Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, Nos 10–12, pp. 1007–1015, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00
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Planar chromatography: current status and future perspectives in pharmaceutical analysis — I. Applicability, quantitation and validation*

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Abstract: The most important features of capillary action planar chromatographic methods are summarized. Certain properties such as ease in operation, sensitivity to experimental conditions, stationary and mobile phase selection, phase system optimization, separation efficiency and detection possibilities are discussed only briefly. Other aspects such as the applicability of planar chromatographic methods in pharmaceutical analysis, quantitation and validation are considered in more detail. The advantages and limitations of different evaluation techniques (visual comparison, spot elution techniques and *in situ* densitometry) are also discussed. Validation steps specific to planar chromatographic methods are emphasized.

Keywords: *Planar chromatography; thin-layer chromatography; pharmaceutical analysis; validation; quantitation.*

Introduction

Chromatography is extensively used in the pharmaceutical industry as a separation tool for quantitative and qualitative analysis. Although the popularity of capillary action planar chromatography has considerably decreased during the past 10 years because of the replacement of several standard thin-layer chromatographic (TLC) separations by high-performance liquid chromatographic (HPLC) methods, it does claim a share of the market of chromatographic techniques because many very difficult analytical problems can be solved even in small laboratories equipped with basic TLC equipment. Traditional TLC is inexpensive, simple to use, and requires minimal instrumentation, laboratory space and maintenance. However, to achieve good precision, accuracy and reproducibility a certain degree of instrumentation is required; the use of densitometric evaluation is necessary at least for quantitation. The purpose of the present reviews is to give a brief outline of planar chromatographic methods with special regard to their applicability in pharmaceutical analysis. In the first part various aspects of the applicability, quantitation and validation of capillary action planar chromatographic methods are considered. In the second part, special techniques and future perspectives are briefly discussed.

Applicability of Planar Chromatography

A literature search of the last 20 years indicates that extensive reviews on TLC systems for pharmaceutical preparations and drug have been published [1–7]. Several monographs, review papers and book chapters deal with the detection and quantitation of the separated compounds [5, 6, 8–12]. Governmental authorities generally require testing of pharmaceuticals for stability and impurity profile before approval is given. Hence the monitoring of the stability of drugs by TLC upon storage [13–15] and under stress [16, 17] is of concern. In addition, determination of bulk drug purity and of the impurity profile has been reported [4, 5, 18, 19].

To illustrate the applicability of planar chromatographic methods in pharmaceutical analysis a brief outline is given here. Some aspects are well known and will be treated briefly below. But some require a more detailed discussion. These aspects are listed in Table 1.

*Presented at the "Fourth International Symposium on Drug Analysis", May 1992, Liège, Belgium.
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Table 1
Separation efficiency of capillary action planar chromatography

Properties	Advantages	Disadvantages
Plate efficiency	Good	
Peak symmetry		Difficult
Irreversible adsorption		Frequently occurs
Solvent purity	No problems	
Spot shape		Dependent
Spot size		Dependent
Development modes		Highly dependent
Vapour phase		Highly dependent

Ease of operation

TLC is simple in instrumentation and in practice. The mobile phase can be easily prepared from organic solvents of conventional purity. The chromatograms can be visually evaluated after colour reaction or under UV light. However, to yield quantitative results densitometric evaluation is usually required. Although great efforts have been made to achieve complete automation, this is difficult and not widely used.

Sensitivity to experimental conditions

Planar chromatographic methods are more or less sensitive to changes in the environmental conditions. Small changes in the eluent composition and/or in temperature and relative humidity during the development may cause dramatic changes in the retention characteristics of the compounds to be separated. This effect is more pronounced and valid if unsaturated chambers are used for the development. Similarly, the quality of any capillary action planar chromatographic separation is also a function of the type and size of the developing chamber.

Stationary phase selection

The most important stationary phases used in pharmaceutical analysis can be found in textbooks [20, 21]. Modifications have been applied to further improve the selectivity and efficiency of precoated layers in TLC. The following examples of modifications could be more generally applied in pharmaceutical analysis: bare silica and alumina; precoated layers suitable for high-performance TLC (HPTLC); combinations of different sorbents on a precoated layer; hydrophobic and hydrophilic chemical modification of bulk sorbents and precoated layers.

Mobile phase selection

The correct choice of mobile phase composition for a given separation constitutes a very important stage in achieving a good separation in TLC. The basic strategy of mobile phase selection in TLC is similar to HPLC regarding solvent classification and elutropic series [22–25]. In general, the selection of a particular solvent to be usable is more simple, it must be at least normally purified having relatively low boiling point and viscosity, cheap and compatible with the sorbent, support plate and binder. Selection of appropriate mobile phase composition is much more difficult. A great variety of factors can be adjusted, e.g. the solvent component, their relative concentration, pH, solvent strength, etc.

In the following part of the paper those aspects of capillary action planar chromatographic methods of special importance in relation to the use of TLC in pharmaceutical analysis are discussed in more detail (Tables 1–5).

Aspects of Special Importance

Separation efficiency

Successful planar chromatographic separation is strongly dependent on several factors. One relates to the quality of sample application. The reproducibility of sample amount and spot size is quite important, but to achieve good chromatographic resolution and sensitivity of detection, the shape of the spots of the applied sample is also of great importance. Techniques and apparatus for sample introduction are reviewed by Fenimore [26, 27], Janchen [28] and Kaiser [29].

In summarizing the practical problems associated with various sampling procedures, the following principles can be considered: there is a very narrow working range for the sample volume load if no focusing step is included into the sample application; sampling in the presence of solvents with a measurable elution strength can start chromatography, resulting in significant loss in separation efficiency; if a solvent used for sample preparation remains in the layer in or around the sampling area, the selectivity and the relative or absolute spot position in the chromatogram may be drastically altered; and if part or all of the sample is solidified or adsorbed on to the stationary phase surface a slow dissolution

effect can cause significant tailing of the spots. The application should be made in the form of spots or streaks. The streak type of sample application which forms very narrow lines of starting shape, results in sharper spots and increased resolution allowing optimum separation efficiency. However, streak-type sample application is less precise than spot-type sample application.

Phase system optimization

The most important aspects of phase system optimization are summarized in Table 2. The methods of optimization are basically similar to those used for HPLC optimization. Here the PRISMA optimization method has been developed by one of the present authors [30–32].

Detection possibilities

The principal methods are outlined in Table 3. A more detailed discussion will be given under *Quantitation* and *Validation*.

Applicability in pharmaceutical analysis

Table 4 summarizes the most important information on the applicability of planar chromatographic methods in pharmaceutical analysis. In general, it can be stated that although HPLC has superseded TLC in many application areas, conventional TLC plays a useful rôle where cost effectiveness is essential. The rapid and accurate identification of the raw materials or finished products is a basic principle in various pharmacopoeias. TLC is widely used for this purpose. Similarly, TLC is widely used as a standard technique for the purity testing of active raw materials and formulated products owing to its simplicity and good resolving power. These are the two most important application areas in various pharmacopoeias. The importance of quantitative TLC methods has considerably decreased and only a few official methods can be found in the pharmacopoeias usually with a spot elution technique for quantitation. In the field of industrial pharmaceutical analysis the situation is different because the instrumentation of TLC has reached a relatively high level. In some special application areas, such as the analysis of the extracts of medicinal plants, fermentation mixtures, modern TLC (precoated or HPTLC layers, densitometric evaluation) has a distinct rôle, because the interference of so-called 'unknown background materials' can be more easily eliminated than with other chromatographic techniques. Many chromatographers working in the pharma-

Table 2
Phase system optimization of capillary action planar chromatography

Properties	Advantages	Disadvantages
Number of variables		
Separation mode		Limited
Stationary phase		Limited
Mobile phase composition	Wide range	
Use of additives	No problems	
pH		Limited
Temperature		Not applicable
Relative humidity		Not applicable
Development mode	Wide range	
Chamber saturation		Limited
Time requirement	Fast	
Dynamic modification		
With organic solvent	Generally used	
With additives	Good alternative	

Table 3
Detection possibilities of capillary action planar chromatography

Properties	Advantages	Disadvantages
In situ detection		
Visual	Qualitative good	Quantitative difficult
Densitometry without derivatization	Widely used	
Densitometry after derivatization	Widely used	
Spot elution		Difficult

Table 4
Applicability of capillary action planar chromatography in pharmaceutical analysis

Properties	Advantages	Disadvantages
Type of solute		
Very polar		Limited
Polar	Good	
Medium polar	Good	
Non-polar	Good	
Structure of solute		
Structurally different compounds	Good	
Related compounds	Good	
Isomers	Good	
Homologous compounds		Limited
Size of solute		
Small	Good	
Medium large		Limited
Large		Limited
Analytical task		
Analysis of starting raw materials		
Plant extracts	Widely used	
Extracts of animal organs	Widely used	
Fermentation mixture	Widely used	
Analysis of intermediates		
Intermediates and crude products		Limited
Reaction mixtures	Applicable	
Mother liquors and secondary products	Applicable	
Analysis of pharmaceutical raw materials		
Identification	Generally used	
Purity testing	Alternative to HPLC	
Assay		Not applicable
Stability testing	Complementary to HPLC	
Analysis of formulated products		
Identification	Generally used	
Purity testing	Alternative to HPLC	
Assay		Not applicable
Stability testing	Complementary to HPLC	
Content uniformity test		Not applicable
Dissolution test		Not applicable
Analysis of drugs and their metabolites in biological media		Limited applicability

ceutical industry prefer to use reversed-phase HPLC in conjunction with normal-phase TLC or HPTLC to analyse raw materials for purity and impurities as well as for stability testing.

Quantitation

A general approach to quantitation in TLC may be made on the basis of methods used for evaluation. Two basic approaches can be distinguished: direct methods in which the separated spots are evaluated *in situ* on the plate; and indirect methods in which quantitative measurements are carried out after elution of the spots on the plate. Although the importance of direct methods has considerably increased, the spot elution technique is also being used, e.g. some assay methods in the U.S.P.

Direct methods can be further divided into two main groups: visual comparison, when the spot intensities are established by visually comparing them with the intensities of simultaneously developed reference spots; and *in situ* densitometry quantifying the chromatogram directly on the plate by measurement of the absorbance of the separated spots.

The semiquantitative estimation of impurities by visual comparison is generally used in different pharmacopoeias for the purity testing of both active raw materials and formulated products. Four purity criteria can be defined.

The first criterion is that the appearance of any spots other than the principal spot on the chromatogram (single spot criterion) is not permitted. In this case a known and prescribed quantity of the samples is transferred on to the plate. This procedure is simple but does not provide information about the impurity profile; in addition evaluation could be dependent on the experimental conditions, such as light intensity of the UV lamp and the plate efficiency.

The second criterion is that the maximum number of impurities and limits of the quantity

of each individual impurity must be specified. Only the presence of those impurities is allowed which show identical R_f values with the simultaneously developed reference compounds. Although this procedure is more accurate compared with the previous one, it requires close conformity of the materials; the impurity profile must be the same, which is the function of the manufacturing process.

The third criterion does not limit the number and quantity of the individual impurities but limits the total intensity of the separated impurity spots in comparison with the intensity of spots of a simultaneously developed reference standard (principal component) applied in known quantity. Although this procedure is generally used for the evaluation of pharmacopoeial purity tests, it suffers from two major limitations: the spot intensity may be the function of the chemical structure of the impurities and of the R_f value as well.

The fourth criterion is that the quantity of each individual impurity and the total amount of impurities must be limited.

Table 5
Validation of quantitative planar chromatography

Properties	Advantages	Disadvantages
Precision		
Method		Difficult
System		Difficult
Accuracy	Good	
Selectivity	Good	
Limit of detection	Good	
Limit of quantitation	Good	
Linearity and range		Limited
Plate loadability	Good	
Sample stability		Difficult
Ruggedness		
Plate to plate variation		Difficult
Sample concentration	Good	
Sample size		Difficult
Spot type		Difficult
Spot size		Difficult
Chamber type		Difficult
R_f value		Difficult
Colour reaction		Difficult
Detection	Good	
Mobile phase composition	Good	
Temperature		Difficult
Running distance	Good	

Table 6
Different validation steps specific to planar chromatography

Sample preparation	
Sample application	Quality of sample application (spot shape and size)
	Drying (sample stability)
	Sample size and concentration
	Standing time prior to running
Chromatographic separation	Plate-to-plate variation
	Position of the spots on the chromatogram
	Position of the spots on the plate
	Mobile phase composition
	Environmental cónditions (saturation, temperature, relative humidity)
Calibration	Single standard with increasing sample volume
	Single standard with increasing sample concentration
	Position of standard and sample spots on the plate
Detection and quantitation	Stability of the formed derivatives on the plate
	Peak height versus peak area measurement
System suitability	R_f or R_M values for two known components obtained from standard dilution
	Desired R_s for two known components obtained from standard dilution
	Precision calculated from five parallel runs of standard solution
	Peak asymmetry calculated for the principal component

Standard dilutions of reference materials are simultaneously applied to the plate and the purity of the material is evaluated by comparison of the spot intensity of the known impurities with those of the standard dilutions of the same compounds; the quantity of other spots different from the reference spots is expressed in terms of the principal component. This evaluation procedure is the most accurate among the procedures based on visual comparison.

Advantages and disadvantages of the spot elution technique can be summarized as follows: the procedure is simple, the quantitation can be performed in several different ways (e.g. colorimetry, UV spectrophotometry, spectrofluorimetry, HPLC); however, simultaneous use of appropriate blank and reference material is required, losses originating from inaccurate location of the spots as well as from insufficient recovery caused by irreversible adsorption may frequently occur.

Advantages of *in situ* densitometry include: independence of chromatographic separation from detection in time and space; the chromatograms can be preserved after measurement; the total amount of the sample can be spotted; the chromatogram is complete; every separated component can be detected; the sensitivity of the detection is higher by about 2 orders of magnitude; by changing the detection wavelength, the selectivity of the separation can be increased; and reflectance spectra can be recorded thus permitting a certain degree of identification.

Disadvantages are: simultaneous running of the appropriate reference standard is required; errors originating from the incomplete distribution of spraying may frequently occur; and separation and quantitation might be influenced by variation in layer thickness, by disturbance of zones caused by solvent demixing and by plate-to-plate variability.

Problems of calibration using *in situ* densitometry may be associated with the method used to evaluate the results.

As with any quantitative method based on the interpretation of detector responses, several methods exist for evaluation of the results. In the case of quantitative TLC, a nonlinear relationship between the detector signal and the amount of substance exists. This nonlinearity is valid for both peak height and peak area measurements. A detailed interpretation of the problems has been published by Ebel *et*

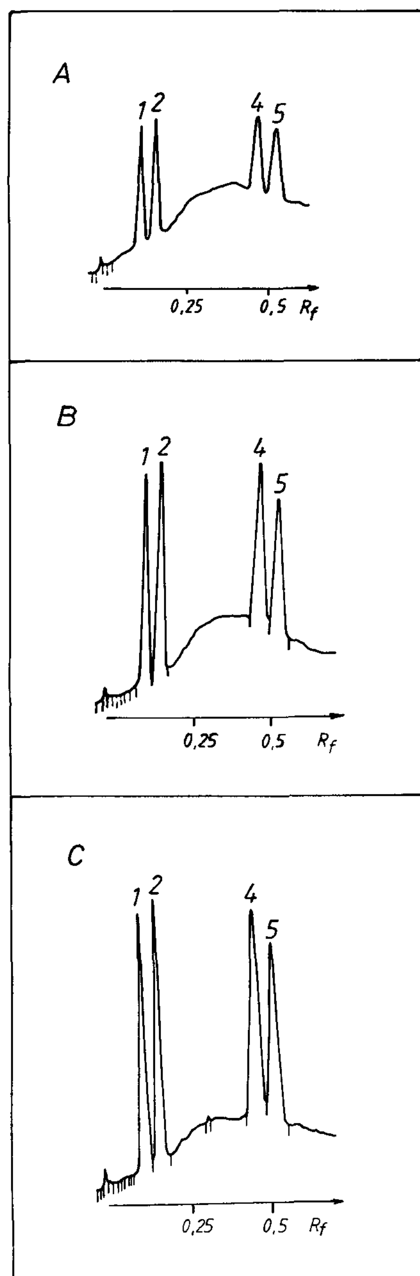

Figure 1
Chromatograms of calibration standards applying 50% (panel A), 100% (panel B) and 150% (panel C) of the expected value in the sample. Chromatoplate: HPTLC silica gel F_{254} (E. Merck, Germany); eluent: methanol–acetonitrile–conc. ammonia solution (514:386:100, v/v/v) containing 5×10^{-3} M ammonium chloride and 8×10^{-2} M ammonium carbonate. Detection: spectrodensitometry in remission mode at 525 nm after colour reaction with Dragendorff reagent. Compounds: by-products and decomposition products of pipecuronium bromide (bisquaternary amino steroid); **1**, 3-acetyl-17-hydroxy-2,16-bisquaternary amino steroid [decomposition product]; **2**, 3,17-dihydroxy-2,16-bisquaternary amino steroid [decomposition product]; **3**, pipecuronium bromide (main component, 3,17-diacetyl-2,16-bisquaternary amino steroid, for separation see Fig. 2); **4**, 3,17-diacetyl,-17-monoquaternary amino steroid (by-product); **5**, 3,17-diacetyl-2-monoquaternary amino steroid.

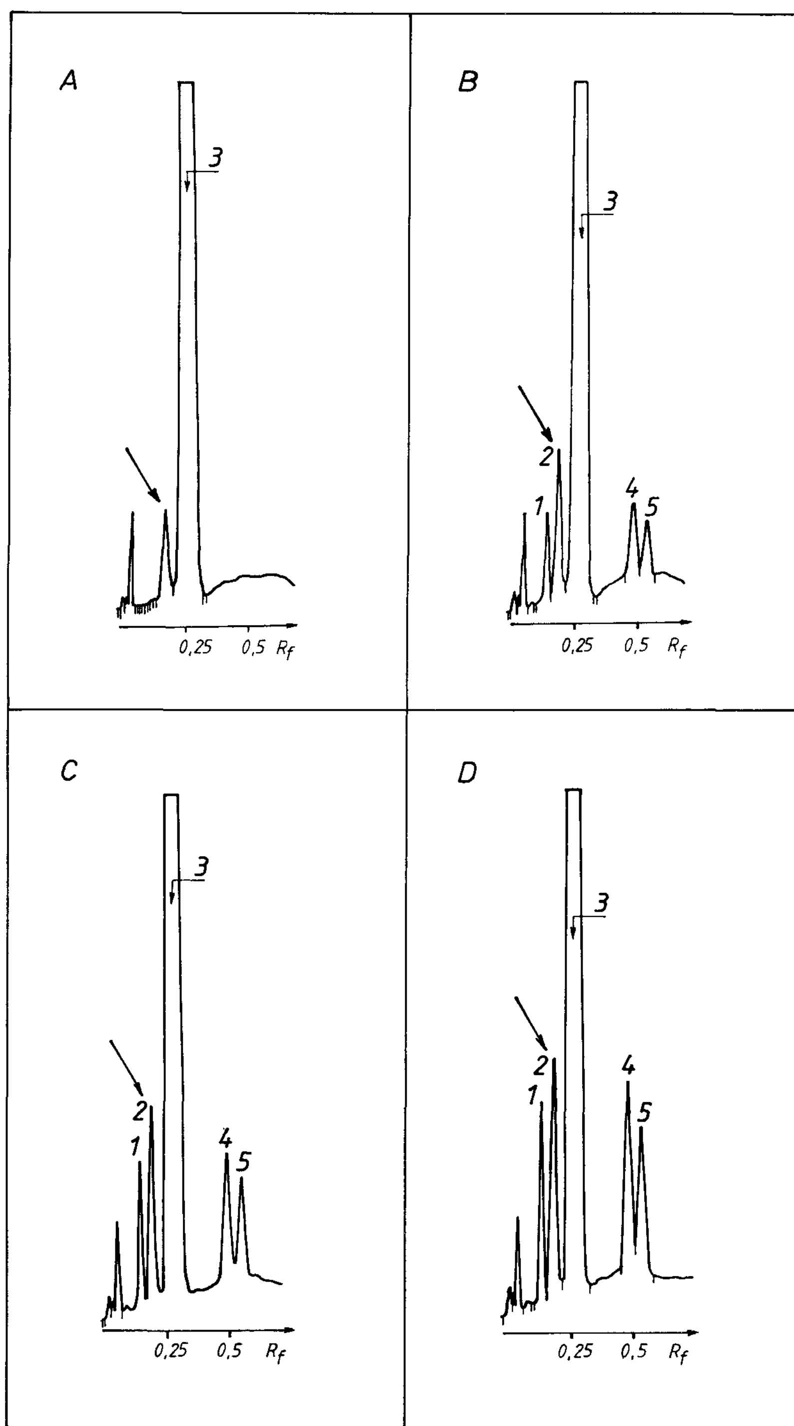

Figure 2
Chromatograms of reference sample with and without spiking with calibration standards. Panel A without spiking; panel B; spiked with calibration standard (50% of the expected value); panel C, spiked with calibration standard (100% of the expected value); panel D, spiked with calibration standard (150% of the expected value). Conditions and compounds as in Fig. 1.

al. [33–38]. Derivative recording using smoothed numerically generated derivatives has been developed to overcome this problem [33].

Validation

Table 5 contains the most important information on the validation of quantitative planar chromatographic methods. An analytical method using TLC may be divided into five distinct steps: sample preparation, including prechromatographic derivatization; sample application; chromatographic separation; calibration; and detection and quantitation inlcuding post-chromatographic derivatization, amplification of detector signal and conversion of detector signal into numerical data. Validation of some steps and procedures are very similar to those used in HPLC, but some aspects are different and specific to planar chromatography (Table 6).

Problems relating to sample application have been mentioned under *Separation efficiency*. A plate-to-plate variation study relates to the variance of R_f values and the reproducibility of detector signals obtained after separation of the same sample performed under identical conditions on two or more different plates. The position of the spots on the chromatogram can also significantly influence the detector signal. Low R_f values result in more concentrated spots (lower detector signal and sensitivity in reflectance mode) whereas at higher R_f values expanded spot areas are obtained. This effect can be minimized by using the transmittance mode. Another important factor is the position of the spots on the plate. This effect is known as the edge effect and is closely associated with the inhomogeneity of the layer. The R_f values of the components spotted at the edges of the plate will change. The recommended system for suitability of the data is outlined in Table 6.

In the authors' laboratory the following procedure is used as a basic working rule: every spot is scanned in triplicate to establish the instrumental error, the mean being used for calculation; every test solution is applied in triplicate, spotting the same volume in each case; calibration standards are applied in triplicate, spotting the same volume containing different amounts (e.g. 80, 100 and 120% of the expected value) of standards. Figures 1 and 2 show a practical example of calibration standards applied in triplicate, spotting the same volume containing different amounts (50, 100 and 150% of the expected value) of reference impurities. As shown in Fig. 2 the peak at the position indicated seems higher than in Fig. 1 owing to the presence of the same impurity in the original sample (spiked sample).

Acknowledgements — One of the authors (S.N.) wishes to thank Desaga (Heidelberg, Germany) for the kind loan of a CD-60 densitometer and an AS-30 sample applicator.

References

[1] A.H. Stead, R. Gill, T. Wright, J.P. Gibbs and A.C. Moffat, *Analyst* **107**, 1106–1110 (1982).
[2] B. Fried and J. Sherma, *Pharmaceuticals in Thin-Layer Chromatography, Techniques and Applications*, Chrom. Sci. Vol. 35. Marcel Dekker, New York (1986).
[3] E.G.C. Clarke, *Isolation and Identification of Drugs*, Vols 1 and 2, Pharmaceutical Press, London (1969; 1975).
[4] G. Szepesi, in *Steroid Analysis in the Pharmaceutical Industry* (S. Görög, Ed.), pp. 103–113. Ellis Horwood, Chichester (1989).
[5] G. Szepesi, in *Quantitative Thin-Layer Chromatography and its Industrial Application* (L. Treiber, Ed.). Marcel Dekker, New York (1986).
[6] T.C. Touchstone, *Quantitative Thin-Layer Chromatography*. Wiley, New York (1973).
[7] L.L. Ng, in *Handbook of Thin-Layer Chromatography* (J. Sherma and B. Fried, Eds), pp. 717–756. Marcel Dekker, New York (1991).
[8] G. Szepesi, in *Modern Thin-Layer Chromatography* (N. Grinberg, Ed.), pp. 249–283. Marcel Dekker, New York (1990).
[9] G. Szepesi, in *Modern Thin-Layer Chromatography* (N. Grinberg, Ed.), pp. 285–311. Marcel Dekker, New York (1990).
[10] J.C. Touchstone and J. Sherma, *Densitometry in Thin-Layer Chromatography*. Wiley, New York (1979).
[11] C.F. Poole, S.K. Poole and T.A. Dean, in *Recent Advances in Thin-Layer Chromatography* (F.A.A. Dallas, H. Read, R.J. Ruane and D. Wilson, Eds), pp. 11–45. Plenum Press, New York (1988).
[12] D.E. Jenchen, in *Handbook of Thin-Layer Chromatography* (J. Sherma and B. Fried, Eds), pp. 113–134. Marcel Dekker, New York (1991).
[13] J. Janicki, W.D. Walking, R.H. Erlich and C.A. Bainbridge, *J. Pharm. Sci.* **70**, 778–783 (1981).
[14] F.D. Sancillo, Proc. 2nd Int. Symp. on Instrumental HPTLC, Interlaken, Switzerland, 1982, pp. 314–320.
[15] M.L. Cheng and C.F. Poole, *J. Chromatogr.* **257**, 140–146 (1983).
[16] H. Fabre, A. Ramiaramanana, M.D. Blanchin and B. Mandrou, *Analyst* **110**, 1289–1295 (1985).
[17] B. Ohner, in *The Quality Control of Medicines* (P.B. Deasy and R.F. Timoney, Eds), pp. 307–334. Elsevier, Amsterdam (1976).
[18] J. Kirschbaum, in *Steroid Analysis in the Pharmaceutical Industry* (S. Görög, Ed.), pp. 265–299. Ellis Horwood, Chichester (1989).
[19] S. Görög, in *Steroid Analysis in the Pharmaceutical Industry* (S. Görög, Ed.), pp. 196–211. Ellis Horwood, Chichester (1989).
[20] S. Gocan, in *Modern Thin-Layer Chromatography*, pp. 5–138. Marcel Dekker, New York (1990).

[21] H.E. Hauck, M. Mack and W. Jost, in *Handbook of Thin-Layer Chromatography*, pp. 87–113. Marcel Dekker, New York (1991).
[22] L.R. Snyder, *J. Chromatogr.* **92**, 223–231 (1974).
[23] L.R. Snyder, *J. Chromatogr. Sci.* **16**, 223–229 (1978).
[24] J.L. Glajch, J.J. Kirkland, K.M. Squire and J.M. Minor, *J. Chromatogr.* **199**, 57–68 (1980).
[25] J.L. Glajch and J.J. Kirkland, *Anal. Chem.* **54**, 1593–1597 (1982).
[26] D.C. Fennimore, in *Instrumental HPTLC* (W. Bertsch, S. Hara, R.E. Kaiser and A. Zlatkis, Eds), pp. 81–95. Hüthig, Heidelberg (1980).
[27] D.C. Fennimore and C.J. Meyer, *J. Chromatogr.* **186**, 555–561 (1979).
[28] D.E. Janchen, in *Instrumental HPTLC* (W. Bertsch, S. Hara, R.E. Kaiser and A. Zlatkis, Eds), pp. 133–164. Hüthig, Heidelberg (1980).
[29] R.E. Kaiser, *J. Planar Chromatogr. — Modern TLC* **1**, 182–187 (1988).
[30] Sz. Nyiredy, C.A.J. Erdelmeier, B. Meier and O. Sticher, *Planta Med.* **51**, 241–246 (1985).
[31] K. Dallenbach-Tolke, Sz. Nyiredy, B. Meier and O. Sticher, *J. Chromatogr.* **365**, 63–72 (1986).
[32] Sz. Nyiredy, K. Dallenbach-Tolke and O. Sticher, *J. Planar Chromatogr. — Modern TLC* **1**, 336–342 (1988).
[33] S. Ebel, Proc. 3rd Int. Symp. on Instrumental TLC, Würzburg, 1985, pp. 387–403.
[34] S. Ebel, D. Albert and U. Schaefer, Proc. 3rd Int. Symp. on Instrumental TLC, Würzburg, 1985, pp. 405–411.
[35] S. Ebel and P. Post, *J. High Resolut. Chromatogr., Chromatogr. Commun.* **4**, 337–343 (1981).
[36] S. Ebel and J. Hocke, *J. Chromatogr.* **126**, 449–456 (1976).
[37] S. Ebel and E. Glaser, *J. High Resolut. Chromatogr., Chromatogr. Commun.* **2**, 36–40 (1979).
[38] S. Ebel and E. Glaser, *J. High Resolut. Chromatogr., Chromatogr. Commun.* **2**, 133–138 (1979).

[*Received for review 5 May 1992; revised manuscript received 10 July 1992*]