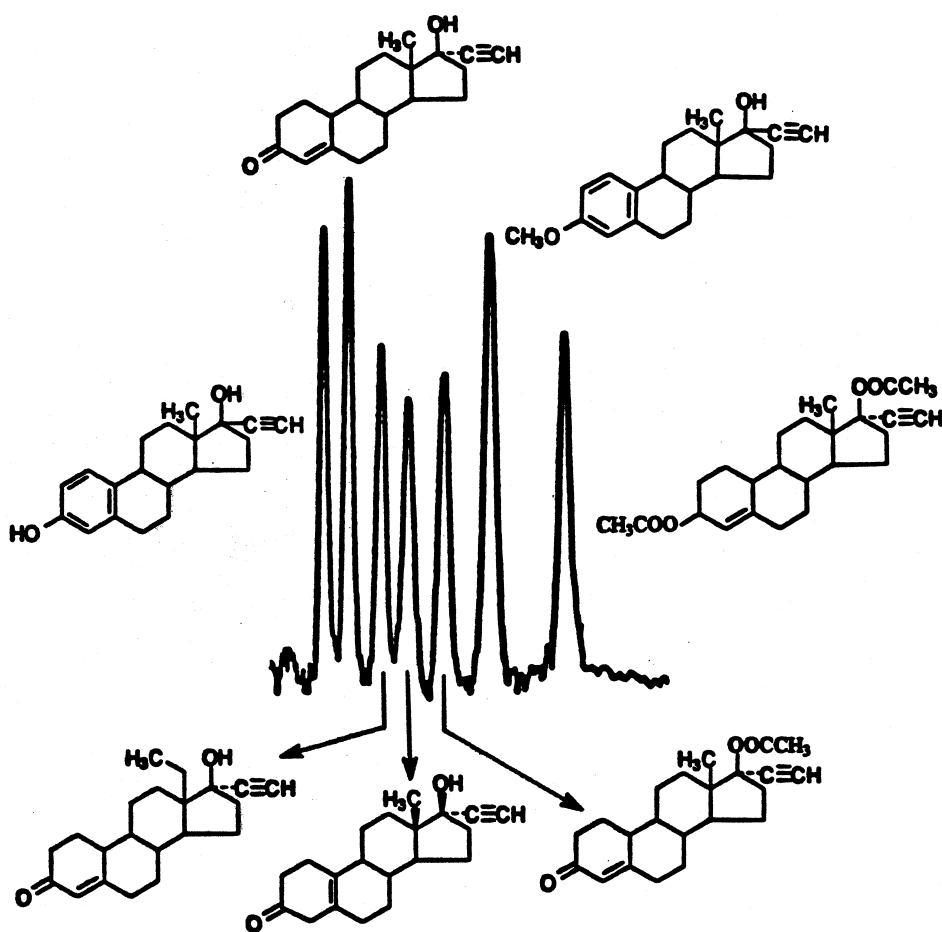

Fig. 2. Separation of ethynyl steroids by modern thin-layer chromatography. Two 15 minute developments with the mobile phase hexane–chloroform–carbon tetrachloride–ethanol (7:18:22:1) on a silica gel 60 HPTLC plate. Chromatogram was recorded by scanning densitometry at 220 nm.

particle layers and scanning densitometry provides a conventional record of the separation in the form of a chromatogram, as well as quantitation of individual components in the mixture after calibration. By way of example, for a typical tablet formulation stated to contain norgestrel at 0.5 mg and ethynodiol diacetate at 2.0 mg per tablet the measured quantities were 0.509 ± 0.008 mg ($n = 10$) and 2.00 ± 0.03 mg ($n = 10$) [4]. Sample preparation involved dissolution and filtration only. The quantitative results are similar in accuracy and precision to other chromatographic techniques and the method is suitable for routine analytical assays. This rather straightforward example indicates that modern TLC should not be neglected as either a selective separation method or for quantitative assays. Some specific reasons for choosing TLC for quantitative analysis are developed below.

2. Complementary features of columns and layers

Separations occur by the same retention mechanisms in column and planar chromatography, and as a consequence, strong similarities in the two approaches to liquid chromatography are to be expected. The characteristic features that distinguishing between the two techniques is set out below.

Kinetic optimization of column separations is achieved by using external force (pressure) allowing small particle size sorbents to be used at (or close to) optimum mobile phase velocities. This results in high theoretical plate numbers and a relatively large separation capacity [5]. The available column inlet pressure and mechanical stability of the stationary phase ultimately limit separation performance measured by these terms [6]. In contrast separations by planar chromatography are normally carried out at atmospheric pressure with capillary forces responsible for the migration of the mobile phase through the layer. Capillary forces are too weak to achieve either an optimum mobile phase velocity or a constant mobile phase velocity as a function of the solvent front migration distance [7–9]. These features result in poor kinetic performance (low numbers of theoretical plates that depend on the migration distance) and a limited separation capacity (low

intrinsic performance coupled with short useful development distances). The achievement of more favorable kinetic separation properties for planar chromatography is an absolute must for improving the separation capacity of planar chromatography. Ways to achieve this are discussed in Section 4. Using current practices more complex separations can be achieved by column chromatography than planar chromatography. For these separations column chromatography is the method of choice.

The stationary phase sorbents for column and planar chromatography are similar, with specific properties individually optimized for their intended use. The format employed for separations, however, is different. The stationary phase in column chromatography is generally inaccessible as it must be housed in a strong container (column) able to withstand usual operating pressures. For planar chromatography the stationary phase is cast as a layer on a supporting structure in the form of a thin cube. The uppermost surface is accessible both during and after development. Because of the dimensions of the layer samples are separated simultaneously in parallel tracks or lanes as opposed to sequentially for column separations. This provides for the possibility of a large increase in sample throughput in planar chromatography as well as the simultaneous separation of samples and standards for identification and calibration. Also, single use of the stationary phase is common practice in planar chromatography. Differences in unit operating costs and the use of sequential methods for solute identification and calibration in column chromatography render this practice impractical. Matrix contaminants are less of a problem in planar chromatography than for column chromatography and in many assays the extent of sample preparation required for analysis can be reduced for planar chromatography compared with column chromatography.

The elution mode is commonly used for separations in column chromatography requiring all solutes to migrate the same distance corresponding to the length of the sorbent bed. Zones are separated in time and detected in the mobile phase as they exit the column. The development mode is the common option for planar chromatography. In this case all solutes have the same migration time during which separated zones migrate different distances. At the

Table 1
Attributes of planar chromatography providing the link to contemporary applications

Attribute	Application
Separation of samples in parallel	·Low-cost analysis and high-throughput screening of samples requiring minimal sample preparation.
Disposable stationary phase	·Analysis of crude samples (minimizing sample preparation requirements) ·Analysis of a single or small number of samples when their composition and/or matrix properties are unknown ·Analysis of samples containing components that remain sorbed to the separation medium or contain suspended microparticles
Static detection	·Samples requiring post-chromatographic treatment for detection ·Samples requiring sequential detection techniques (free of time constraints) for identification or confirmation
Storage device	·Separations can be archived ·Separations can be evaluated in different locations or at different times ·Convenient fraction collection for multimodal column/layer chromatography
Sample integrity	·Total sample occupies the chromatogram not just that portion of the sample that elutes from the column

end of the chromatography the mobile phase is evaporated and the separation immobilized. Detection, therefore, takes place in the presence of the stationary phase, is free of time constraints since the separation is immobilized, and the separated zones are simultaneously accessible. This provides great flexibility in the choice of detection strategies and even the possibility of archiving separations for sequential detection processes performed at different times or different locations. Several detection techniques can be employed sequentially, provided that they are nondestructive, and detection techniques employing chemical reactions can be optimized free of time constraints. Since the total sample occupies the chromatogram and not just the portion which elutes from the column, the integrity of the analysis is guaranteed in planar chromatography but can only be implied in column chromatography.

The above differences in characteristic features provide for the existence of planar chromatography as a complementary technique to column liquid chromatography. It is important to emphasize the complementary rather than competitive aspects of the two separation techniques. Both approaches to liquid chromatography are no more than separation tools for which the job at hand is responsible for selecting the preferred technique. There is no doubt that column chromatography provides a higher separation capacity required for the routine separation of some complex mixtures. At the present time it also provides a wider range of stationary phases and separation mechanisms than available to planar chromatography and a higher level of automation for unattended and out of working hours operation. It also offers a wider range of detection possibilities and is generally more successful in the hands of operators with limited training. Situations for which we turn to planar chromatography include the analysis of large numbers of samples that require minimum sample preparation, when using planar chromatography permits a reduction in the number of sample preparation steps, and when post-chromatographic reactions are required for detection. In other words when the attributes of planar chromatography, as summarized in Table 1, appear at the top of our list of desirable features for the analysis and the deficiencies of the technique are considered less influential in making this choice than the attributes. In the next section we will highlight some of the features of planar chromatography that illustrate its correct use in separation science.

3. Why use thin-layer chromatography at all?

3.1. Whole sample is always evaluated

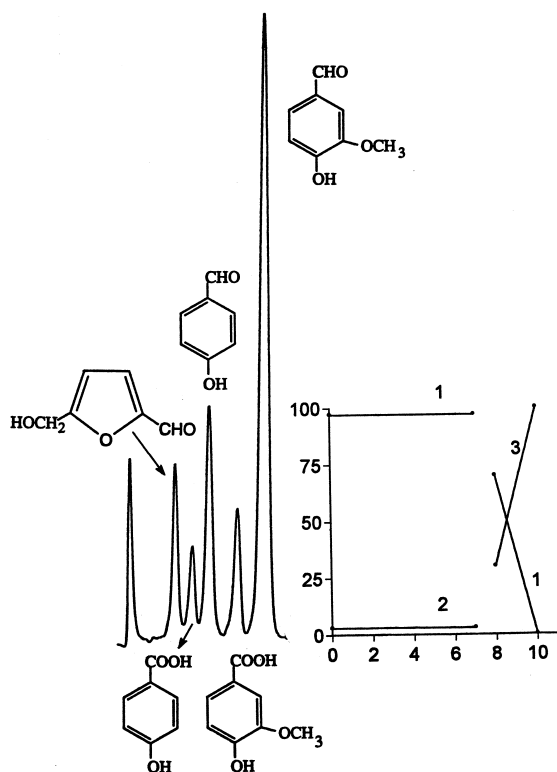The dominant trend in TLC is to use normal-phase

Fig. 3. Separation of the principal aromatic flavor compounds in natural vanilla extracts by thin-layer chromatography on silica gel HPTLC plates using automated multiple development. Mobile phase gradient starting from chloroform (1) and ethyl acetate–acetic acid–propan-1-ol (1:1:1) (2) for the first 7 developments then chloroform (1) to hexane (3) in 3 developments.

characterized mixtures has at times revealed new and unexpected information about the sample. The determination of the flavor potential and authenticity of vanilla is based in part on the concentration ratios of the principal polar aromatic flavor compounds determined by reversed-phase liquid chromatography [10]. It was quite straightforward to develop a TLC method for high-throughput screening of vanilla extracts that minimized sample preparation steps [11,12]. A typical separation is shown in Fig. 3. An additional peak was observed in the normal-phase TLC separation that was absent from the reversed-phase column separation. This compounds was isolated and identified as 5-(hydroxymethyl)-2-furfural, and was confirmed as a common component of vanilla extracts [13]. This compound was buried in the large matrix peak eluting close to the column hold-up volume in the reversed-phase column separations and its contribution to understanding the mechanism of the development of the characteristic vanilla flavor overlooked. As a further example of the complementary use of chromatographic techniques we developed a solvent-assisted supercritical fluid extraction method for the isolation of flavor compounds from cinnamon that provided extracts sufficiently clean for direct analysis by gas chromatography [14]. The complex gas chromatographic profiles provided suitable information for flavor assessment and identification of the botanical origin of the various cinnamons of commerce. Separation of the acids-containing fraction by TLC produced a very simple chromatogram, particularly when recorded in the fluorescence mode, Fig. 4 [10,15]. The chromatographic properties of the main peak did not

methods for separations and in liquid column chromatography to use reversed-phase methods. This complementary approach for analysis of even well
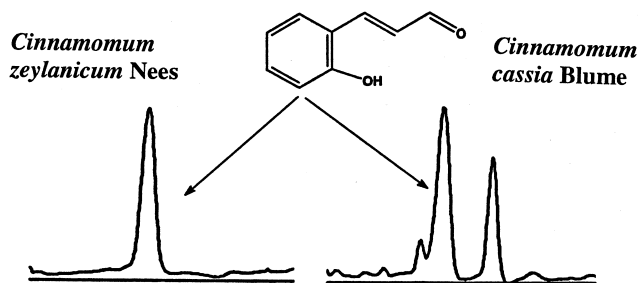

Fig. 4. Separation of solvent-assisted, supercritical-fluid extracts of cinnamon by thin-layer chromatography and fluorescence (λ_{ex}=365 nm and λ_{em}>400 nm) scanning densitometry. Separations were obtained on DIOL HPTLC layers with a single 15 min development using chloroform–hexane–methanol (59:39:2).

correspond to any of the known compounds iden-
tified by gas chromatography. This compound was
isolated by preparative TLC and identified as 2-
hydroxycinnamaldehyde [16]. In spite of decades of
research on cinnamon, this compound had not been
isolated before, even though it is found in reasonable
concentration (0.02 to 0.5 mg/g). Thermal re-
arrangement of 2-hydroxycinnamaldehyde results in
the formation of coumarin, a natural component of
some botanical sources of cinnamon, and so was not
detected by gas chromatography. Separations by
normal-phase column liquid chromatography of cin-
namon extracts are unpopular because the extracts
tend to be dirty and foul columns. Also, liquid
column chromatography lacks the separation capaci-
ty of gas chromatography for detailed profile analy-
sis. Using gas chromatography and TLC in tandem is
the best solution to characterizing cinnamon extracts
[10,14,17].

3.2. Simultaneous sample cleanup and separation

On a number of occasions we have turned to TLC
for the analysis of target compounds in complex
matrices when we can achieve sample cleanup and
separation simultaneously. Since the stationary phase
is used once only matrix components that are
immobilized at the origin and do not move into the
chromatogram cause no problems, while these would
be a disaster for column experiments, and demand
the use of additional sample preparation steps prior
to separation. Natural and artificial vanilla flavor is
added to chocolate to reinforce its characteristic
chocolate taste. For quality control purposes the
concentration of vanillin in chocolate is of impor-
tance. Analysis of vanillin in chocolate is quite
straightforward by TLC [16]. Sample preparation
involves sonication of chocolate in 95% (v/v) etha-
nol and application of the supernatant to the TLC
plate after filtration. A typical result is shown in Fig.
5. The automated multiple development technique is
used to ensure efficient extraction of vanillin from
the matrix components in the first few development
steps with a polar mobile phase as well as to obtain
focused bands in the separation. Vanillin is well
separated from the matrix components and can be
quantified easily by scanning densitometry. The true
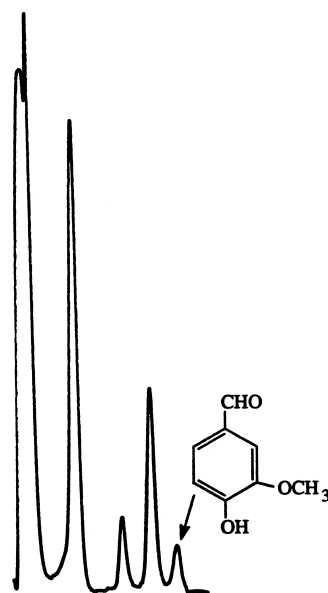extent of the matrix burden is not indicated by the

Fig. 5. Separation of vanillin from a solvent extract of chocolate
by thin-layer chromatography on silica gel HPTLC plates using
automated multiple development and a similar mobile phase
gradient to that indicated in Fig. 3.

scanned chromatograms. Visually, the tall peak at the
origin of the chromatograms appear as small choco-
late bars on the layer with dimensions corresponding
roughly to the sample application zone. Simple
sample preparation requirements, the ability to sepa-
rate samples in parallel, and combining sample
cleanup with separation results in a high throughput
method suitable for quality control applications.

3.3. Screening technique in surveillance programs

The appropriate legal and health authorities have
set up surveillance programs to control food con-
taining unacceptable levels of drug residues, to
ensure an adequate and safe drinking water supply,
and to control the use of recreational and perform-
ance enhancing drugs. Improving the quality and
effectiveness of these programs rests on the use of
practical and affordable methods of analysis. There
are two general strategies in use, broadly classified
as multiresidue methods and the pyramidal approach.
Multiresidue methods allow detection and quantita-
tion of as many different analytes as possible in a
single analysis. The increasing number of substances

abused or potential contaminants makes the development of suitable multiresidue methods difficult. Most of these methods, in any case, tend to be single class procedures while multiclass analysis is becoming of increasing importance. They also tend to be target substance directed, in general, and inefficient for analysing samples, for example, with biological activity, when no obvious compound class is defined as the cause. River water, for example, could be contaminated with any number of crop-protecting agents belonging to different compound classes.

The pyramidal approach is based on the distinct steps of screening, to identify suspect samples, and confirmation, to establish the contaminant level of the suspect samples only. This approach requires the use of a low cost, high sample throughput screening method combining sensitivity (defined by established minimum allowed residue limits) and a low level of false positive with no false negative results. The screening methods may be qualitative indicators of contamination since samples identified as containing contaminants subject to control are then analyzed in a second confirmatory method by an approach that is optimized for the analysis of the suspect contaminant. Planar chromatography is well suited to the demands of a screening method. Single use of the stationary phase minimizes sample preparation requirements, parallel separations enhance sample throughput, ease of post-chromatographic derivatization by selected chemical reactions (singularly or in sequence) improves selectivity and specificity of the analysis, and several screening protocols for different analytes can be carried out simultaneously. Some examples will follow to demonstrate the value of planar chromatography as a screening method in large-number sampling programs.

Cost and sample throughput are always important considerations in surveillance programs. In the real world restricted budgets dictate the extent to which a program can be implemented to protect the public health. Also, in surveillance programs it is usual for the majority of samples to contain no contaminants and it has to be asked whether the resources devoted to establishing negative results might be better spent discovering more positive results. In practice, solvent management is important, not only as a direct cost, but because concentrating sample extracts and changing solvents in a method sequence are general-

ly time consuming and sources of method variability. This dictates the use of small samples, for example, 1–5 g of tissue, requiring only a few milliliters of solvent for extraction and favors the use of solid-phase extraction for isolation, concentration, and solvent exchange. Solid-phase extraction requires low solvent volumes for analyte recovery which can be applied in total (if sample splitting for sequential screening is not required) to the TLC plate. In addition, solid-phase extraction allows parallel sample processing, and like TLC is a high sample throughput method. Using small sample sizes, even if most of the extract is taken for analysis, does mean that sensitive detection techniques are required for identification since minimum allowed residue limits are often set at very low amounts (low parts per billion based on sample concentration). Many of the planar chromatography methods rely on fluorescence detection to achieve the required identification limits, and since only a small fraction of regulated contaminants are naturally fluorescent, ease of exploiting sensitive and selective derivatization reactions is of considerable importance.

Modern farming methods employ veterinary drug treatments to prevent or cure animal diseases or to promote growth. Sulfonamides [18–21] and quinolones [21] are effective for treating various bacterial infections in food-producing animals but should be used in such a manner that prevents significant residues from appearing in food for human consumption. The tolerance limit for sulfamethazine in pig meat in several countries, for example, is set at 0.1 mg/kg [18]. To detect sulfamethazine, tissue samples (5 g) are homogenized in water, the sulfonamides extracted by reversed-phase solid-phase extraction, and extracts cleaned up on a tandem acidic alumina and anion-exchange minicolumn. After separation by TLC sulfamethazine is visualized by reaction with fluorescamine. This method is rapid (one analyst can complete 12 samples in 8 h), uses little solvent (about 20 ml per sample for extraction and chromatography), and is sensitive (detection limit is about 0.25 ppb with a linear range for quantitation of about 2–15 ppb). Fig. 6 illustrates the detection of sulfamethazine at the 2.2 ppb level in pork. Abjean has compared the results for a pyramidal approach to the determination of sulfonamides in animal tissues with the results from
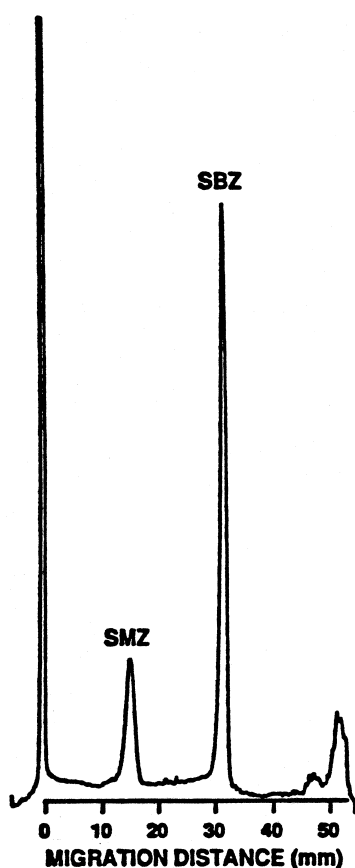
Fig. 6. Determination of sulfamethazine (SMZ) in pork tissue by aqueous extraction and solid-phase isolation and cleanup before thin-layer chromatography with fluorescence detection (λ_{ex}=366 nm and λ_{em}>400 nm) of the derivative formed by postchromatographic treatment with fluorescamine. Sulfabromomethazine (SBZ) is used as an internal standard (10 ppb). (From Ref. [18]; © Association of Official Analytical Chemists International.)

a multiresidue method using HPLC [19]. Three hundred samples were analyzed of which eight were found to contain residue levels above the regulatory limit. Six samples per day could be processed by the multiresidue method requiring 50 days to analyse all samples. Using TLC for screening and the HPLC method for confirmation, 30 samples per day could be analysed and all samples processed in 12 days. Using the combination of TLC screening and HPLC confirmation of positive results no relevant information was lost and positive samples were identified for 20% of the cost of the multiresidue method. In

addition, the combination of sample screening followed by confirmation by a second method provides two independent indications of contaminant identity.

The simultaneous identification of chloramphenicol, nitrofurans and sulfonamides in pork or beef provides an example of a multiclass screening method by TLC [20]. The drugs were identified by homogenization and extraction from 1 g of tissue with ethyl acetate, cleanup of the extract on a silica gel solid-phase extraction cartridge, and separation by TLC. Spraying the plate with pyridine allowed the nitrofurans to be visualized as yellow fluorescent spots on a purple background when visualized at 366 nm. After evaporation of excess pyridine, chloramphenicol and sulfonamides were visualized with fluorescamine as yellow spots on a purple background when visualized at 366 nm. This procedure allows the analysis of 20 samples per day per analyst for three residue classes in a single method. Quinolones are a new class of antimicrobial agents efficacious in the treatment of bacterial infections in poultry and pigs [21]. These drugs can be isolated from tissue by selective solvent extraction, small volume liquid-liquid distribution to remove nonpolar matrix co-extractants and finally solid-phase extraction. Quinolones are naturally fluorescent but treatment with terbium chloride after separation by TLC enhances the sample detection limits to allow identification at the regulatory limit of μg/kg.

The use of anabolic steroids as growth promoters for cattle fattening is prohibited in most countries but this illegal practice is still all too common [22,23]. Extraction, with or without enzymatic digestion to improve recoveries, followed by solid-phase extraction for isolation and sample cleanup (required in some methods) precedes separation by TLC and post-chromatographic derivatization for detection. Diazonium dyes are used for the selective detection of estrogen-like compounds (minimum detection limits of about 12.5 ng or 25 ppb) and acid-catalyzed fluorescence induction for the identification of other steroids [22–24]. Anabolic steroids are identified by comparison of R_F values and the different colored products formed by fluorescence induction.

In forensic toxicology a broad range screening method is required for the rapid identification of individual drug residues in biological fluids from the hundreds of potentially toxic compounds in common

use [25,26]. Drugs are identified by simultaneous TLC separations in standardized separation systems employing (as far as possible) complementary separation mechanisms and by the use of sequential post-chromatographic derivatization reactions providing compound class or functional group information. For example, de Zeeuw et al. [27] have summarized data for over one thousand toxicologically relevant substances in ten standardized TLC systems. The reproducibility of R_F values is improved by co-chromatography of standard substances with unknowns. Substance identification is made by searching substance libraries using a process such as the mean list length to maximize the probability of identification [28]. All substances that migrate in a R_F window that might be confused are ranked and compared across a number of separation systems. If the separation systems have complementary properties the list of possible substances that might be confused should become shorter as an increasing number of substances fall outside the identification window for the unknown. Eventually only a handful of possible substances should remain on the list and identification can then be confirmed by a suitable selective separation method. The certainty of identification can be improved by matching in situ UV spectra with spectral libraries and by using visualization reactions in conjunction with R_F values. Two different TLC systems with low mutual correlation, automated library matching of UV spectra, and appropriately chosen sequential visualization reactions are sufficient in many cases for broad scale screening of drug residues in urine [26,29]. A TLC method has been proposed as a quick screening method for the identification of 13 amphetamine derivatives for official drug control [30]

Ground, raw and drinking water may be contaminated by a large number of crop-protecting agents used in modern agriculture. Not more than 4 or 5 contaminants are likely to be found in individual samples but their identification is difficult because the potential number of compounds and their metabolites is very large. Potential contaminants belong to several different compound classes covering a wide polarity range. It is unlikely that a single multiresidue procedure for routine monitoring of water could be developed in this case. Solid-phase extraction followed by TLC using automated multiple

development is an established screening method for crop-protecting agents in water in Europe [31,32] and has been issued as a German standard [33]. The TLC portion of the method has been adapted for the screening of pesticides in soil [34] and a related method developed for the determination of explosives and their biodegradation products in contaminated soil and water from former ammunition plants [35]. The use of polar solvent gradients together with silica gel column chromatography is difficult because of the long equilibration time required between separations. This is not a problem using incremental multiple development with solvents of increasing strength for each development as implemented in the automated multiple development technique. In addition to selective chemical reactions for substance identification, biomonitoring for toxicity evaluation is straightforward [36,37]. The TLC plate is simply dipped or sprayed with cholinesterase and a substrate to determine cholinesterase inhibition as white spots on a violet background or dipped into a suspension of luminescent bacteria with toxic substances revealed as dark zones resulting from the reduced bioluminescence. Detection limits from nanograms to picograms per separated zone are obtained. Combining biomonitoring with chromatography is becoming increasingly important for evaluating the toxicity of complex mixtures as a means of focussing the analytical effort on the relevant components of the mixture.

The general procedure for the identification of crop-protecting agents employs a 25 to 35 step gradient with both acid and base modified solvents. Twelve extracts are separated simultaneously along with six different standard mixtures for identification purposes. A typical solvent gradient is shown in Fig. 7 together with a separation of a standard mixture of common contaminants. Multiple-wavelength scanning is commonly used to improve the certainty of identification based on simultaneously matching migration distances and absorbance ratios to those of known substances. Full in situ UV spectra can be searched as well against appropriate libraries or matched to standards run on the same plate. Typical limits of detection are at the low tens of nanograms per sample zone corresponding to a concentration limit of about 50 ng/l for a 500-ml water sample. Screening for 265 pesticides and common metabo-
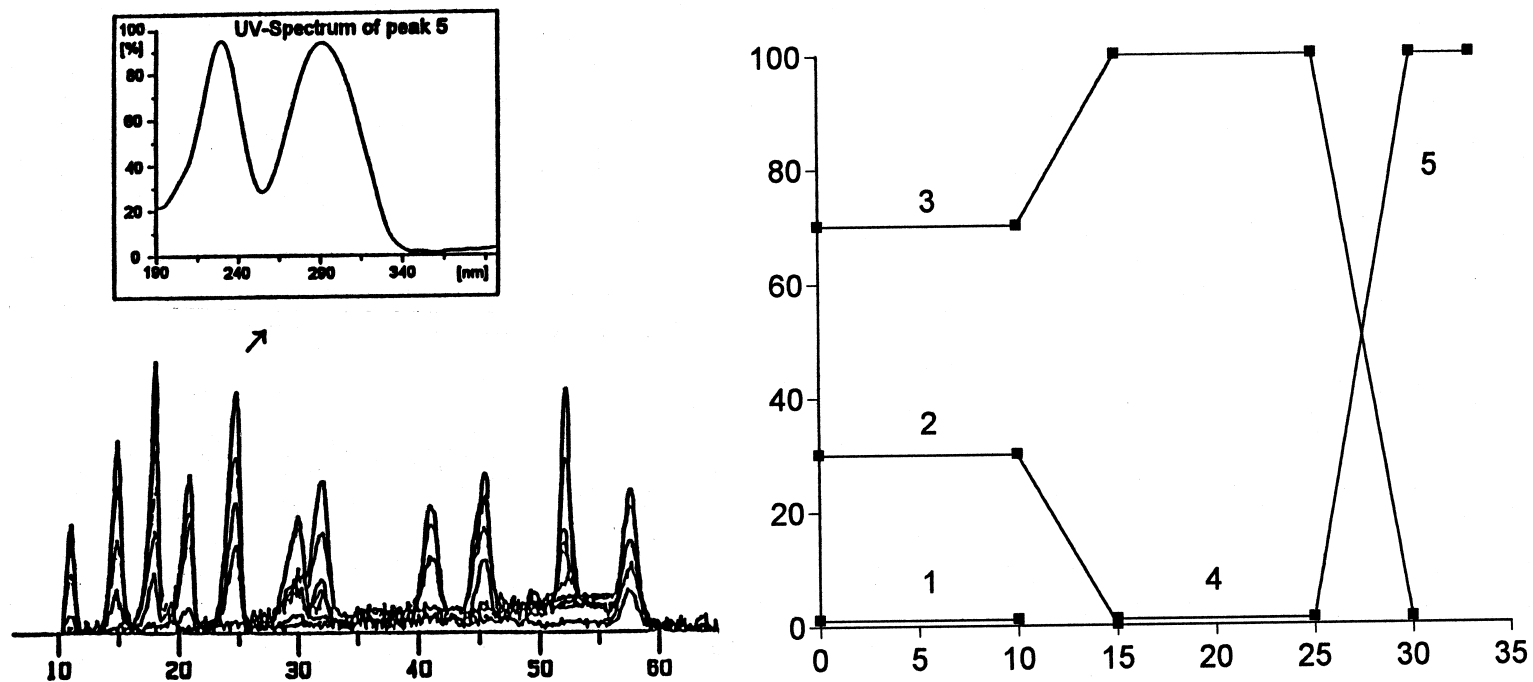
Fig. 7. Separation of a mixture of polar crop-protecting agents (50 ng per component) on silica gel by automated multiple development. The figure shows the gradient profile used for the separation, the use of multiple wavelength scanning for detection and an in situ UV spectra for one peak. Solvent compositions for gradient: 1=aqueous ammonia; 2=acetonitrile; 3=dichloromethane; 4=formic acid; and 5=hexane. (After Ref. [32]; © American Chemical Society.)

lites in water is possible with the standard procedure [32]. Additional solvent gradients are available for the confirmation of substances detected in the screening gradient.

3.4. Use in the pharmaceutical industry

Planar chromatography is the method of choice in the pharmaceutical industry for the analysis of complex and dirty samples with poor detection characteristics or containing impurities that remain adsorbed to the stationary phase [38,39]. Planar chromatography is also useful for the analysis of samples in large numbers that are unsuitable for automated column liquid chromatography, such as studies of content uniformity or stability testing. Renger [40] has demonstrated that the typical costs

for the assay of theophylline tablets and for content uniformity of methscopolamine tablets by HPLC exceed those of TLC by a factor of 6 to 8, and requires three times the number of analysts. A number of authors have demonstrated the validation of pharmaceutical assays conforming to regulatory requirements, including system suitability and ruggedness tests [41–45].

Another classical field of application of planar chromatography is assays and purity tests of plant extracts (phytopharmaceuticals) [40,46,47]. Fig. 8 illustrates the separation of forskolin derivatives isolated from the roots of the *Colens forskohlii* Briq family using automated multiple development and post-chromatographic treatment with chlorosulfonic acid for absorbance detection at 365 nm [47]. This method is used for the assay of crude *Coleus forskohlii* extracts to establish their pharmacological

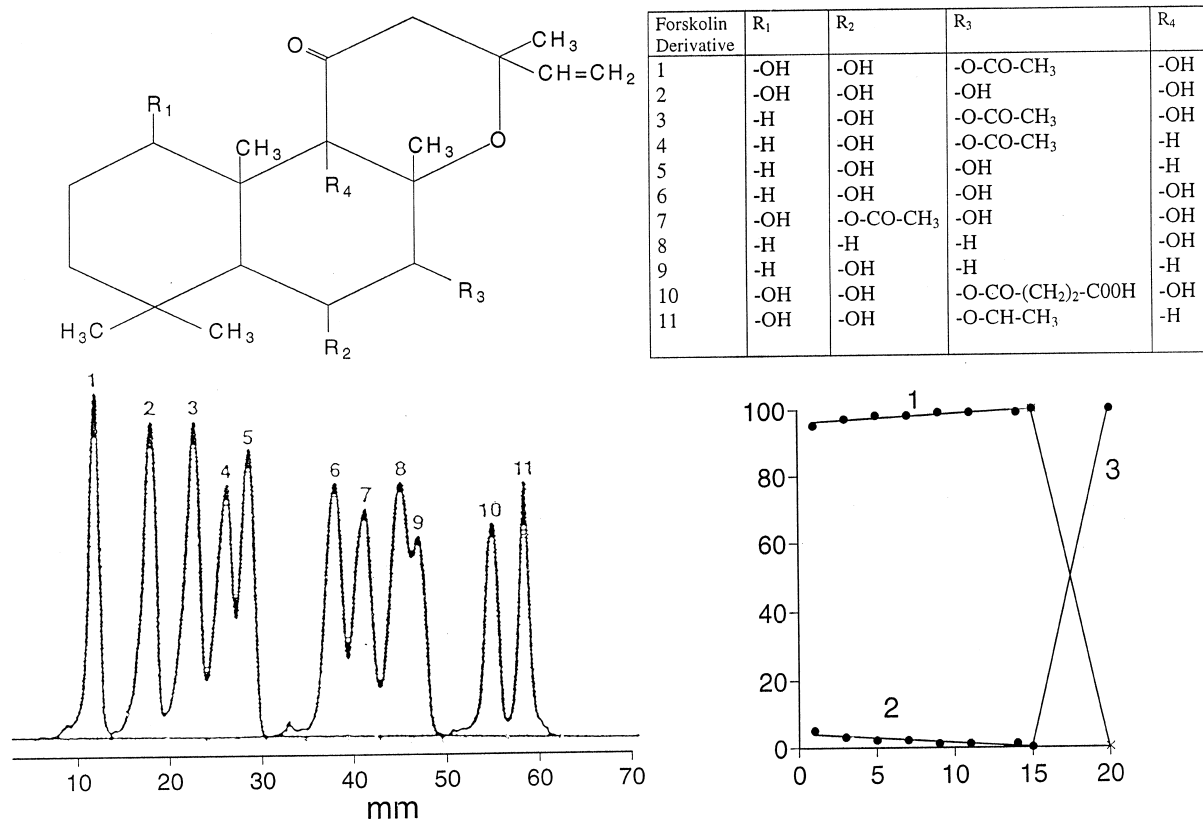| Forskolin Derivative	R₁	R₂	R₃	R₄
1	-OH	-OH	-O-CO-CH$_3$	-OH
2	-OH	-OH	-OH	-OH
3	-H	-OH	-O-CO-CH$_3$	-OH
4	-H	-OH	-O-CO-CH$_3$	-H
5	-H	-OH	-OH	-H
6	-H	-OH	-OH	-OH
7	-OH	-O-CO-CH$_3$	-OH	-OH
8	-H	-H	-H	-OH
9	-H	-OH	-H	-H
10	-OH	-OH	-O-CO-(CH$_2$)$_2$-COOH	-OH
11	-OH	-OH	-O-CH-CH$_3$	-H

Fig. 8. Separation of forskolin derivatives on a silica gel HPTLC plate by automated multiple development. The solvents for the gradient are: 1=dichloromethane; 2=methanol; and 3=hexane. (After Ref. [47]; © Elsevier.)

potency. The increasing use of herbal products as over-the-counter remedies and folk medicines has increased the use of TLC for the determination of active ingredients and to establish content uniformity and potency. Planar chromatography is unmatched in its ability to provide finger print profiles of complex crude botanical extracts yielding suitable information for quality control purposes. Re-evaluation of the chromatogram under different conditions and the use of selective derivatization reactions enhance the information available. Detection by image analyzers has increased in popularity because of their ability to provide suitable records for archiving, comparison and retroactive searches under software control. The ease of coupling planar chromatography with in situ bioassays for potency or activity testing and for efficient activity-guided isolation of natural products is also a significant trend.

Lipids are used as multifunctional excipients or active ingredients in pharmaceutical products. They are complex mixtures of wide polarity analyzed by class fractionation, most commonly, and occasionally by interclass separations for speciation. Most lipids have no convenient UV chromophore and one advantage of planar chromatography is the availability of simple and suitable detection techniques. Dipping or spraying with a phosphoric acid solution of copper (II) sulfate followed by heating produces brown-violet spots that can be quantified by scanning densitometry [48]. Automated multiple development techniques have lead to improved separations of lipid classes and are in the process of replacing conventional one and two-dimensional methods [48–51]. The complexity of natural lipid samples will never yield to a single analytical method but TLC should retain its prominent position as one of several common tools for their characterization and for quality control of formulated products.

An increasing number of enantiomeric pharmaceutical compounds have been separated by TLC using chiral stationary phases, phases modified by coating with a chiral selector, and chiral mobile phases [52–55]. Methods employing mobile phase additives such as β-cyclodextrins and bovine serum albumin are simple to apply and interest is growing in the use of chiral stationary phases based on modified celluloses. Compared to column liquid chromatography the proportion of enantiomer sepa-rations performed by TLC is small but the potential for increasing use has been demonstrated.

3.5. Substrate for spectroscopy

There are three factors of particular interest in the use of TLC plates as substrates for spectroscopy. First of all there is the need of TLC itself for efficient in situ detection and identification techniques to support its general use in analysis. Secondly, there is the opportunity to exploit spectroscopic techniques that cannot be used in liquid chromatography because of time constraints, solvent incompatibility, or special needs. Lastly, there is the possibility of coupling column chromatography and TLC with the layer acting as a storage device for the column chromatogram for off-line spectroscopic analysis. Note that in this latter case the TLC plate is not used for separation but functions as a repository of the chromatographic information from the column chromatogram. Truly multimodal separations incorporating separation of the stored column chromatogram by TLC are discussed in Section 6. The particular merits of TLC as a substrate for spectroscopy are that after separation or deposition the whole chromatogram is immobile and available for analysis free of time constraints. Spectroscopic evaluation takes place in the absence of solvent (but in the presence of sorbent of course) and provided that the spectroscopic techniques are nondestructive, multiple evaluations under different conditions, techniques or even different locations, are possible without having to repeat the separation. Expensive instruments need not be dedicated to a single project but can be shared among projects more efficiently because the separation and detection step are performed off-line with the layer acting as a storage device for the chromatographic separation until the spectroscopic instrument is available. A more detailed account of spectroscopic evaluation of TLC chromatograms is available in recent review articles [55–57] and only some brief highlights will be discussed here.

Modern slit-scanning densitometers provide access to the complete UV–visible absorption spectrum of compounds separated by TLC. The amount of substance required for an interpretable spectrum depends on the chromatographic conditions and the absorp-

tion coefficient for the compound. Typically this will be in the range of 0.01–1.0 μg. Absorption spectra are useful for confirming the possible identity of expected substances by matching to library spectra under software control and as a means of determining the presence of interfering co-migrating substances. Absorption spectra, however, contain little structural information and are not generally useful for elucidating the structure of unknown compounds. Fluorescence is a more selective technique, and for compounds capable of fluorescence, interpretable spectra can be recorded from subnanogram amounts. Slit-scanning densitometers, however, are designed for sensitivity and not spectral resolution. Optical filters are commonly used for isolation of the emission spectrum [58,59]. This provides limited opportunities for structural elucidation. Full excitation and emission spectra of TLC zones can be obtained using conventional fluorescence spectrometers equipped with a plate-scanning accessory. Fluorescence line narrowing spectroscopy provides vibrationally resolved fluorescence emission spectra suitable for distinguishing between isomeric and structurally similar fluorescent compounds [57,60,61]. Fluorescence line narrowing spectroscopy requires cryogenic temperatures (<30 K) and is a slow scanning technique unsuitable for direct coupling to column liquid chromatography. Deposition of the column chromatogram onto the TLC layer, with or without further separation, provides a suitable mechanism for spectral measurements.

The on-line coupling of column chromatography and Fourier transform infrared spectroscopy (FT-IR) is limited by the strong absorption of common chromatographic solvents and has poor sensitivity because cells with a short optical pathlength are required. Signal averaging to improve sensitivity is not possible because of the short residence time of the compound in the flow cell. The spectral information that can be obtained is restricted generally to spectral windows where interference from strong solvent absorption bands is absent. Solid-phase spectra recorded after solvent elimination are preferred for structural elucidation [3]. In situ infrared spectra can be obtained from TLC plates using a commercially available scanning diffuse reflectance Fourier transform infrared (DRIFT) spectrometer [62–64]. The inherent absorption of silica gel results in

intense interference bands located between 3700–3100 cm^{-1} and 1600–800 cm^{-1} superimposed on to the DRIFT spectra of the absorbing substances. The mid-infrared absorption band is absent in weakly absorbing metallic oxides which, in turn, tend to have higher infrared reflectivity than silica gel but poor sorption properties for chromatography. A new layer containing a mixture of magnesium tungstate and silica gel improves detection limits about 3 fold due to its higher reflectivity and improves the quality of DRIFT spectra in the mid-infrared region extending the operating window down to about 1270 cm^{-1} [65]. Due to interactions between analyte and the TLC layer, small but significant changes in position and shape of bands may occur. Some of these changes can be related to proton-transfer interactions with poly(acrylate) binders [66]. Reliable identification usually requires comparison to reference libraries containing spectra for the adsorbed species. Usually a microgram or less of substance will provide an adequate spectrum for identification purposes and low nanogram amounts for detection based on absorption by characteristic functional groups.

The Raman spectrum of a substance is derived from fundamental molecular vibration modes. Like the more familiar infrared spectra, it can be interpreted to provide structural and functional group information. Typical TLC sorbents, such as silica gel, have favorable properties for in situ recording of Raman spectra of adsorbed compounds. On the other hand, the normal Raman signal is very weak and the sensitivity too low for regular use. In certain cases, however, it is possible to significantly enhance the Raman signal by making use of resonance and surface effects. Surface enhanced resonance Raman (SERRS) spectra can be obtained from nanogram and lower amounts of adsorbed substances [67–69]. This requires matching the excitation laser with the absorption properties of the compound combined with application of a silver sol to the substance adsorbed on the layer. Signal averaging is another method applicable to improving spectral quality from small amounts of adsorbed substances. Recording SERRS spectra in a column effluent is fraught with problems and transferring the column chromatogram to a TLC layer prior to application of the silver sol and spectral recording is a better approach [57,69].

Just about two decades of effort have resulted in routine instruments for on-line coupling of column liquid chromatography and capillary electrophoresis with mass spectrometry. By comparison the coupling of TLC with mass spectrometry has not reached the same level of maturity [55,56,70–73]. The obstacles to the construction of a routine scanning mass spectrometer for TLC are different to those encountered for column separations. Dynamics are not important because the sample is static and there is no need to separate analytes from the liquid phase. As part of the changing face of coupling liquid phase separations to mass spectrometry the solvent has shifted from being the principal problem to part of the solution. On the other hand substance zones in TLC are separated in space, are immobile, and are adsobed to the stationary phase. For true detection and identification two-dimensional spatial imaging of the layer and sample transfer to the vapor phase without degrading the chromatographic resolution or mass spectrometer sensitivity are required. Current methods of interfacing TLC to mass spectrometry accomplish this to various levels of satisfaction using fast atom bombardment (FAB), liquid secondary-ion mass spectrometry (SIMS), matrix-assisted laser desorption/ionization (MALDI) [71], surface-assisted laser desorption/ionization (SALDI) [72], and electrospray [73]. These techniques provide relatively low sample ionization efficiency. To compensate for this the interfaces employed attempt to provide high sample extraction efficiency by use of a mobile transfer matrix to concentrate the analyte at the surface of the layer. Viscous solvents or low melting point solids applied to the layer prior to spectral recording are used for this purpose. Sputtering processes are then employed to promote the sample into the vapor phase. When a solid transfer matrix is used the energy from the sputtering process is used to melt the solid in the region selected for analysis while the remainder of the chromatogram is stabilized by the solid transfer matrix. The liquid matrix (or melted solid) improves sensitivity and persistence of the ion current for long periods by continuously replenishing the surface concentration of the analyte. In the case of electrospray a surface probe and a hydrophobic barrier are used to extract the sorbed analyte from a defined area of the layer (corresponding to part of a single separated zone)

and transport the solution to the electrospray ionization region. All these ionization techniques are soft ionization methods, producing molecular ions or molecular adduct ions with little accompanying fragmentation. Structural information for identification purposes is obtained by tandem mass spectrometric techniques that are now routine procedures [74]. Further work is needed to provide the type of routine instrumentation suitable for general laboratory use but progress made over the last few years has been impressive and it seems only a matter of time before these demands are met. Coupling of TLC to mass spectrometry has already been applied to a wide range of sample types including drugs and their metabolites, pesticides, natural products, synthetic chemicals and dyes [56]. In most cases suitable spectra for identification purposes were obtained from sample amounts in the range of 1–1000 ng depending on the instrument and interface used. It is also a notable achievement that a significant number of the samples analyzed have been those for which conventional detection by scanning densitometry is difficult due to weak analyte UV absorption. In time, mass spectrometry may provide a complementary approach to optical detection for general use in TLC as well as the main tool for identification purposes.

4. Approaches to kinetic optimization and increased zone capacity

It is now widely appreciated that separation efficiency and separation capacity in modern TLC are primarily limited by the inadequate velocity of the mobile phase driven solely by capillary forces [7,8,75]. Two features of this phenomenon are that the mobile phase velocity is spatially dependent and declines (approximately quadratically) with migration distance of the solvent front and that the maximum velocity attainable at any position on the plate is less than the velocity required for optimum efficiency. For common solvents and normal development distances the mobile phase velocity will be between 0.02 and 0.005 cm/s while the optimum mobile phase velocity for modern HPTLC plates is about 0.05 cm/s [76]. The consequences of inadequate mobile phase velocity are that zone broadening is largely dominated by molecular diffu-

sion and useful development distances for a separation are established by the range of acceptable mobile phase velocities.

A further consequence of the quadratic decrease in mobile-phase velocity in TLC is that zones are forced to migrate through regions of different local efficiency and the plate height for the layer must be specified by an average value. A plot of the average plate height as a function of the solvent front migration distance, Fig. 9, reveals several important features relating to the optimum separation conditions [7]. Firstly, there is a dominant relationship between the average particle size, development length, and zone capacity under capillary flow controlled conditions. High performance layers (nominally 5-μm particle size) produce more compact zones provided that the solvent front migration distance does not exceed 5–6 cm. At longer solvent front migration distances the mobile phase velocity declines to the point where zone broadening exceeds zone center migration. Longer solvent front migra-

tion distances require layers with a larger particle size to obtain a reasonable range of mobile phase velocities but because their intrinsic efficiency is less than that of the fine particle layers the total number of theoretical plates produced is lower, if not by a large amount. The virtue of the HPTLC layer is that it requires shorter migration distances to achieve a given efficiency, resulting in faster separations and more compact zones that are easier to detect by scanning densitometry. In reality separations obtained under capillary flow controlled conditions are limited to a maximum of about 5000 theoretical plates (the actual number of theoretical plates encountered by a zone depends on its migration distance and is always less than the maximum value). The zone capacity for baseline separated peaks in the chromatogram is about 12–14, and this is not strongly dependent on the average particle size of the layer. Given that the flow resistance and packing structures of modern layers can be judged as adequate for optimum performance [77,78] as long
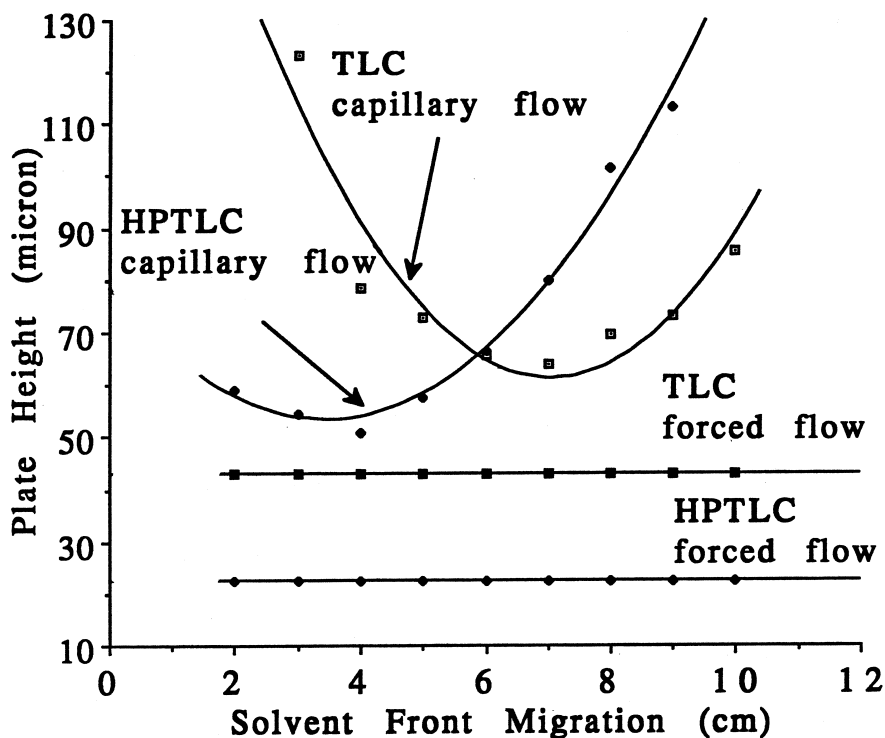

Fig. 9. Variation of the average plate height as a function of the solvent front migration distance for conventional and high-performance TLC layers with capillary controlled flow and optimized constant flow (forced flow) development. (From Ref. [7]; © Elsevier.)

as capillary forces are responsible for controlling the movement of the mobile phase, the only way forward is to explore procedures that circumvent the limitations of capillary forces. Conceptually an obvious solution is forced flow development with control of the mobile phase velocity established by pneumatic or electroosmotic movement of the mobile phase through the layer. A further viable approach is to retain capillary forces for migration of the mobile phase but employ a zone refocusing mechanism to counteract natural zone broadening. This is the basis of high-performance separation methods using multiple development techniques. Lastly, optimization of the zone capacity can be achieved by using two-dimensional development in which different sequential retention mechanisms are applied in orthogonal directions to spread the separation over the surface of the layer. A comparison of these approaches follows.

4.1. Forced flow development

For forced flow development an external force is required to move the mobile phase through the layer, preferably at a constant velocity corresponding to the optimum mobile phase velocity, or any other selected velocity. This is achieved by enclosing the open side of the layer by a membrane forced into intimate contact with the layer by an external pressure source together with a metering pump to force mobile phase through the layer, much like in column liquid chromatography [55,79–82]. Equipment for this purpose falls into two categories depending on the external force used to maintain contact between the membrane and the layer. A polymeric membrane and a hydraulic water cushion at up to 50 bar is used in the personal OPLC chamber while in other systems using a metal membrane and a hydraulic press, pressures up to 500 bar have been used [83–86]. The alternative to pneumatic force is centrifugal force embodied in rotation planar chromatography [87,88]. Rotational planar chromatography has proven popular for preparative-scale applications but generated only limited interest for analytical separations.

Fig. 10 illustrates the variation of the reduced plate height as a function of the reduced mobile phase velocity for a column and a typical HPTLC layer with plate height measurements made in the on-line mode (much the same way as column
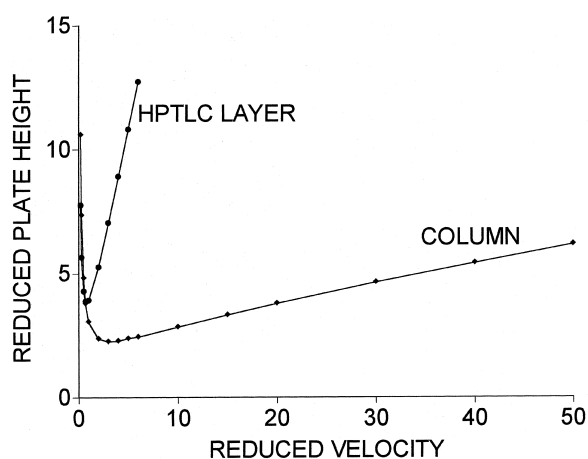

Fig. 10. Plot of the reduced plate height against the reduced mobile phase velocity for a typical high-performance TLC layer and HPLC column.

measurements are made). The optimum reduced mobile phase velocity is shifted to a lower value compared to the column and the minimum in the reduced plate height ($\approx$3.5) is higher than the best column values ($\approx$2.0–2.5). Also, at higher reduced mobile phase velocities the reduced plate height for the layer is significantly larger than the values observed for a typical column. Contributions to the plate height from flow anisotropy for HPTLC layers are similar to column values, suggesting a good packing structure, with the main difference being the contribution to the plate height from resistance to mass transfer [75,77]. The latter is typically an order of magnitude larger for layers than for columns. The larger value for the resistance to mass transfer contribution to the plate height might be due to either restricted diffusion within the porous particles or different rates of sorption for the silica gel and silica gel coated with binder. As a consequence, separations by forced flow development will be slower than those achieved with columns and fast separations at high flow rates will be much less efficient. Some expectations for separations by forced flow development for different scenarios are summarized in Table 2. For a typical development distance of 18 cm a modest increase in performance (a maximum of 8000 theoretical plates) in a credible time of 9 min is achieved for forced flow development compared to results for capillary flow controlled systems. Really

Table 2
Performance characteristics of forced flow TLC. Assumptions: viscosity$=3.5\cdot10^{-4}$ N s/m^2 and solute diffusion coefficient$=2.5\cdot10^{-9}$ m^2/s[a]

Development time (min)	Pressure drop (atm)	N_{max}	Development length (cm)
(a) *HPTLC (optimum conditions $h=3.75$, $\nu=0.8$, $\phi=800$ and $d_p=6$ μm)*			
4	2.1	3550	8
9	4.7	8000	18
25	12.9	22 200	50
50	25.8	44 400	100
(b) *HPTLC (fast development option $h=9$ and $\nu=5$)*			
0.6	12.9	1480	8
1.4	29.1	3330	18
4.0	80.7	9250	50
8.0	161.0	18 500	100
(c) *HPTLC ($d_p=3$ μm; other parameters as in (a))*			
2.0	16.5	7610	8
4.5	37.2	17 100	18
12.5	103	47 600	50
25.0	207	95 200	100
(d) *Conventional TLC ($h=4.5$ and $\nu=0.8$, $\phi=600$ and $d_p=9$ μm)*			
6	0.44	1980	8
13.5	1.03	4450	18
37.5	2.9	12 350	50
75	5.7	24 700	100

[a] $h=$reduced plate height, $\nu=$reduced mobile phase velocity, $\phi=$flow resistance parameter, and $d_p=$average particle size, 1 atm$=101\ 325$ Pa.

significant increases in efficiency are achieved only by the use of longer bed lengths at the expense of separation of time. This can be achieved by the serial coupling of conventional size layers arranged in a stack with a special tail to head vertical connection between layers [89–91]. The rather low optimum mobile phase velocity means that long development lengths are possible with relatively low and practical pressure requirements. As expected, fast development with modern layers results in relatively low efficiency and high pressures are required if long development lengths are used. These conditions are not useful for most separations, especially since layers permit separations to be carried out in parallel, so the relative time per separation is not adversely impaired compared to column techniques by the use of lower mobile phase velocities. Reducing the average particle size for the layer from 5 μm to 3

μm provides a significant improvement in efficiency with a favorable separation time but is more demanding in terms of operating pressure. To provide a realistic increase in separation capacity layers with a smaller particle size are required, and if long development distances are to be employed, then instrumentation capable of operating at higher pressures than those commercially available will also be needed. Layers prepared from 3 μm particles were available at one time but proved unattractive for general use when capillary controlled flow was used for development [85,92]. Alternatively, new layers with lower resistance to mass transfer properties would result in a significant improvement in separation time and a useful increase in efficiency. This requires a greater understanding of the influence of the binder on the kinetic properties of layers and perhaps the use of higher quality silica gel for layer preparation. New layers prepared from spherical silica particles have been announced but have not been fully evaluated so far [93].

4.2. Electroosmotic flow

Electroosmotic flow represents an alternative mobile phase transport mechanism to pneumatic forced flow development for TLC. Early studies by Pretorious et al. [94] went largely unnoticed until electroosmotic flow was demonstrated to be a viable transport mechanism for column liquid chromatography [95,96]. Driving this interest is the possibility of exploiting columns with a higher intrinsic operating efficiency and of longer lengths than is possible for pressure driven flow. Electroosmotic forces provide a plug flow profile reducing transaxial contributions to peak broadening associated with pressure driven flow. In addition, since the mobile phase velocity is independent of particle size (in the absence of double layer overlap) it should be possible to sustain an optimum mobile phase velocity in longer columns than is possible for pressure driven flow. The ultimate performance of the chromatographic system is set by radial dispersion resulting from Joule heating. Theoretical calculations show that similar advantages should exist for TLC with instrument and operating requirements that are not extreme [86]. Preliminary studies of electroosmotic flow in TLC have produced mixed results [97,98].

Only a moderate increase in mobile phase velocity above that expected for capillary flow was obtained and, in addition, the mobile phase velocity was not constant but declined with migration distance. Although improved performance was demonstrated this was a lot less than predicted by theory. More work is required to truthfully evaluate the possibility of exploiting electroosmotic flow as a general transport mechanism in TLC. Success in this endeavor would provide the simplest and preferred solution to optimizing the kinetic characteristics of TLC separations.

4.3. Unidimensional multiple development

Multiple development is a complementary approach to forced flow development for increasing the separation performance of TLC [7,80,99–101]. All unidimensional multiple development techniques, Table 3, employ successive repeated development of the layer in the same direction with removal of mobile phase between developments. Approaches differ in the changes employed at each development, such as the solvent front migration distance and the mobile phase composition, and in the total number of successive development steps employed. Adequate theory allows the calculation of the migration posi-

tion and zone width as a function of the number of development steps [101–103]. Capillary forces are responsible for migration of the mobile phase but a zone refocusing mechanism counteracts the normal zone broadening. Each time the solvent front traverses the stationary sample in unidimensional multiple development it compresses the zone in the direction of development. The compression occurs because the mobile phase first contacts the bottom edge of the zone where the sample molecules start to move forward before those molecules still ahead of the solvent front. Once the solvent front has reached beyond the zone, the refocused zone migrates and is broadened by diffusion in the usual way. Under optimum conditions a balance is struck between the zone refocusing and zone broadening mechanisms. In this case it is possible to migrate a zone a considerable distance without significant zone broadening beyond that observed in the first few developments, Fig. 11. Unidimensional multiple development with changes in the mobile phase composition at each or several of the development steps is the basis of automated multiple development. Theory and practice indicate a zone capacity for automated multiple development between 30 and 40, which is comparable to expected values for forced flow development, Table 4. Automated multiple

Table 3
Multiple development techniques

| Method	Features
Multiple chromatography	· Fixed development length · Same mobile phase for each development · The number of developments can be varied
Incremental multiple development	· Variable development length (a) First development is the shortest (b) Each subsequent development is increased by a fixed distance (c) Last development length corresponds to the maximum useful development distance · Same mobile phase for each development · The number of developments can be varied
Increasing solvent strength gradients	· Fractionates sample into manageable subsets · Optimizes separation of each subset · Complete separation of all components is not achieved at any segment in the development sequence
Decreasing solvent strength gradients	· Uses incremental multiple development · First development employs the strongest solvent with a weaker solvent for each subsequent step · Final separation recorded as a single chromatogram
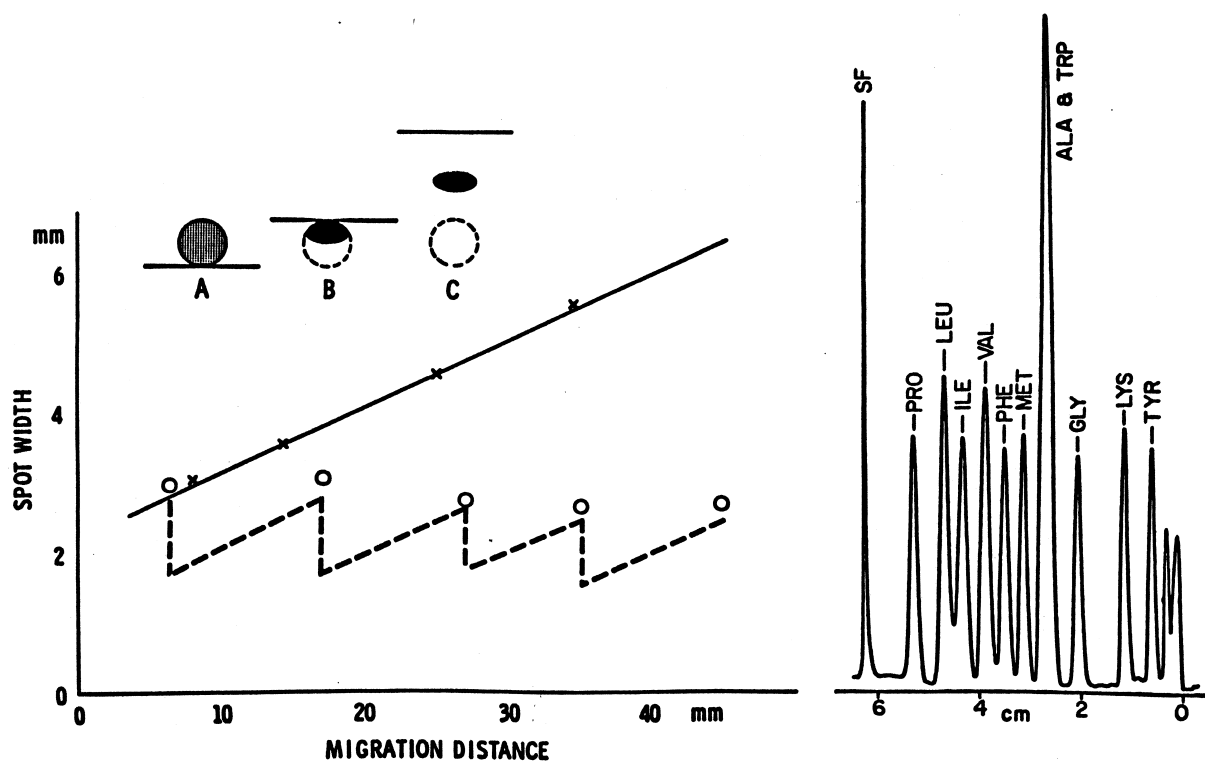
Fig. 11. Illustration of the zone refocusing mechanism (left) and its application to the separation of a mixture of phenylthiohydantoin-amino acids (right). The broken line on the left-hand side of the figure represents the change in spot size due to the expansion and contraction stages in multiple development and the solid line depicts the expected zone width for a zone migrating the same distance in a single development. (After Ref. [99]; © Research Institute for Medicinal Plants.)

Table 4
Zone capacity calculated or predicted for different conditions in TLC

Development	Dimensions	Zone capacity
(i) Predictions from theory		
Capillary controlled flow	1	<25
Forced flow	1	<80 (up to 150 depending on pressure limit)
Capillary controlled flow	2	<400
Forced flow	2	several thousand
(ii) Based on experimental observations		
Capillary controlled flow	1	12–14
Forced flow	1	30–40
Capillary controlled flow (AMD)	1	30–40
Capillary controlled flow	2	≈100
(iii) Predictions based on results in (ii)		
Forced flow	2	≈1500
Capillary flow (AMD)	2	≈1500

development under forced flow conditions could provide a significant improvement in zone capacity with shorter separation times, but remains to be tested. Using two successive forced flow developments with different mobile phase compositions indicated the possibility of reducing the separation time [104] but a significant increase in zone capacity would require a larger number of development steps with a mobile phase containing segments of migrating and immobilizing solvents.

4.4. Two-dimensional development

Two-dimensional separations in planar chromatography are rather trivial to perform even if the general results obtained so far have not matched predictions from theory [7,105–107]. Theoretical results are somewhat inflated because assumed values for layer properties in the models are not in agreement with measured values. We have taken care of this in Table 4 by presenting the theoretical values and then some estimates based on observed values for mainly one-dimensional systems. Even if the estimated values seem to fall far short of the theoretical values the increase in zone capacity for two-dimensional systems is significantly larger than for a single, unidimensional development. The realization of a more efficient separation system implies that the resolved sample should be distributed over the entire surface of the layer. This can be achieved only if the selectivity of the separation mechanism is complementary in the orthogonal directions. The general difficulty in devising separation systems of high zone capacity has been the problem of identifying suitable complementary retention mechanisms for the two separation dimensions. Most results found in the literature employ different solvent systems for the two dimensions resulting in differences in intensity rather than true orthogonality. In these cases sample components become distributed along the diagonal between the two development directions and a significant portion of the separation space remains unused. There are approaches that should be more suitable using bilayer plates and chemically bonded layers that adapt to different retention mechanisms depending on the properties of the mobile phases employed for the separation [7].

The zone capacity would be even greater in two-dimensional planar chromatography if zone broadening was minimized by using forced flow development and multiple development techniques exactly as outlined for unidimensional development. Early pioneering work on forced flow two-dimensional development was carried out by Guiochon and co-workers [108,109] using layers without binders, which the authors referred to as bidirectional chromatography. The complexity of the apparatus and the difficulty of on-line detection in the elution mode (since zones are now separated in time and space) resulted in diminished interest in this approach. Advances in technology since that time would make the detection process less formidable and it may be time to explore this approach again. There are fewer technological problems in combining two-dimensional development with the overpressured development chamber and in situ detection. The probable zone capacity would be lower but still sufficiently large to represent a significant advance over one-dimensional separations, Table 4. Indeed, several results have been reported for the separation of mixtures best classified as complex because of the structural similarity of their components rather than because of the total number of components they contain [110–113]. Even less work has been done using the multiple development zone refocusing mechanism to enhance the zone capacity of two-dimensional TLC [114,115]. Whether performed manually or automated, separations will be slow because of the large number of development and intermediate drying steps usually required, but the potential for a significant increase in the zone capacity is apparent, Table 4.

The acceptance of two-dimensional planar chromatography for quantitative analysis is very much dependent on providing a convenient method for in situ quantitative detection and data analysis. Conventional slit-scanning densitometers are designed for evaluation of separations contained within a single track. Adaptation to scanning two-dimensional separations has been demonstrated using normal scanning operations with small steps between scans or by zigzag scanning. Special software is required to map the whole surface of the layer and define zone dimensions as x and y coordinates, and optical density, as z coordinate, as a three-dimensional plot or contour diagram [7,116,117]. Mechanical scan-

ning of the TLC plate is slow by whatever means adopted, and it is unclear whether meaningful quantitative data can be obtained for routine analysis [7]. Liquid SIMS [118] and radioimaging detectors [119–121] for analytes containing radioactive elements, have also been used to record two-dimensional separations. These approaches require special equipment that is not found in most analytical laboratories and, in the case of radioimaging, is limited to samples that are not commonly encountered in everyday work. They could serve to progress the optimization of two-dimensional separation techniques. The awaited breakthrough in general detection for two-dimensional planar separations is likely to come from optical imaging systems using a video camera or similar device [7,93,117,122,123]. Unlike mechanical scanning techniques, electronic scanning enables images of a surface to be obtained rapidly and individual images to be summed to improve the signal-to-noise ratio. There have been enormous developments in this technology over the past decade but the main limitations for two-dimensional TLC remain the difficulty of evenly illuminating the whole layer with monochromatic light and low sensitivity in the UV region. At the moment, for one-dimensional separations image analysers are less sensitive, even in the visible region, and provide lower resolution for chromatogram recording than modern slit-scanning densitometers. The main attractions of image analysis for detection in TLC are fast data acquisition, simple instrument design, the absence of moving parts, and unique software approaches for archiving and comparing chromatographic images. Significant advances in electronic scanning are expected in years to come and image analysis will likely become the preferred method of densitometric evaluation for one- and two-dimensional TLC separations at some time in the future.

5. Multimodal separation techniques

Multimodal techniques provide another route to multidimensional separations by combining two complementary separation techniques using different separation methods. In this section we will limit our discussion to the coupling of column separation techniques with planar chromatography. Discussion is further limited to automated on-line coupling procedures that provide the added benefits of time and labor saving and minimize sample contamination [7,55,57,107]. The specific reasons for coupling column separations to planar separations in addition to increased separation capacity are to take advantage of the attributes unique to planar separations summarized in Table 1 and discussed in Section 3. The TLC plate functions as a separation and storage device retaining information from the column separation and TLC separation in an immobilized format. This allows the sample components to be further investigated free of time constraints. This is advantageous for biomonitoring, sample components that require derivatization for convenient or selective detection, for sequential scanning using different detection principles or to preserve and transport the separation to different locations for detection, and for applications employing solid-phase spectroscopic identification techniques (see Section 3.5).

Coupling gas chromatography to TLC is not particularly difficult but has been little used since the late 1960s when several interfaces were described [7,107]. Many of the problems for which these instruments were used are solved by gas chromatography–mass spectrometry today and contemporary interest in GC–TLC has declined. In the late 1970s Stahl and co-workers [124,125] described an apparatus for supercritical fluid extraction with deposition of the fluid extract onto a moving TLC plate. More recently Keller and co-workers [126,127] described a suitable interface for direct coupling of supercritical fluid chromatography and TLC. Decompression of the supercritical fluid at a small orifice occurs with rapid cooling, favoring the deposition process while not inhibiting the removal of the fluid as a gas. Efficient transfer to the layer usually requires addition of a solvent to the fluid, however, to avoid loss of high-speed sample particles. Wet particle deposition being much more efficient than dry particle deposition. The coupling of capillary electrophoresis to a substrate for mass spectrometry has been briefly described [57], and presumably could be adapted to TLC. Current interest, however, is largely in the coupling of column liquid chromatography to TLC, which has reached a reasonable level of maturity and commercialization.

5.1. On-line coupling of column liquid chromatography to thin-layer chromatography (LC–TLC)

The most general interface for coupling column liquid chromatography to TLC is based on various modifications of the spray jet sample applicator [7,55,57,128–134]. At flow rates typical for narrow-bore columns (5–100 μl/min) the whole column eluent can be applied to the layer. For higher column flow rates a splitter in the transfer line to the spray jet applicator is required. The column eluent is nebulized by mixing with (heated) nitrogen gas and sprayed as an aerosol onto the layer. The spray head can be moved horizontally on one line within a defined bandwidth or, better, can be made to deposit the spray over a defined rectangular area (e.g. 8×6 mm) to promote more effective solvent evaporation, Fig. 12. In the latter case zone refocusing prior to chromatography is required, such as the mechanism employed in the automated multiple development technique. Multimodal separations can be performed in the profiling or target compound mode. In the former case, the whole column chromatogram as

volume fractions is sequentially transferred to the layer as a series of bands that are subsequently developed in parallel. Scanning individual tracks on the TLC plate corresponding to each transferred fraction can reveal an immense amount of information about sample complexity, Fig. 13. In the target compound mode, fractions identified using the column detector or from elution windows established by marker compounds, are transferred to the layer and stored there until all available space for column fractions is occupied by fractions transferred from different samples.

The main limitations of the spray jet interface are its restricted flow capacity when coupled to conventional diameter columns and inability to handle certain mobile phase compositions containing large amounts of involatile ionic additives, such as buffers and ion-pair reagents. Such problems can be overcome by adding an on-line solid-phase extraction step to concentrate column fractions and to exchange the solvent composition prior to transfer to the TLC layer. Muller and Jork [135] have described a suitable on-line interface for this purpose using an automated solid-phase extraction module compatible
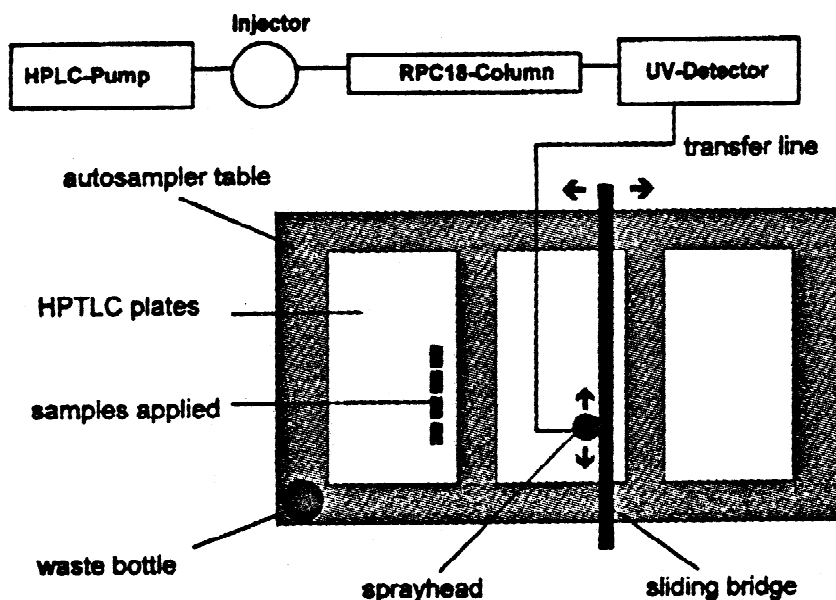

Fig. 12. Schematic diagram of the on-line coupling of a narrow-bore column to TLC using a spray jet interface. (From Ref. [134]; © Elsevier.)
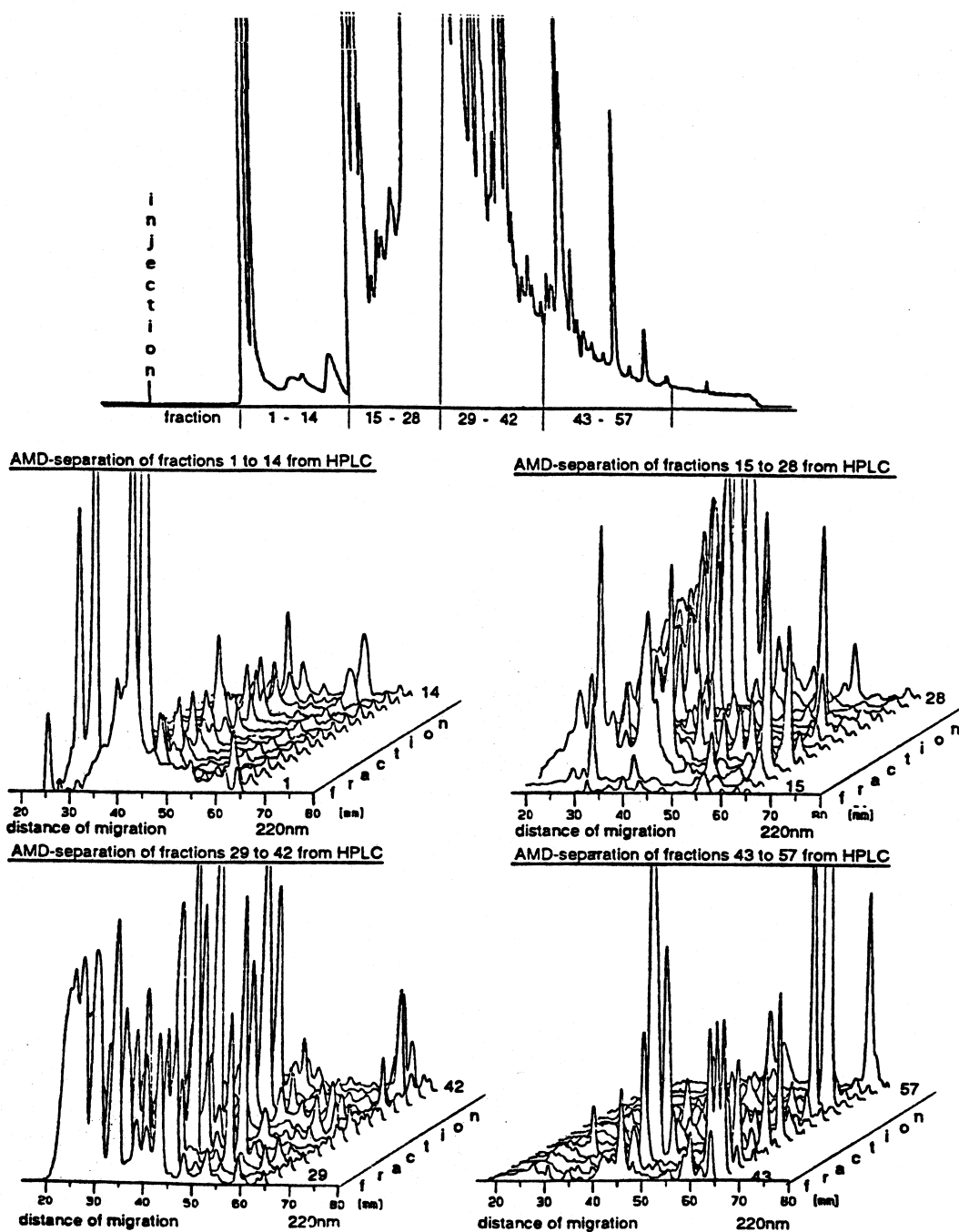
Fig. 13. Separation of contaminants in a wastewater sample by on-line coupling of reversed-phase column gradient liquid chromatography and normal-phase automated multiple development TLC. (From Ref. [67]; © Marcel Dekker.)

with parallel sample processing, Fig. 14. The interface was used for the determination of 4(5)-methylimidazole in caramel, a common coloring agent in foodstuffs. 4(5)-Methylimidazole was isolated from interfering high-molecular-mass ionic compounds by separation on a hydrophobic size-exclusion column with a sodium perchlorate-containing mobile phase. The fraction containing 4(5)-methylimidazole was concentrated by solid-phase extraction on an ion-exchange sorbent, desalted, and transferred to the spray-jet applicator in a small volume of organic solvent containing aqueous ammonia. The solid-phase extraction module is fully automated so that cartridge exchange, conditioning, fractionation, and elution can be carried out in a sequential fashion coordinated with the operation of the liquid chromatograph and spray-jet applicator. An example of the determination of 4(5)-methylimidazole in a cola beverage is shown in Fig. 15.
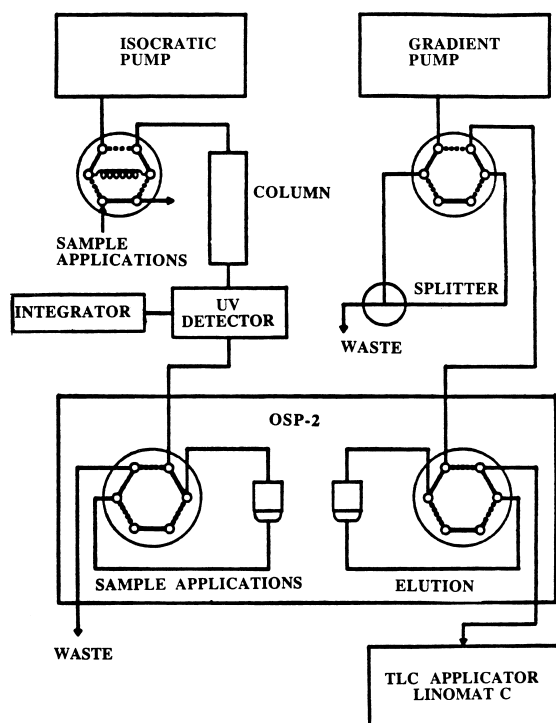

Fig. 14. Instrumental configuration for automated on-line coupling of column chromatography to thin-layer chromatography by a solid-phase extraction interface and spray jet sample applicator. (From Ref. [135]; © Research Institute for Medicinal Plants.)

6. Looking into the crystal ball

Progress in separation science is a combination of revolution followed by enhancements. Most revolutions are difficult to predict but enhancements should be more amenable to crystal ball gazing. In a mature technique like planar chromatography enhancements are more likely than revolutionary changes. Probably the most significant enhancement for planar chromatography today would be wider acceptance of the role it can play in the separation sciences and its re-introduction into the mainstream of separation science to promote wider knowledge of the technique and access to instrumentation.

The performance limit in modern TLC is a product of the inadequacy of capillary forces as a mobile phase transport mechanism. There are no indications that adjusting layer properties can solve this problem and the solution must lie in controlling zone broadening by other means. Zone refocusing by multiple development is already exploited to its maximum as far as we know in techniques such as automated multiple development. The alternative is to explore avenues that enhance capillary flow. Electoosmotic flow is an attractive and experimentally favorable mechanism but has not been adequately demonstrated for this purpose. It should figure strongly in the mind and work of planar chromatographers in the next decade. As we are starting to learn in column electrochromatography success may require the development of new sorbents with properties tailored to providing a reliable flow mechanism. The fall back position remains pneumatic forced flow development. To take this technique further convenient and user friendly instrumentation is a must. Layers with a smaller average particle size to improve performance and separation speed and layers with improved mass transfer characteristics to allow higher optimum mobile phase velocities are required. The unfavorable mass transfer characteristics are not a significant problem as long as capillary forces are responsible for the migration of the mobile phase. But to fully exploit the benefits of forced flow development an understanding of the factors contributing to the unfavorable resistance to mass transfer properties of layers is required. A notable difference between layers and columns is that layers contain a significant amount of polymeric binder to
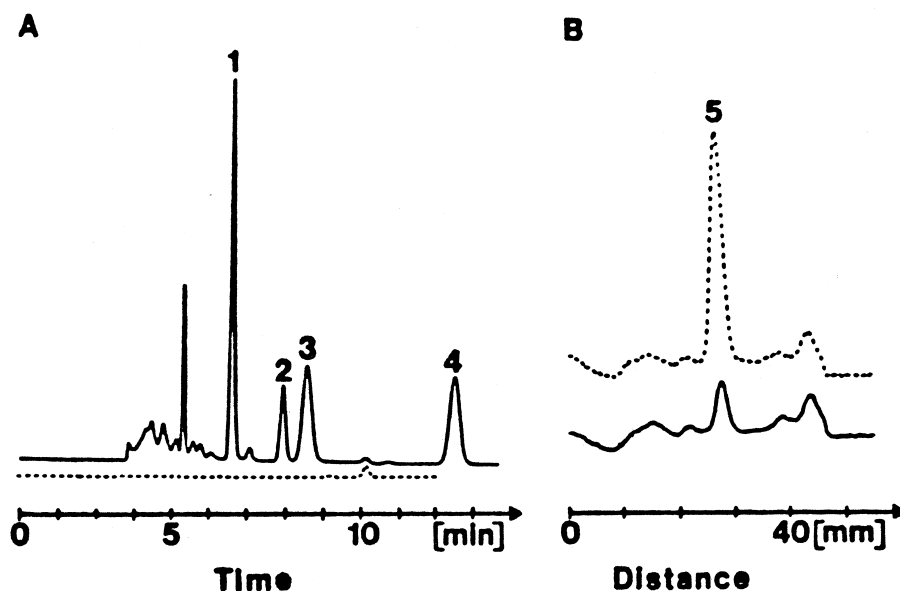
Fig. 15. On-line coupling of size-exclusion column chromatography and TLC for the determination of 4(5)-methylimidazole in a cola beverage using the interface shown in Fig. 14. Part A shows the size-exclusion column separation with the peak in the dotted line indicating the elution position of 4(5)-methylimidazole. Part B shows the separation of 4(5)-methylimidazole by TLC after transfer of the 4(5)-methylimidazole fraction from the size-exclusion column with the peak in the dotted line indicating the migration distance for 4(5)-methylimidazole. Peaks: 1=sodium benzoate; 2=sodium saccharin; 3=aspartame; 4=caffeine; and 5=4(5)-methylimidazole. (From Ref. [135]; © Research Institute for Medicinal Plants.)

stabilize the layer structure. The influence of the binder on the kinetic properties of layers needs to be understood and it is time to commence work on this problem.

With the exception of cellulose layers the properties of modern TLC layers have been optimized for the separation of small molecules. If TLC is to have a role in developing research areas in the life and material sciences then biocompatible and wide pore sorbents are needed. Planar electrophoretic separations are already common place in the life sciences and a role for TLC in the separation of neutral biopolymers should be possible. Developments in column liquid chromatography have completely overshadowed TLC in this area and not even preliminary results are available to demonstrate the possible role of TLC except for the use of layers prepared from handmade, unstable swollen gels. With the exception of precipitation chromatography, that uses standard layer configurations and the selective solubility of polymers in a mobile phase gradient as the basis for separation, TLC is un-

explored for the profiling of synthetic polymers. The separation of biopolymers and synthetic polymers are too large a general research area in modern science to ignore and efforts should be made to define a role for TLC in this area based on layers prepared with appropriate sorbent chemistry.

We are probably somewhere close in time to the replacement of slit-scanning densitometry by image analyzers. Problems remain for the time being but TLC is likely to be a beneficiary of the extensive research effort in this field unrelated to the separation sciences. Whether figures of merit are improved vis-a-vis scanning densitometry is less important than the possibilities created by electronic data analysis. This may revitalize interest in two-dimensional TLC for complex mixture analysis, particularly if it can be coupled with advances in the separation performance of layers as discussed above. Software developments should provide for new approaches to integration, multiple-wavelength scanning and comparison of chromatographic images for rapid characterization of complex mixtures.

The complementary properties of TLC and column separations suggest a wider role for TLC in multimodal separations. If normal-phase gradient separations are employed as one dimension then there is a real advantage in using TLC compared to columns. The flexible detection strategies associated with layer techniques, including biomonitoring, and the possibility of using surface-enhanced spectroscopies for identification, are other favorable properties.

My last image in the crystal ball is of a modest-cost, layer scanning mass spectrometer for compound identification and as an alternative to optical detection for recording chromatograms. As far as routine use goes this may be further over the horizon than some of my earlier projections, but steady progress has been made over the last decade, and the cost and flexibility of quadrupole and ion-trap mass spectrometers continues to decline, while ruggedness and reliability improve. The interface requirements are entirely different to those of column liquid chromatography and thin-layer chromatography requires its own customized solution. Now that coupling liquid chromatography to mass spectrometry is showing some maturity the coupling of thin-layer chromatography to mass spectrometry may become the next technology holy grail.

References

[1] F. Kreuzig, J. Planar Chromatogr. 11 (1998) 322.
[2] E. Stahl, Thin-Layer Chromatography, Springer Verlag, New York, 1969.
[3] J.G. Kirchner, Thin-Layer Chromatography, Wiley, New York, 1978.
[4] J.A. Berndt, C.F. Poole, J. Planar Chromatogr. 1 (1988) 174.
[5] C.F. Poole, S.K. Poole, Chromatography Today, Elsevier, Amsterdam, 1991.
[6] J.E. MacNair, K.C. Lewis, J.W. Jorgenson, Anal. Chem. 69 (1997) 983.
[7] C.F. Poole, S.K. Poole, J. Chromatogr. A 703 (1995) 573.
[8] C.F. Poole, W.P.N. Fernando, J. Planar Chromatogr. 5 (1992) 323.
[9] F. Geiss, Fundamentals of Thin Layer Chromatography (Planar Chromatography), Hüthig, Heidelberg, 1987.
[10] S.K. Poole, W. Kiridena, K.G. Miller, C.F. Poole, J. Planar Chromatogr. 8 (1995) 257.
[11] S.K. Poole, S.L. Daly, C.F. Poole, J. Planar Chromatogr. 6 (1993) 129.
[12] M.T. Belay, C.F. Poole, Chromatographia 37 (1993) 365.
[13] W.R. Kiridena, S.K. Poole, C.F. Poole, J. Planar Chromatogr. 7 (1994) 273.
[14] K.G. Miller, C.F. Poole, T.M.P. Chichila, J. High Resolut. Chromatogr. 18 (1995) 461.
[15] S.K. Poole, C.F. Poole, Analyst 119 (1994) 113.
[16] W. Kiridena, K.G. Miller, C.F. Poole, J. Planar Chromatogr. 8 (1995) 177.
[17] K.G. Miller, C.F. Poole, T.M.P. Pawlowski, Chromatographia 42 (1996) 639.
[18] J. Unruh, D.P. Schwartz, R.A. Barford, J. Assoc. Off. Anal. Chem. Int. 76 (1993) 335.
[19] J.P. Abjean, J. Planar Chromatogr. 6 (1993) 147.
[20] J.P. Abjean, J. Assoc. Off. Anal. Chem. Int. 80 (1997) 737.
[21] M. Juhel-Gaugain, J.P. Abjean, Chromatographia 47 (1998) 101.
[22] F. Smets, Ch. Vanhoenackere, G. Pottie, Anal. Chim. Acta 275 (1993) 147.
[23] M.B. Medina, N. Nagdy, J. Chromatogr. B 614 (1993) 315.
[24] J. Hoebus, E. Daneels, E. Roets, J. Hoogmartens, J. Planar Chromatogr. 6 (1993) 269.
[25] I. Ojanpera, Trends Anal. Chem. 11 (1992) 222.
[26] I. Ojanpera, K. Goebel, E. Vuori, J. Liq. Chromatogr. Rel. Technol. 22 (1999) 161.
[27] R.A. de Zeeuw, J.P. Frank, F. Degel, G. Machbert, H. Scutz, J. Wijsbeek (Eds.), Thin-Layer Chromatographic R_F Values of Toxicologically Relevant Substances on Standardized Systems, VCH, Weinheim, 1992.
[28] P.G.A.M. Schepers, J.P. Franke, R.A. deZeeuw, J. Anal. Toxicol. 7 (1983) 272.
[29] I. Ojanpera, J. Nokua, E. Vuori, P. Suniba, E. Sippola, J. Planar Chromatogr. 10 (1997) 281.
[30] Z. Fater, G. Tasi, B. Szabady, S. Nyiredy, J. Planar Chromatogr. 11 (1998) 224.
[31] G.E. Morlock, J. Chromatogr. A 754 (1996) 423.
[32] S. Butz, H.-J. Stan, Anal. Chem. 67 (1995) 620.
[33] German Standard DIN 38 407, Part II, Beuth Verlag, Berlin, 1994.
[34] R. Koeber, R. Niessner, Fresenius J. Anal. Chem. 354 (1996) 464.
[35] C. Steuckart, E. Berger-Preiss, K. Levsen, Anal. Chem. 66 (1994) 2570.
[36] C. Weins, H. Jork, J. Chromatogr. A 750 (1996) 403.
[37] G. Eberz, H.-G. Rast, K. Burger, W. Kreiss, C. Weisemann, Chromatographia 43 (1996) 5.
[38] B. Renger, J. Assoc. Off. Anal. Chem. Int. 76 (1993) 7.
[39] C. Weins, H.E. Hauck, LC·GC Mag. 14 (1996) 456.
[40] B. Renger, J. Assoc. Off. Anal. Chem. Int. 81 (1998) 333.
[41] G. Szepesi, J. Planar Chromatogr. 6 (1993) 187.
[42] G. Szepesi, J. Planar Chromatogr. 6 (1993) 259.
[43] Z. Szikszay, Z. Vegh, K. Ferenczi-Fodor, J. Planar Chromatogr. 11 (1998) 428.
[44] I. Vovk, A. Golc-Wondra, M. Prosek, J. Planar Chromatogr. 10 (1997) 416.
[45] K. Ferenczi-Fodor, A. Nagy-Turak, Z. Vegh, J. Planar Chromatogr. 8 (1995) 349.
[46] K. Hostettmann, C. Terreaux, A. Marston, O. Potterat, J. Planar Chromatogr. 10 (1997) 251.

[47] F. Bonte, P. Pinguet, A. Saunois, J.-M. Chevalier, A. Meybeck, J. Chromatogr. A 791 (1997) 231.

[48] S. Zellmer, J. Lasch, J. Chromatogr. B 691 (1997) 321.

[49] U. delaVigne, D.E. Janchen, Inform 1 (1990) 477.

[50] F. Bonte, P. Pinguet, J.M. Chevalier, A. Meybeck, J. Chromatogr. B 664 (1995) 311.

[51] J. Muthig, H. Ziehr, J. Chromatogr. B 687 (1996) 357.

[52] L. Lepri, J. Planar Chromatogr. 10 (1997) 320.

[53] M. Mack, H.E. Hauck, J. Planar, Chromatogr. 2 (1989) 190.

[54] M. Mack, H. Hauck, Chromatographia 26 (1989) 197.

[55] J. Sherma, B. Fried (Eds.), Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, 1991.

[56] G.W. Somsen, W. Morden, I.D. Wilson, J. Chromatogr. A 703 (1995) 613.

[57] G.W. Somsen, C. Gooijer, U.A.Th. Brinkman, Trends Anal. Chem. 17 (1998) 129.

[58] H.T. Butler, M.E. Coddens, C.F. Poole, J. Chromatogr. 290 (1984) 113.

[59] C.F. Poole, S.K. Poole, J. Chromatogr. 492 (1989) 539.

[60] S.J. Kok, R. Posthmus, I. Bakker, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst, Anal. Chim. Acta 303 (1995) 3.

[61] R.J. vandeNesse, I.H. Vinkenberg, R.H.J. Jonker, G.Ph. Hoornweg, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst, Appl. Spectrosc. 48 (1994) 788.

[62] G. Glauninger, K.-A. Kovar, V. Hoffmann, Fresenius J. Anal. Chem. 338 (1990) 710.

[63] O. Frey, K.-A. Kovar, V. Hoffmann, J. Planar Chromatogr. 6 (1993) 93.

[64] S. Stahlmann, K.-A. Kovar, J. Chromatogr. A 813 (1998) 145.

[65] G.K. Bauer, A.M. Pfeifer, H.E. Hauck, K.-A. Kovar, J. Planar Chromatogr. 11 (1998) 94.

[66] G.K. Bauer, K.-A. Kovar, J. Planar Chromatogr. 11 (1998) 30.

[67] E. Koglin, J. Planar Chromatogr. 3 (1990) 117.

[68] G.W. Somsen, P.G.J.H. terRiet, C. Gooijer, N.H. Velthorst, U.A.Th. Brinkman, J. Planar Chromatogr. 10 (1997) 10.

[69] G.W. Somsen, S.K. Coulter, C. Gooijer, N.H. Velthorst, U.A.Th. Brinkman, Anal. Chim. Acta 349 (1997) 189.

[70] K.L. Busch, Trends Anal. Chem. 11 (1992) 314.

[71] J.T. Mehl, A.I. Gusev, D.M. Hercules, Chromatographia 46 (1997) 358.

[72] Y.-C. Chen, J. Shiea, J. Sunner, J. Chromatogr. A 826 (1998) 77.

[73] R.M. Anderson, K.L. Busch, J. Planar Chromatogr. 11 (1998) 336.

[74] P. Martin, W. Morden, P. Wall, I.D. Wilson, J. Planar Chromatogr. 5 (1992) 255.

[75] C.F. Poole, S.K. Poole, Anal. Chem. 66 (1994) 27A.

[76] W.P.N. Fernando, C.F. Poole, J. Planar Chromatogr. 4 (1991) 278.

[77] C.F. Poole, W.P.N. Fernando, J. Planar Chromatogr. 6 (1993) 357.

[78] W.P.N. Fernando, C.F. Poole, J. Planar Chromatogr. 3 (1990) 389.

[79] C.F. Poole, S.K. Poole, Anal. Chem. 61 (1989) 1257A.

[80] S. Nyiredy, G. Szepesi, J. Pharm. Biomed. Anal. 10 (1992) 1017.

[81] M. Mazurek, Z. Witkiewicz, Chem. Anal. (Warsaw) 42 (1997) 767.

[82] E. Tyihak, E. Mincsovics, J. Planar Chromatogr. 4 (1991) 288.

[83] G. Flodberg, J. Roeraade, J. Planar Chromatogr. 8 (1995) 10.

[84] R.E. Kaiser, R.I. Reider, J. Am. Oil Chem. Soc. 66 (1989) 79.

[85] G. Flodberg, J. Roeraade, J. Planar Chromator. 6 (1993) 252.

[86] C.F. Poole, I.D. Wilson, J. Planar Chromatogr. 10 (1997) 332.

[87] S. Nyiredy, L. Botz, O. Sticher, J. Planar Chromatogr. 2 (1989) 53.

[88] M. Mazurek, Z. Witkiewicz, Chem. Anal. (Warsaw) 43 (1998) 529.

[89] L. Botz, Sz. Nyiredy, O. Sticher, J. Planar Chromatogr. 3 (1990) 352.

[90] L. Botz, Sz. Nyiredy, O. Sticher, J. Planar Chromatogr. 4 (1991) 115.

[91] E. Tyihak, G. Katay, Z. Ostorics, E. Mincsovics, J. Planar Chromatogr. 11 (1998) 5.

[92] S.K. Poole, H.D. Ahmed, C.F. Poole, J. Planar Chromatogr. 3 (1990) 277.

[93] E. Hanh-Deinstrop, A. Koch, M. Muller, J. Planar Chromatogr. 11 (1998) 404.

[94] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.

[95] M.M. Dittmann, G.P. Rozing, G. Ross, T. Adam, K.K. Unger, J. Cap. Electrophoresis 5 (1997) 201.

[96] L.A. Colon, Y. Guo, A. Fermier, Anal. Chem. 69 (1997) 461A.

[97] M. Pukl, M. Posek, R.E. Kaiser, Chromatographia 38 (1994) 83.

[98] D. Nurok, M.C. Frost, C.L. Pritchard, D.M. Chenoweth, J. Planar Chromatogr. 11 (1998) 244.

[99] C.F. Poole, S.K. Poole, W.P.N. Fernando, T.A. Dean, H.D. Ahmed, J.A. Berndt, J. Planar Chromatogr. 2 (1989) 336.

[100] C.F. Poole, M.T. Belay, J. Planar Chromatogr. 4 (1991) 345.

[101] C.F. Poole, S.K. Poole, M.T. Belay, J. Planar Chromatogr. 6 (1993) 438.

[102] B. Szabady, M. Ruszinko, S. Nyiredy, Chromatographia 45 (1997) 369.

[103] B. Szabady, M. Ruszinko, Sz. Nyiredy, J. Planar Chromatogr. 8 (1995) 279.

[104] A. Kovacs, L. Simon-Sarkadi, E. Mincsovics, J. Planar Chromatogr. 11 (1998) 43.

[105] G. Guiochon, A.M. Siouffi, J. Chromatogr. 245 (1982) 1.

[106] M. Zakaria, M.-F. Gonnord, G. Guiochon, J. Chromatogr. 271 (1983) 127.

[107] C.F. Poole, S.K. Poole, in: H.J. Cortes (Ed.), Multidimensional Chromatography. Techniques and Applications, Marcel Dekker, New York, 1990, pp. 29–73.

[108] G. Guiochon, M.-F. Gonnord, M. Zakaria, L.A. Beaver, A.M. Siouffi, Chromatographia 17 (1983) 121.

[109] M.-F. Gonnord, A.M. Siouffi, J. Planar Chromatogr. 3 (1990) 206.

[110] P. Harmala, L. Botz, O. Sticher, R. Hiltunen, J. Planar Chromatogr. 3 (1990) 515.

[111] M. Mazurek, Z. Witkiewiez, J. Planar Chromatogr. 4 (1991) 379.

[112] H. Kalasz, M. Bathori, L.S. Ettre, B. Polyak, J. Planar Chromatogr. 6 (1993) 481.

[113] Z. Fater, B. Szabady, S. Nyiredy, J. Planar Chromatogr. 8 (1995) 145.

[114] S.K. Poole, M.T. Belay, C.F. Poole, J. Planar Chromatogr. 5 (1992) 16.

[115] N.-K. Olah, L. Muresan, G. Cimpan, S. Gocan, J. Planar Chromatogr. 11 (1998) 361.

[116] H. Yamamoto, K. Nakamura, D. Nakatani, H. Terada, J. Chromatogr. 543 (1991) 201.

[117] M. Petrovic, M. Kostelan-Macan, S. Balic, J. Planar Chromatogr. 11 (1998) 353.

[118] K.L. Busch, J. Planar Chromatogr. 5 (1992) 72.

[119] H. Filthuth, J. Planar Chromatogr. 2 (1989) 198.

[120] I. Hazal, I. Ormos, I. Klebovich, J. Planar Chromatogr. 8 (1995) 92.

[121] J. Szunyog, E. Mincsovics, I. Hazal, I. Klebovich, J. Planar Chromatogr. 11 (1998) 25.

[122] I. Vovk, M. Prosek, J. Chromatogr. A 768 (1997) 329.

[123] I. Vovk, M. Prosek, J. Chromatogr. A 779 (1997) 329.

[124] E. Stahl, J. Chromatogr. 142 (1977) 15.

[125] E. Stahl, W. Schild, Z. Fresenius', Anal. Chem. 280 (1976) 99.

[126] U. Keller, I. Flament, Chromatographia 28 (1989) 445.

[127] L. Wunsche, U. Keller, I. Flament, J. Chromatogr. 552 (1991) 539.

[128] J.W. Hofstraat, S. Griffioen, R.J. vandeNesse, U.A.Th. Brinkman, C. Gooijer, N.H. Velthorst, J. Planar Chromatogr. 1 (1988) 220.

[129] D.E. Jaenchen, H.J. Issaq, J. Liq. Chromatogr. 11 (1988) 1941.

[130] K. Burger, Analusis 18 (1990) 113.

[131] C.T. Banks, J. Pharm. Biomed. Anal. 11 (1993) 705.

[132] O.R. Queckenberg, A.W. Frahn, J. Planar Chromatogr. 6 (1993) 55.

[133] U. Wippo, H.-J. Stan, Dtsch. Lebensm. Rdsch. 5 (1997) 144.

[134] H.-J. Stan, F. Schwarzer, J. Chromatogr. A 819 (1998) 35.

[135] E. Muller, H. Jork, J. Planar Chromatogr. 6 (1993) 21.