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Overpressured layer chromatography in comparison with thin-layer and high-performance liquid chromatography for the determination of coumarins with reference to the composition of the mobile phase

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Abstract

The retention behaviour of fifteen closely related coumarins in normal-phase overpressured layer chromatography (OPLC) was studied with the aim of comparing the retentions with those in normal-phase thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) when optimization of the mobile phase was carried out according to the PRISMA system. The mobile phase optimization was carried out on TLC plates in unsaturated chambers. The resulting mobile phases were transposed to off-line, non-equilibrated OPLC and further to HPLC. The retention in TLC was measured at 37 selectivity points and in OPLC and HPLC at 13 points. Capacity factors (k') and separation factors (α) were calculated in order to study the retention behaviour in the different systems. Two- and three-dimensional evaluations of k' against selectivity points showed similar retention behaviours for the coumarins in TLC, OPLC and HPLC. The α values for TLC, OPLC and HPLC showed similar patterns in the three-dimensional evaluations. The retention behaviour at different solvent strengths was also examined. According to quadratic regression, k' showed a dependence on the change in solvent strength. OPLC, which can be considered as a "planar column" technique, and TLC are closely related methods, whereas HPLC shows a different behaviour in the elution process with regard to solvent strength.

1. Introduction

Based on the paper by Kirkland and Glajch [1] and the solvent classification of Snyder [2], the PRISMA optimization model was developed for thin-layer chromatography (TLC) and for high-performance liquid chromatography (HPLC) [3,4]. The model has since been applied to overpressured layer chromatography (OPLC) [5] and the different types of rotational planar chromatography (RPC) [6,7].

The basic concept of the PRISMA system [4] is first to optimize the solvent strength and subsequently the selectivity in a triangular mixture solvent diagram. The three selectivity-adjusting solvents are selected from ten solvents used in preliminary experiments and diluted, if necessary, by the solvent strength-adjusting solvent. The optimum solvent mixture is found by testing appropriate solvent mixtures located at "selectivity points". Transfer of the optimized

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mobile phase between various planar and column chromatographic methods is one of the concepts used in the PRISMA system [8,9].

To assess the possibilities of transfer between methods, their specific features have to be taken into account. In conventional TLC, the mobile phase moves by means of capillary forces through the porous thin layer always as an unsaturated flow, and the mobile phase content (and correspondingly the phase ratio) varies with the distance from the source to the front [10]. OPLC is a planar chromatographic technique in which the vapour space is eliminated above the sorbent layer by means of overpressure, and the mobile phase must be forced through the stationary phase using pressure [11,12]. The separation time in this kind of forced-flow technique is usually short, the diffusion effects can be reduced and the bands of compounds are small and compact. Because OPLC can be used as an equilibrated or non-equilibrated "planar column" technique, it may be employed as a pilot technique for the transfer of the optimized TLC mobile phase to the various column chromatographic (CLC) methods [13]. CLC is a closed system in which no vapour phase is present and the eluent is forced through by external pressure. HPLC, which is the most established form of CLC nowadays, is used equilibrated with the mobile phase.

In this work, the retention behaviours of fifteen closely related coumarins was studied using three different methods (TLC, OPLC and HPLC) in order to investigate the transfer of the optimized mobile phase from one method to another according to the PRISMA system. Transferring data from the TLC separation to HPLC has been discussed earlier [14]. The retention measurements were performed at 37 selectivity points for TLC and 13 points for OPLC and HPLC, and also at the middle selectivity point 333 using different solvent strengths of the optimized mobile phase. The aim of the study was to determine if the dependences between the capacity factors and/or separation factors at the selectivity points show similar behaviours in the methods when the same mobile phase solvent composition is transferred from one system to another. Therefore, two- and three-dimensional evaluations were carried out. The solvent strength behaviour of the retentions of the investigated coumarins was also described mathematically and compared between methods. As OPLC is considered as a "column separation in plane" owing to the forced flow of solvent, it was also of interest to discuss which system is actually closer to OPLC.

2. Experimental

2.1. Apparatus

A Linomat IV TLC spotter (Camag, Muttenz, Switzerland) was used to apply the samples on TLC and OPLC plates, and a CS-9000 dual-wavelength flying-spot scanner (Shimadzu, Kyoto, Japan) for the densitometric evaluations. OPLC separations were performed on a Chrompres-25 chromatograph (Factory of Laboratory Instruments, Budapest, Hungary) using a 20-bar external pressure. A pump from Haenni (Switzerland) was used for solvent delivery. Each development was preceded by a prerun with *n*-hexane.

A model 425 HPLC gradient former and a model 420 pump (Kontron Instruments, Rotkreuz, Switzerland), equipped with an ERC-7210 UV detector (ERMA Optical Works, Tokyo, Japan) and a Shimadzu C-R1B integrator were used. The HPLC system was connected to an Olivetti (Ivrea, Italy) model M24 personal computer.

2.2. Chemicals

The coumarins (umbelliferone, herniarin, psoralen, osthol, 2′-angeloyl-3′-isovaleryl vaginate, angelicin, bergapten, oxypeucedanin, ostruthol, isobergapten, sphondin, xanthotoxin, imperatorin, pimpinellin and isopimpinellin) and solvents were obtained as described previously ([15]; see also molecular structures).

2.3. Chromatographic conditions

The coumarins were applied in the form of spots (1 μl of solute in chloroform) in four

groups on the TLC and OPLC plates. The TLC separations were performed on 4 × 10 cm plates in the ascending one-dimensional mode in 6 × 22 cm unsaturated N-chambers (Camag) at ambient temperature. The OPLC separations were performed on 10 × 20 cm plates. The plates were prepared by impregnating all sides with a polymer suspension (Factory of Laboratory Instruments). For the development two channels were scraped in the silica coating, one for the solvent inlet and the other, at a distance of 18 cm, for the solvent outlet. The assays were carried out on alufoil TLC plates coated with silica gel 60 F_{254} (average particle size 10 μm) (Merck, Darmstadt, Germany). The migration distance and the solvent front were measured with a densitometer at 320 nm.

The column for the HPLC separations was LiChrosorb Si 60 (average particle size 10 μm) (250 × 4 mm I.D.) (Merck) at ambient temperature. The flow-rate was 1.0 ml/min and detection was effected at 320 nm. The solvent peak was treated as the dead volume.

2.4. Calculation of retardation data obtained from the analysis

The capacity factors for HPLC (k'_c) were calculated from the equation $k'_c = (t_R - t_0)/t_0$, where t_R is the retention time of the compound and t_0 is the dead time. The retardation values obtained from the TLC plate without correction [$(R_F)_{obs}$] were converted into capacity factor values (k'_p) using the equation $(1/(R_F)_{obs}) - 1$ [10,14].

Calculations and statistical evaluations were performed with a StatView II v1.03 program on a Macintosh IIsi computer. For three-dimensional evaluation of the retardation data, Systat v5.1 software was used.

3. Results and discussion

In preliminary experiments, optimization of the mobile phase was performed using the PRISMA model on normal-phase TLC plates in unsaturated chambers as described [14]. Ethyl acetate (solvent strength $S_i = 4.4$), chloroform ($S_i = 4.1$) and tetrahydrofuran ($S_i = 4.0$) in *n*-hexane ($S_i = 0.0$) were found to give the best separation of these coumarins. Retention measurements were performed by TLC at 37 selectivity points (P_S; see Fig. 1). The solvent strength was adjusted to $S_T = 2.0$ to give retardation factor (R_F) values between 0.2 and 0.8 for the solutes in the TLC assays at the middle selectivity point 333.

OPLC retention measurements were performed at thirteen selectivity points (Fig. 1) using the same solvents and *n*-hexane as the S_T regulator as in the TLC runs. Air and/or gas possibly adsorbed on the surface of the stationary phase was eliminated by a prerun with *n*-hexane. In this case, the application distance from the solvent inlet was adjusted to 5 mm to avoid a decisive influence on the separation of the multi-front formations [16]. S_T was adjusted to 2.2 and the flow-rate to 0.65 at $P_S = 333$ in order to keep the spots of the analysed coumarins as sharp bands. The R_F values for the solutes were also kept between 0.2 and 0.8, when the runs were made in the non-equilibrated mode.

HPLC retention measurements were performed at the same selectivity points as for the OPLC runs. The chosen S_T of 1.2 at $P_S = 333$ gave for the last-eluting compound a capacity factor (k'_c) of less than 20.

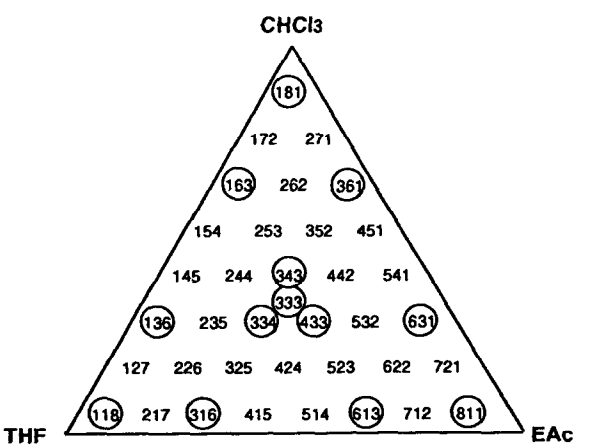

Fig. 1. The selectivity points (P_S) describing the horizontal plane in the PRISMA model (TLC, all; OPLC and HPLC, circled). EAc = Ethyl acetate; $CHCl_3$ = chloroform; THF = tetrahydrofuran.

3.1. Dependence of capacity factors and selectivity points

Regression functions of different order for the measured two-dimensional retention data were compared at constant solvent strength (for S_Ts, see above). The capacity factors of the coumarins at selectivity points along one edge, *i.e.*, 118–811, 811–181 or 181–118, of the PRISMA showed dependences as quadratic regressions of the type $k' = A(P_S)^2 + B(P_S) + C$ (r^2 = 0.98–0.83 for TLC, r^2 = 1.00–0.79 for OPLC and r^2 = 1.00–0.90 for HPLC). The k' value with this mobile phase system was highest at P_S = 181, as can be seen in Fig. 2. The three representative compounds were chosen according to the elution order, *i.e.*, one from the beginning (angelicin), one from the middle (2′-angeloyl-3′-isovaleryl vaginate) and one from the end (ostruthol). The mobile phase composition had a similar effect on the elution of all the coumarins in TLC, OPLC and HPLC. In reversed-phase (RP) HPLC, similar findings for retention were obtained by Nyiredy *et al.* [17]. Outinen *et al.* [18] found that the linear and quadratic functions were insufficient to describe the retention of dansylamides in RP-HPLC. They selected cubic regression functions to describe the dependence.

The three numerical values in P_S were plotted on x–y coordinates against a fourth parameter

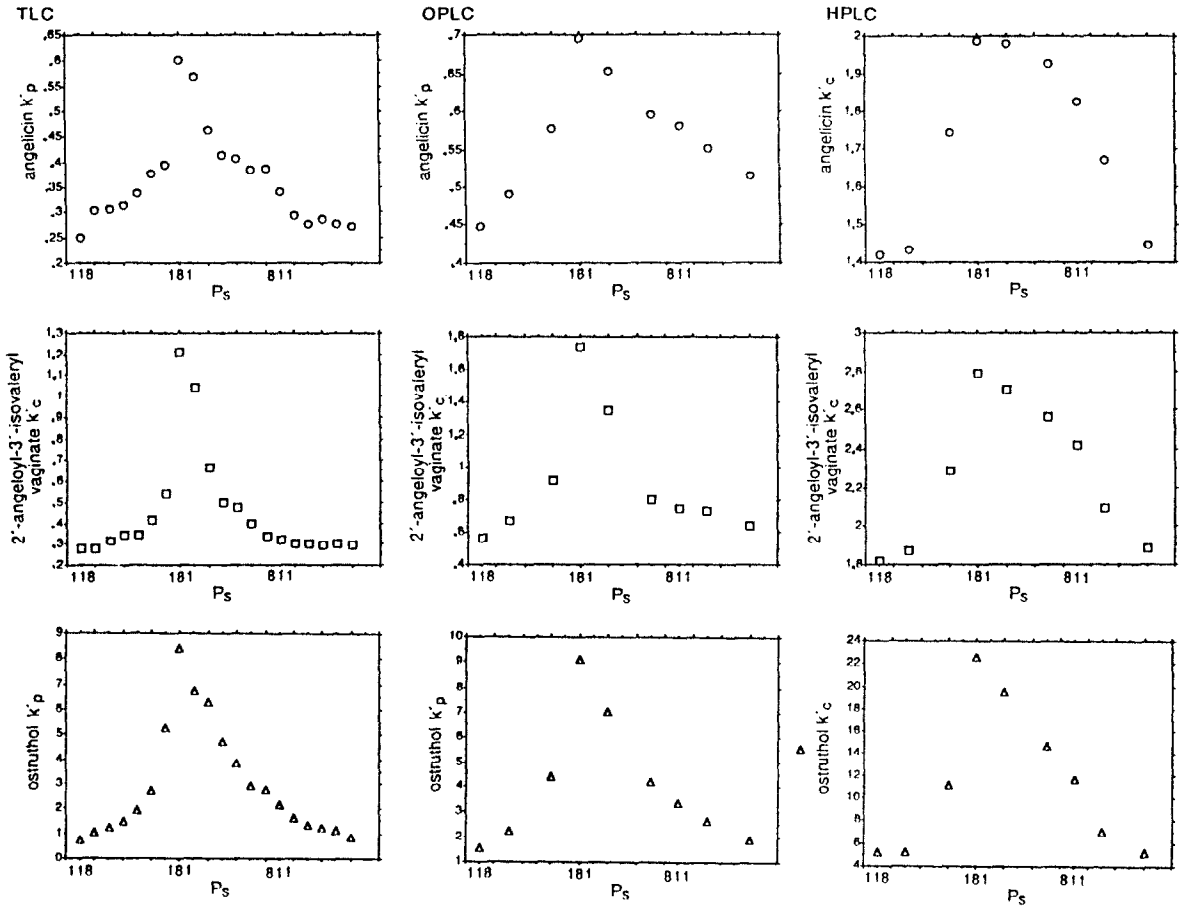

Fig. 2. Dependences between the k' values of angelicin, 2′-angeloyl-3′-isovaleryl vaginate and ostruthol and selectivity points (P_S) between 118–811–181–118.

(z-coordinate: k') in order to obtain three-dimensional figures of the P_S in the prism. Coumarins have similar three-dimensional surfaces in TLC, OPLC and HPLC, as demonstrated by the three representative compounds in Fig. 3. Selectivity point 181 gives the highest capacity factor values, falling to the corner of 118 with the lowest k' values and the surface follows this decreasing trend fairly smoothly. The corner 811 k' values are about half of the maximum values for each compound in TLC and HPLC. OPLC shows a different trend for the middle selectivity compared with the other two methods.

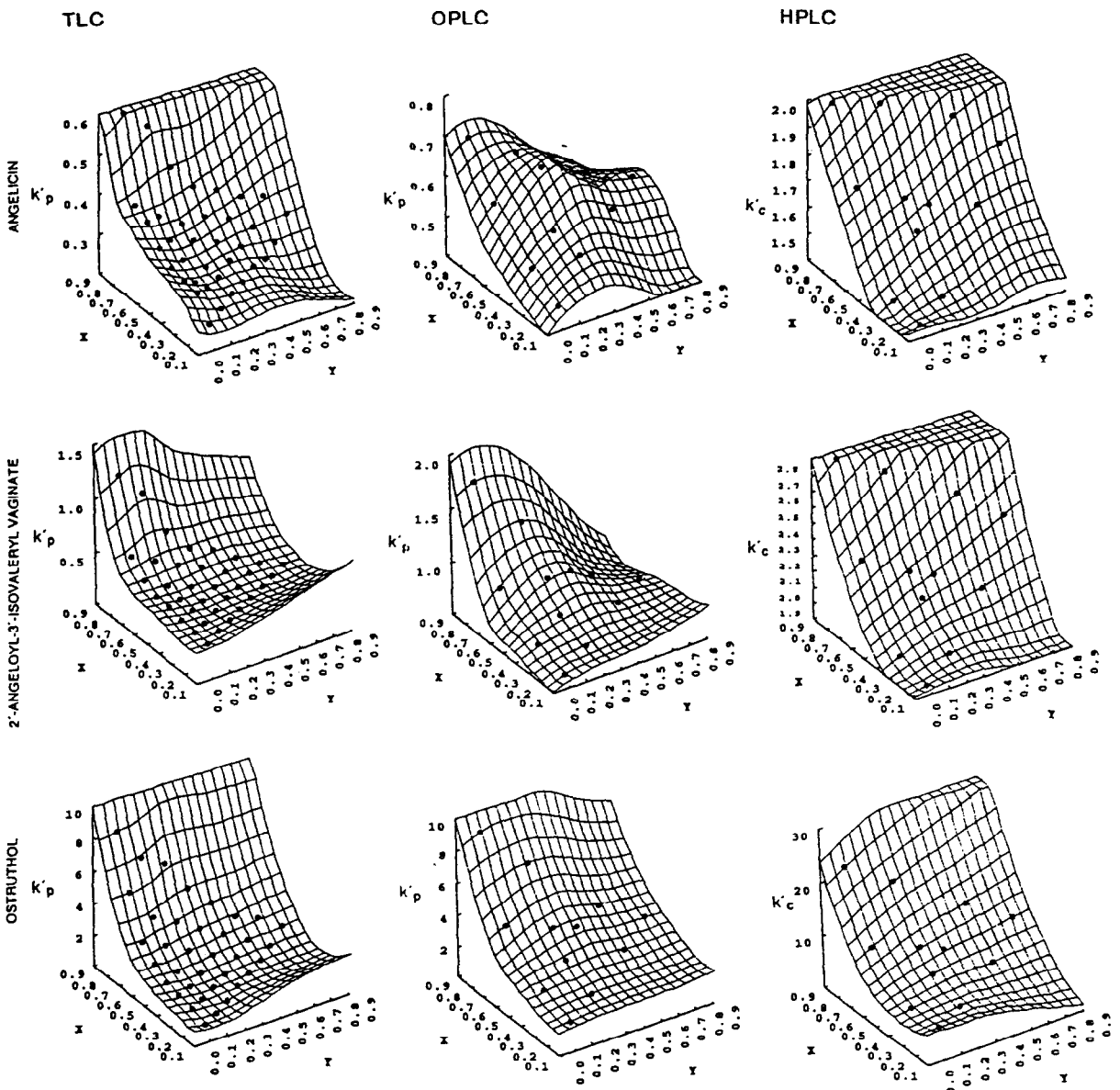

Fig. 3. Three-dimensional k' surfaces of the three representative compounds. $P_S = 118$ (front), 181 (top) and 811 (right).

3.2. Dependence of separation factors and selectivity points

The three-dimensional α (separation factor, $\alpha = k'_2/k'_1$, where k'_1 and k'_2 are the capacity factors of the last- and first-eluted compounds, respectively) surfaces were constructed for the fifteen coumarins at all selectivity points. The behaviour of α is shown in ref. 14 for TLC and HPLC for the first two and last two eluting coumarins, and an average value for seven coumarins eluting in the middle of the run. In OPLC the same kind of behaviour was obtained, *i.e.*, for the first ($1.0 < \alpha < 1.5$) and last ($1.0 < \alpha < 1.8$) α values the surfaces decreased fairly smoothly from P_S 181 down to the corner of 118, and for the compounds eluting in the middle ($1.0 < \alpha < 1.15$) the surface started to rise along the side of P_S 118–811. The range of α values also remained fairly constant in the methods. This indicates that the separation of the coumarins would be similar if the conditions for the analysis are the same in these methods.

3.3. Influence of solvent strength

Solvent strength values of 1.4–2.2 in TLC, 1.4–2.6 in OPLC and 0.8–1.6 in HPLC (S_T steps of 0.2 in all methods) were examined at selectivity point 333. The capacity factors of the coumarins were calculated and are plotted against the solvent strengths demonstrated by angelicin, 2'-angeloyl-3'isovaleryl vaginate and ostruthol in Fig. 4. The capacity factors of the fifteen coumarins showed dependences in all three methods as a quadratic regression of the type $k' = A(S_T)^2 + B(S_T) + C$ ($r^2 = 1.00$–0.96). This result is in accordance with that of Vuorela *et al.* [19] for six investigated coumarins in TLC ($0.45 < S_T < 1.15$). Outinen *et al.* [20] also demonstrated that the dependence between the k' values of seventeen dansyl amides and the rate of change in the gradients at solvent strength $S_T = 0.5 \rightarrow 2.6$ in RP-HPLC followed a quadratic regression function ($r^2 = 1.00$–0.98). The dependences for the coumarins were not linear over the investigated k' range and the solvent strengths used for OPLC in this study.

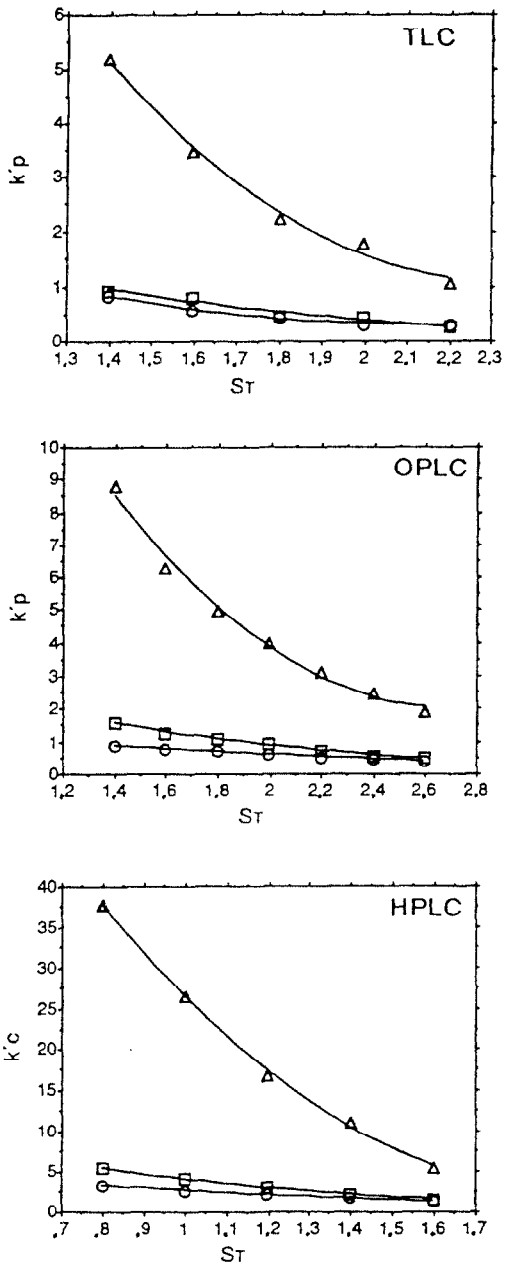

Fig. 4. Dependences between the k' values of (○) angelicin, (□) 2'-angeloyl-3'-isovaleryl vaginate and (△) ostruthol and the solvent strengths (S_T) tested in TLC, OPLC and HPLC at $P_S = 333$.

The behaviour of S_T was investigated further. In order to compare the changes in retention with different S_T values in these methods, the k'_p

and k'_c values at the joint S_T value of 1.4 were plotted against k'_p and k'_c values at the other S_T, and regression analysis was carried out (Table 1). The slopes of the curves (A in Table 1) were further plotted against various S_T (Fig. 5). The curves obtained for TLC and OPLC compared with HPLC had clearly different slope values, whereas the functions for TLC and OPLC showed similar S_T behaviour. Changing S_T in HPLC causes a much larger change in the retention behaviour of the coumarins. This indicates that, for unsaturated TLC and off-line, non-equilibrated OPLC, a change in S_T causes a similar change in the retention behaviour of the compounds and these methods are therefore more closely related to each other than to HPLC.

When a multi-component mobile phase is used, a mobile phase gradient might arise along the migration distance due to mobile phase demixing. Moreover, precoating the stationary phase with molecules of the mobile phase can modify the surface properties, a phenomenon that might affect the separation. Petrović and Acanski [21] have shown that small changes of the silica surface affect only the phase ratio of the TLC system, whereas adsorption of acetic acid vapour by the silica surface has a significant influence on both the equilibrium constant of a solute and the phase ratio. A short "equilibration" of a few minutes can, but need not, lead to

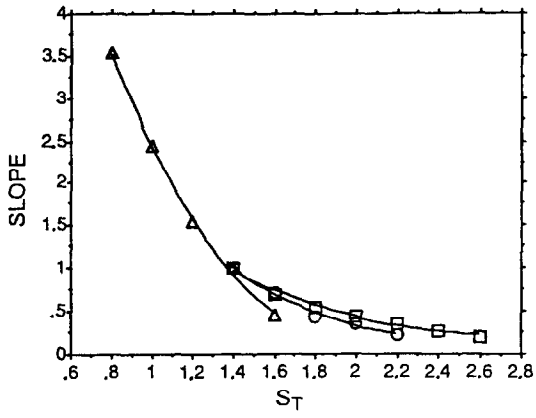

Fig. 5. Plot of slope values against various S_T. ○ = TLC; □ = OPLC; △ = HPLC.

sorptive saturation [22]. Further, there are differences in the evaporation of the different solvents from the absorbent layer. For instance, chloroform, which has a high solvent strength value (4.1) in normal-phase systems, evaporates more readily from the normal-phase adsorbent layer than, *e.g.*, *n*-hexane ($S_i = 0.0$) [23], making the separation process complex. This causes changes in the solvent strength and the selectivity of the mobile phase in use. OPLC pre-equilibrated with the mobile phase might be considered closer to equilibrated CLC, because the selectivity of the mobile phase is considered to be the same all over the adsorbent [13]. These phenomena might be responsible for the differ-

Table 1
Slopes (A), intercepts (B) and correlation coefficients (r) for equations obtained from $k'_{S_{Tx}} = Ak'_{S_{T1.4}} + B$ for TLC, OPLC and HPLC from fifteen coumarins

S_T	TLC			OPLC			HPLC		
	A	B	r	A	B	r	A	B	r
0.8							3.53	−3.53	0.99
1.0							2.46	−1.86	0.99
1.2							1.54	−0.54	1.00
1.4	1.00	0	1.00	1.00	0	1.00	1.00	0	1.00
1.6	0.72	−0.01	0.98	0.71	0.22	1.00	0.46	0.77	0.97
1.8	0.45	0.10	0.99	0.55	0.31	0.99			
2.0	0.35	0.06	0.99	0.44	0.35	0.99			
2.2	0.22	0.13	0.94	0.34	0.32	0.98			
2.4				0.27	0.31	0.97			
2.6				0.20	0.28	0.96			

ences in the separation of compounds between TLC, OPLC and HPLC.

4. Conclusions

Using a multi-component eluent results in the same kind of behaviour with regard to the capacity factors of the fifteen coumarins. In the two- and three-dimensional evaluations of the capacity factors in TLC, OPLC and HPLC, similar behaviour of the coumarins occurred when the mobile phase selectivity was changed, *i.e.*, retention of the compounds is similarly dependent on the mobile phase composition. A similar, three-dimensional figure for the α values is obtained with the three methods, which indicates similar separations of compounds when transferring analytical conditions between the methods. The selectivity of the mobile phase seems to remain the same in the methods, whereas a change in solvent strength in TLC and OPLC has a different effect on the retention behaviour of the compounds to that for a change in S_T in HPLC. This should be taken into consideration when transferring the mobile phase composition from TLC via OPLC to HPLC.

5. Acknowledgement

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6. References

[1] J.J. Kirkland and J.L. Glajch, *J. Chromatogr.*, 255 (1983) 27.
[2] L.R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.
[3] S. Nyiredy, C.A.J. Erdelmeier, B. Meier and O. Sticher, *Planta Med.*, 51 (1985) 241.
[4] S. Nyiredy, B. Meier, C.A.J. Erdelmeier and O. Sticher, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 186.
[5] K. Dallenbach-Tölke, S. Nyiredy, B. Meier and O. Sticher, *J. Chromatogr.*, 365 (1986) 63.
[6] S. Nyiredy, C.A.J. Erdelmeier, B. Meier and O. Sticher, *GIT Suppl. Chromatogr.*, 4/85 (1985) 24.
[7] S. Nyiredy, K. Dallenbach-Tölke and O. Sticher, in: F.A.A., Dallas, H. Read, R.J. Ruane and I. Wilson (Editors), *Recent Advances in Thin Layer Chromatography*, Plenum Press, New York, 1988, pp. 45–54.
[8] S. Nyiredy, K. Dallenbach-Tölke and O. Sticher, *J. Planar Chromatogr.*, 1 (1988) 336.
[9] G.C. Zogg, *Thesis*, No. 8780, ETH Zurich, Zurich, 1989.
[10] F. Geiss, *Fundamentals of Thin Layer Chromatography (Planar Chromatography)*, Hüthig, Heidelberg, 1987.
[11] E. Tyihák, E. Mincsovics and H. Kalász, *J. Chromatogr.*, 174 (1979) 75.
[12] S. Nyiredy, *Application of the "PRISMA" Model for the Selection of Eluent Systems in Over-Pressure Layer Chromatography (OPLC)*, Labor MIM, Budapest, 1987.
[13] S. Nyiredy, K. Dallenbach-Tölke, G.C. Zogg and O. Sticher, *J. Chromatogr.*, 499 (1990) 453.
[14] P. Härmälä, H. Vuorela, E.-L. Rahko and R. Hiltunen, *J. Chromatogr.*, 593 (1992) 329.
[15] P. Härmälä, *J. Planar Chromatogr.*, 6 (1991) 460.
[16] S. Nyiredy, C.A.J. Erdelmeier and O. Sticher, in E. Tyihák (Editor), *Proceedings of the International Symposium on TLC with Special Emphasis on Overpressured Layer Chromatography (OPLC), Szeged, Hungary*, Labor MIM, Budapest, 1986, pp. 222–231.
[17] S. Nyiredy, K. Dallenbach-Tölke and O. Sticher, *J. Liq. Chromatogr.*, 12 (1989) 95.
[18] K. Outinen, H. Vuorela and R. Hiltunen, *Acta Pharm. Fenn.*, 101 (1992) 11.
[19] H. Vuorela, K. Dallenbach-Tölke, R. Hiltunen and O. Sticher, *J. Planar Chromatogr.*, 1 (1988) 123.
[20] K. Outinen, V.-M. Lehtonen, R. Hiltunen and H. Vuorela, *XII Helsinki University Course in Drug Research, Abstract Book*, The Finnish Pharmaceutical Society, Helsinki, 1993, p. 43.
[21] S.M. Petrović and M. Acanski, *J. Planar Chromatogr.*, 6 (1991) 439.
[22] F. Geiss, *J. Planar Chromatogr.*, 2 (1988) 102.
[23] P. Merkku, J. Yliruusi, H. Vuorela and R. Hiltunen, *J. Planar Chromatogr.*, submitted for publication.