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Retention behaviour of closely related coumarins in thin-layer chromatographic preassays for high-performance liquid chromatography according to the "PRISMA" model

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ABSTRACT

The retention behaviour of fourteen closely related coumarins in normal-phase thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) was studied with the aim of testing the suitability of TLC as a preassay for HPLC when the optimization of the mobile phase has been carried out according to the "PRISMA" system. The retention (retardation) in TLC and HPLC was measured at 37 and 13 selective points, respectively. The retention behaviour at different solvent strengths was also examined. Capacity factors (k') and separation factors (α) were calculated to study the retention behaviour in the two systems. Two- and three-dimensional evaluations of k' against selectivity points showed similar retention behaviour for the coumarins in TLC and HPLC. According to quadratic regression, k' showed a dependency on the change in solvent strength. Similar behaviour of α values for TLC and for HPLC was demonstrated in three-dimensional evaluations.

INTRODUCTION

Coumarins are widely distributed in plants, especially in the Apiaceae and Rutaceae families. Coumarins usually contain many heteroatoms. The number and position of the various substituents in the coumarin molecule significantly influence their adsorption behaviour in thin-layer chromatography (TLC) and column chromatography [1].

Glowniak and Bieganowska [1,2] have studied the retention behaviours of coumarins using both normal- and reversed-phase TLC and high-performance liquid chromatography (HPLC). They studied the effects on retention of solvent composition and the individual substituents in the solute molecules. They found a linear relationship between the experimentally obtained retentions and the concentration of the organic modifier. The most selective mobile phase with respect to the effects of a substituent on retention was chosen by plotting the retention values of the coumarins against the mobile phase.

Some theoretical aspects concerning the use of TLC as a pilot technique for column liquid chromatography (CLC) have been presented by Różylo and Janicka [3]. Their work utilizes a thermodynamic description of adsorbent–binary solution–solute systems to characterize a given chromatographic process, and they investigated the effect of the chromatographic technique employed on the thermodynamic description of the chromatographic system. Różylo and Janicka [3] concluded that retention data obtained from sandwich chambers described adsorption from solutions in the same way as measurements from column liquid chromatography.

Nyiredy *et al.* [4] have presented strategies of mobile phase transfer from thin-layer to medium-pressure liquid column chromatography (MPLC) with silica as the stationary phase. The major advantage of the strategies was that mobile phase transfer started from a TLC separation in which the whole R_F range was used, in contrast to the general rule [5] that all zones should be below $R_F = 0.3$ in TLC.

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However, the prediction of the final MPLC result was improved when overpressured layer chromatography (OPLC) was used as a pilot method.

The "PRISMA" optimization model assists in the selection of optimal eluent systems for both planar chromatographic techniques and column chromatographic techniques [6]. The PRISMA model can be visualized as a graphic representation of the solvent strengths (S_T) and the proportions of the solvents (P_S). The prism described by PRISMA consists of an unlimited number of triangular solvent diagrams (horizontal functions; P_S) in which every triangular plane corresponds to a different solvent strength (vertical function; S_T) [7]. The PRISMA optimization system consists of three parts. In the first part the basic parameters such as the stationary phase and the solvents are selected. In the second part of the system the optimal combination of the selected solvents is achieved using the actual PRISMA model. The third part includes selection of a suitable method and transfer of the optimized mobile phase to the various chromatographic techniques.

The aim of this study was to test TLC as a preassay for HPLC when optimization of the mobile phase has been carried out according to the PRISMA system [6], as demonstrated by fourteen closely related coumarins. A total of 37 selectivity points (P_S) in the PRISMA model were examined using TLC, and 13 selectivity points were examined using HPLC. The retention was measured at five solvent strengths (S_T) at $P_S = 333$. The capacity factors (k'), and the separation factors (α) of the two methods were calculated and compared. Regression analysis and three-dimensional evaluations were performed in order to study the predictability of the HPLC behaviour and optimization on the basis of the TLC experiments.

EXPERIMENTAL

Apparatus

A Linomat IV TLC spotter (Camag, Muttenz, Switzerland) was used to apply the samples to TLC plates, and a CS-900 dual-wavelength flying-spot scanner (Shimadzu, Kyoto, Japan) was used for the densitometric evaluations. A 425 HPLC gradient former and 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 M24 personal computer (Olivetti, Ivrea, Italy).

Chemicals

The coumarins (Fig. 1) bergapten, imperatorin, osthol, ostruthol, oxypeucedanin, psoralen, 2′-angeloyl-3′-isovaleryl vaginate and xanthotoxin were isolated from *Angelica archangelica* L. at the Pharmacognosy Division, Department of Pharmacy, University of Helsinki. Angelicin, herniarin and umbelliferone were obtained from Roth (Karlsruhe, Germany). Isobergapten, pimpinellin and sphondin were isolated from *Heracleum sphondylium* L. and identified at the Department of Pharmacy, ETH Zürich, Switzerland. The *n*-hexane was of technical grade (Oy Exxon Chemicals; Espoo, Finland) and was filtered before use. 1,4-Dioxane, methyl ethyl ketone, 2-propanol and toluene were of reagent grade and they were obtained from Merck (Darmstadt, Germany). Diethyl ether and acetic acid of analytical quality were from May & Baker (Dagenham, UK). The chloroform stabilized with ethanol of analytical quality was from RP Normapur (Paris, France), and absolute ethanol was from Alko (Helsinki, Finland). All other solvents, *i.e.* dichloromethane, tetrahydrofuran and ethyl acetate, were of HPLC quality (Rathburn, Walkerburn, UK).

Chromatographic conditions

The TLC separations were performed on 10 × 4 cm plates in ascending one-dimensional mode in 22 × 6 cm unsaturated N-chambers (Camag) at ambient temperature. The solvent volume was 5 ml and the migration distance was 8.5 cm. The assays were carried out on alufoil, silica gel 60 F_{254} (average particle size 10 μm) TLC plates (Merck, Germany). The migration distances and the solvent fronts were measured with the densitometer at 320 nm.

The column for the HPLC separations was a 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.

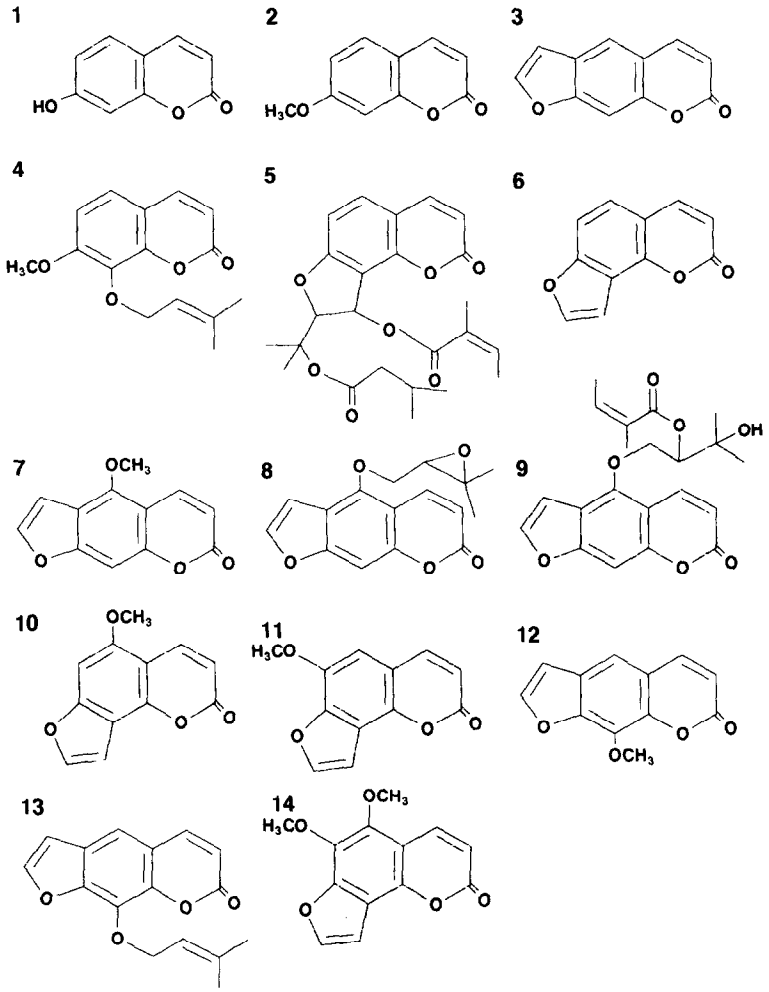

Fig. 1. Structures of the coumarins used in the study in the order according to the classification of Murray *et al.* [8]. 1 = Umbelliferone; 2 = herniarin; 3 = psoralen; 4 = osthol; 5 = 2′-angeloyl-3′-isovaleryl vaginate; 6 = angelicin; 7 = bergapten; 8 = oxypeucedanin; 9 = ostruthol; 10 = isobergapten; 11 = sphondin; 12 = xanthotoxin; 13 = imperatorin; 14 = pimpinellin.

Correlation between retention data obtained from TLC and HPLC

Calculation of the correlations and the regression analysis were performed with Stat View SE + Graphics software on a Macintosh SE computer. The Systat procedure was used for three-dimensional evaluation of the retention data and the separation factors.

RESULTS AND DISCUSSION

Ethyl acetate ($S_T = 4.4$), chloroform ($S_T = 4.1$) and tetrahydrofuran ($S_T = 4.0$) in *n*-hexane ($S_T = 0$) were selected according to the PRISMA model [6] to give the best separation of the fourteen coumarins in unsaturated chambers with normal-phase TLC plates. Retention measurements were performed in TLC at 37 selectivity points (P_S) using the three selected solvents, *n*-hexane serving as the solvent strength (S_T) regulator (Fig. 2). P_S describes the proportions of the selected solvents for the mobile phase. For example, a mobile phase characterized by $P_S = 181$ and $S_T = 2.0$ is a combination consisting of 4.5% ethyl acetate, 39.0% chloroform and 5.0% tetrahydrofuran adjusted with 51.5% *n*-hexane. The influence of S_T at the middle selectivity

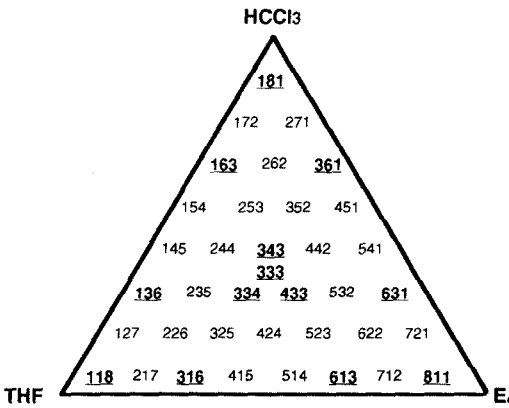

Fig. 2. The solvents selected for the analysis and selectivity points (P_S) studied (TLC, all; HPLC, underlined). EAC = Ethyl acetate; THF = tetrahydrofuran.

point, $P_S = 333$, was tested by varying S_T from 1.4 to 2.2. Solvent strength was adjusted to $S_T = 2.0$, on the basis of these experiments, to give retardation factor (R_F) values between 0.2 and 0.8 for the solutes in the TLC assays.

HPLC retention measurements were performed at thirteen selectivity points (Fig. 2) using the same solvents and *n*-hexane as the S_T regulator, as in the TLC runs. The influence of S_T at the middle selectivity point, $P_S = 333$, was tested by varying S_T from 0.8 to 1.6. For HPLC analysis S_T was adjusted to $S_T = 1.2$ to give the last-eluting compound a capacity factor (k') of less than 20.

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 volume. The R_F values from TLC were transformed so as to obtain capacity factor values (k'_p) similar to k'_c values in HPLC, $k'_p = [1/(R_F)_{obs}] - 1$, using the observed values $(R_F)_{obs}$ from the plate without correction [9,10].

The obtained experimental capacity factors were plotted against the selectivity points along the edges of the prism. Three representative compounds are shown in Fig. 3. The compounds were chosen according to elution, *i.e.* one from the beginning (pimpinellin; 14), one from the middle (herniarin; 2) and one from the end (umbelliferone; 1). The mobile phase composition has a similar effect on the elution of all the coumarins in both TLC and HPLC. The k' value is highest at $P_S = 181$.

The possible existence of a mathematical dependency between the capacity factor and P_S was also investigated. Regression functions of different order for the measured two-dimensional retention data were compared at constant S_T, *i.e.* 2.0 for TLC and 1.2 for HPLC. The capacity factors of the coumarins at selectivity points along one edge, *i.e.*, 118-181, 181-811 or 811-118, of the PRISMA showed high dependences, with quadratic regressions of the type $k' = A(P_S)^2 + B(P_S) + C$ ($r = 0.99$–0.91 for TLC and $r = 1.00$–0.95 for HPLC). In reversed-phase HPLC similar findings for retention have been obtained by Nyiredy *et al.* [11].

Three-dimensional k' surfaces of each compound were constructed at all the investigated tertiary selectivity points. The three numerical values of P_S were plotted on an x–y coordinate against a fourth parameter (z-coordinate; k') in order to obtain three-dimensional figures of the P_S in the prism. Coumarins have similar three-dimensional surfaces in both TLC and HPLC, as demonstrated by pimpinellin (14), herniarin (2) and umbelliferone (1) in Fig. 4. Selectivity point 181 gives the highest capacity factor values, falling to the corner of 118 with the lowest k' values. The surface follows this decreasing trend rather smoothly, the corner 811 k' values being about half of the maximum values for each compound.

The influencec of solvent strength in TLC was compared with that in HPLC. S_T values of 1.4, 1.6, 1.8, 2.0 and 2.2 in TLC were examined and 0.8, 1.0, 1.2, 1.4 and 1.6 in HPLC at selectivity point 333. The capacity factors (k'_p and k'_c) of the coumarins were calculated and plotted against the solvent strengths, as demonstrated by pimpinellin, herniarin and umbelliferone (Fig. 5). The capacity factors in both methods showed high dependences, with quadratic regression of the type $k' = A(S_T)^2 + B(S_T) + C$ ($r = 1.00$–0.99). In this study the dependences for the coumarins were not linear over the investigated k' range and the solvent strengths used, which is in accordance with the findings of Vuorela *et al.* [12].

The behaviour of S_T in these two methods was studied further. In order to compare the changes in retention with different S_T values in the two methods, 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

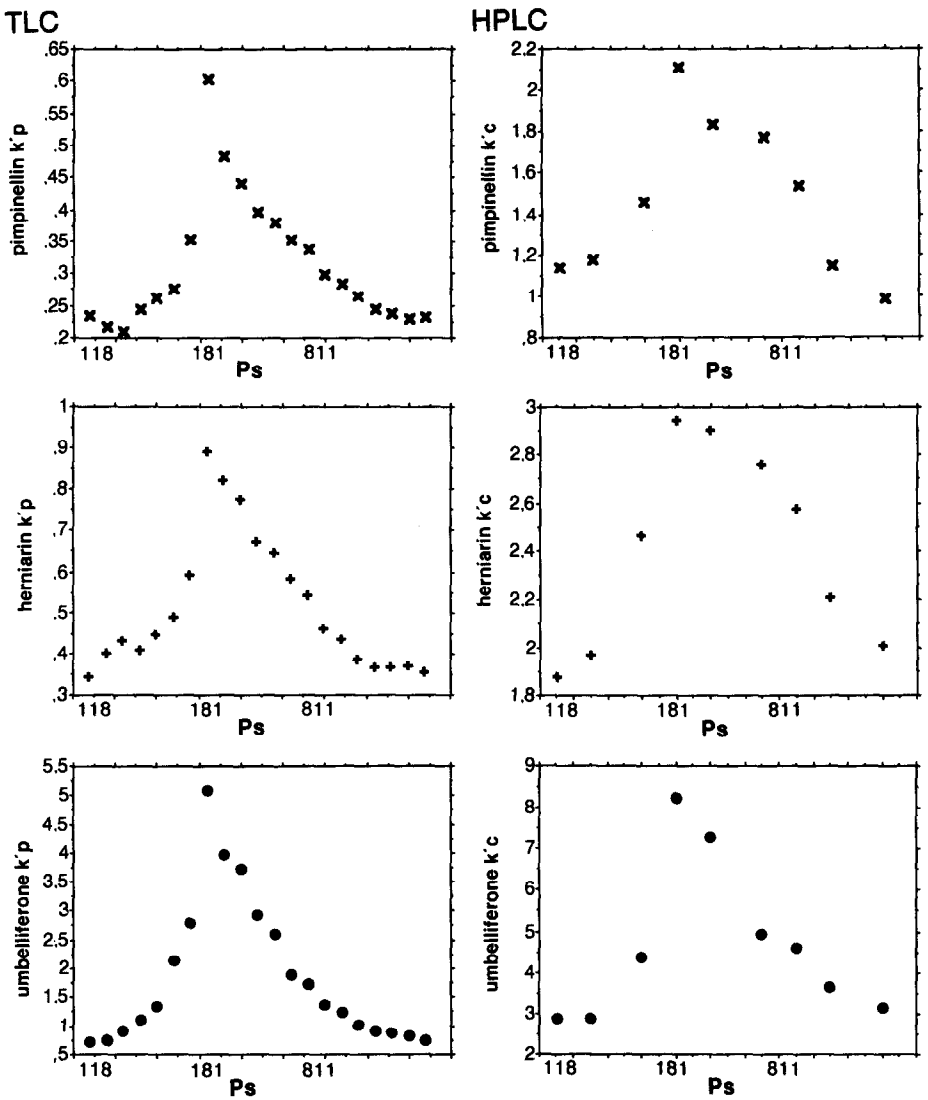

Fig. 3. The capacity factors (k') of three coumarins in P_S along the edges, *i.e.* 118, 181 and 811.

I). The slopes of the curves (A in Table I) were further plotted against S_T (Fig. 6). Two curves for TLC and HPLC were obtained with different slope values. The functions for TLC and HPLC showed clearly different S_T behaviour. This indicates that a change in S_T causes a different change in the retention behaviour of the compounds in TLC and HPLC. This has to be taken into consideration when transferring the mobile phase from TCL to HPLC.

The possibility of predicting the retention in HPLC from TLC experiments at the change in S_T was tested. TLC $S_T = 2.0$ capacity factors were plotted against HPLC $S_T = 1.2$. Fitted k'_c values for HPLC at $S_T = 1.2$ k'_c were calculated using the regression function (Stat View SE + Graphics software). The fitted and experimental k'_c values in HPLC at $S_T = 1.2$ were compared (Fig. 7). A high dependency ($r = 0.93$) was obtained between the fitted and experimental k'_c, thus making it possible to

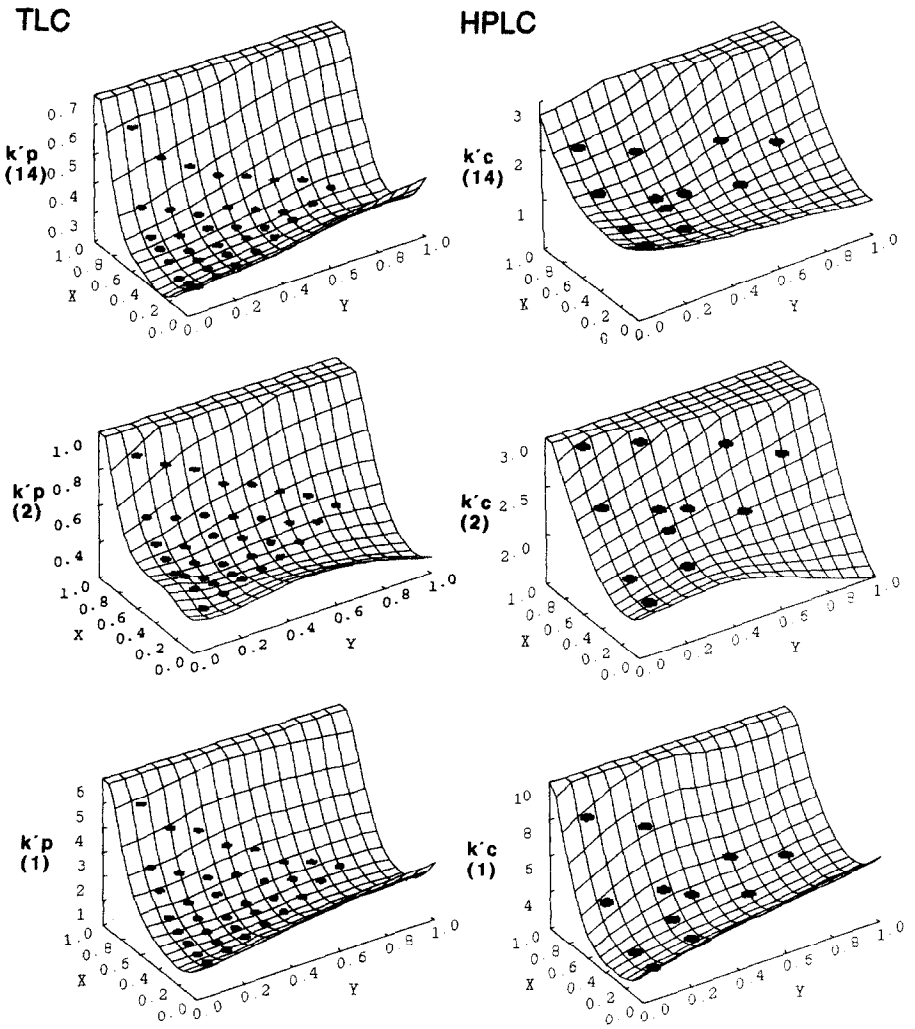

Fig. 4. The three-dimensional k' surfaces in TLC and HPLC for the three representative compounds ($P_S = 118$ at the front, $P_S = 181$ at the top, $P_S = 811$ on the right).

evaluate the effect of S_T on HPLC from TLC runs.

The separation factor (α) was defined as k'_2/k'_1, where k'_2 is the capacity factor for the second-eluting peak and k'_1 that for the first-eluting peak. The three-dimensional α surfaces for each compound at all P_S values were constructed as described for the three-dimensional k' surfaces. Fig. 8 shows the behaviour of α as demonstrated by the two first- (α_1) and two last- (α_{13}) eluting coumarins, and an average value (α_x) for seven coumarins eluting in the middle of the run. The average value was calculated because the coumarins elute close to each other over a k' range of less than 1.25, and their relative elution order changed from one selectivity point to another.

The α_1 values give a starting inverted saddle for TLC and an inverted saddle for HPLC. In α_x the saddles are no longer so pronounced. in α_{13} the surfaces decrease quite smoothly from P_S 118 down to the corner of 118 in TLC and HPLC. The α surfaces behave alike in the two methods. This is in agreement with the fact that the distances between the

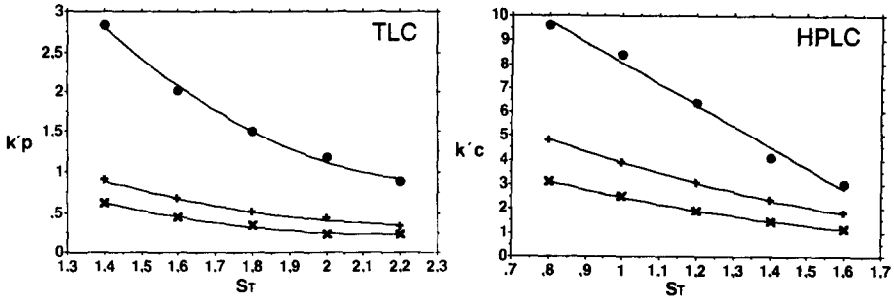

Fig. 5. The capacity factors plotted against the five solvent strengths (S_T) tested in TLC and HPLC at $P_S = 333$. × = Pimpinellin; + = herniarin; ● = umbelliferone.

TABLE I

SLOPES (A), INTERCEPTS (B) AND CORRELATION COEFFICIENTS (r) FOR EQUATIONS OBTAINED FROM $k'_{S_{Tx}} = Ak'_{S_{T1.4}} + B$ FOR TLC AND HPLC AT $P_S = 333$

S_T	TLC			HPLC		
	A	B	r	A	B	r
0.8				3.47	−3.00	0.99
1.0				2.43	−1.58	0.99
1.2				1.53	−0.46	1.00
1.4	1.00	0	1.00	1.00	0	1.00
1.6	0.87	−0.19	0.99	0.47	0.66	0.97
1.8	0.43	0.11	0.99			
2.0	0.35	0.08	0.95			
2.2	0.20	0.14	0.95			

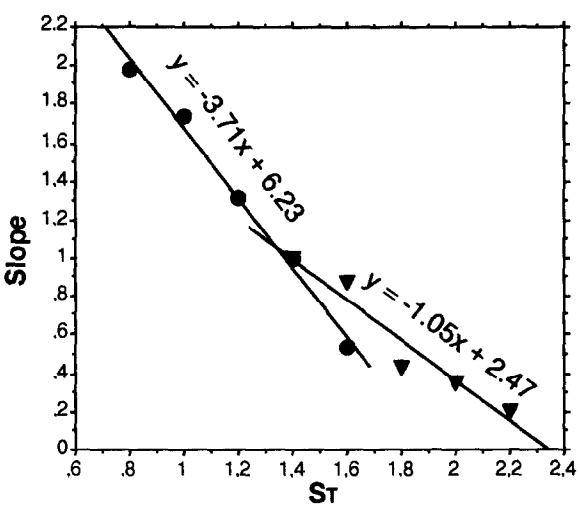

Fig. 6. The slope values plotted against various S_T. ▼ = TLC; ● = HPLC.

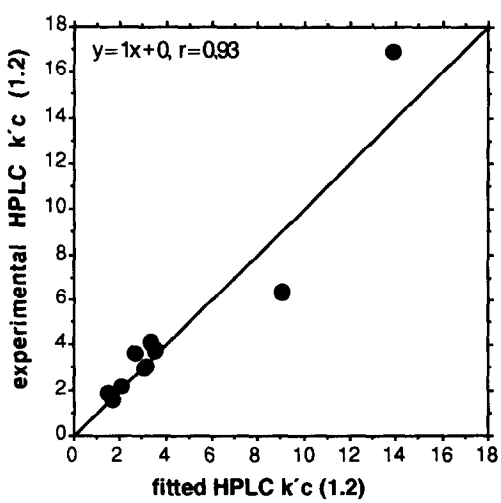

Fig. 7. Comparison of fitted k'_c values and experimentally obtained k'_c values.

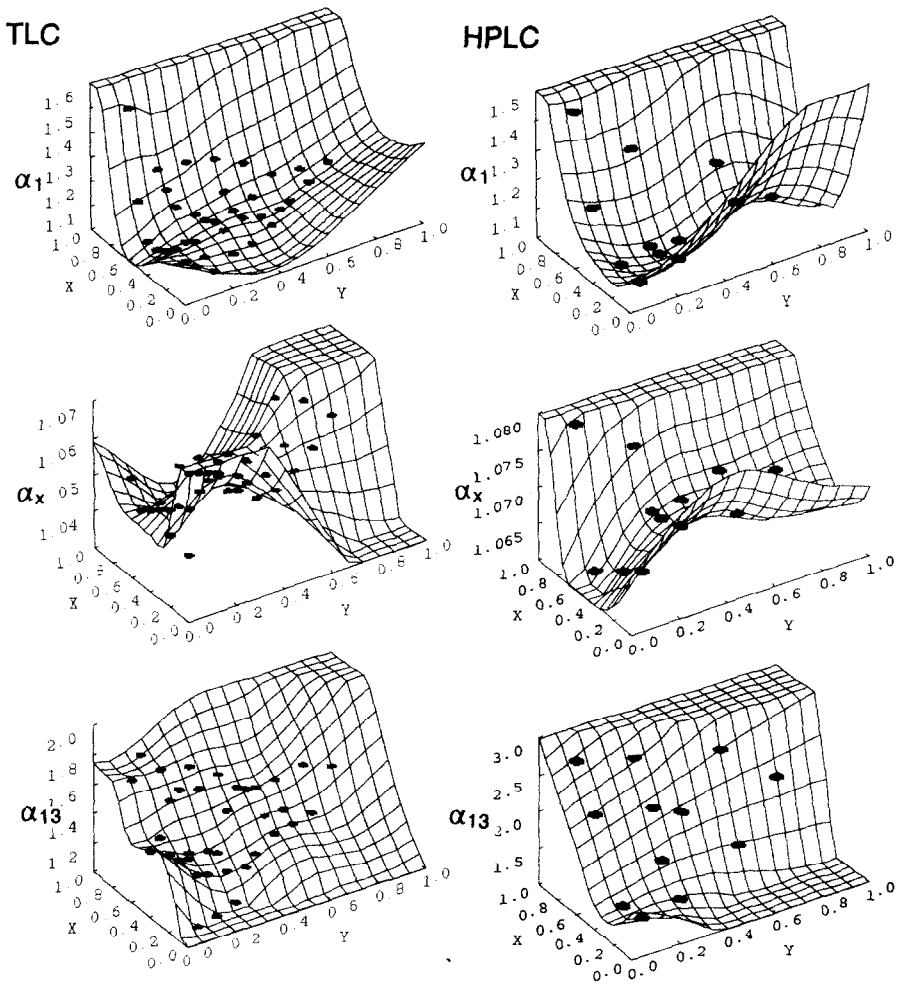

Fig. 8. The separation factors constructed as α surfaces of the PRISMA ($P_S = 118$ at front, $P_S = 181$ at the top, $P_S = 811$ to the right).

peaks follow an opposite order of detection in TLC and HPLC. It should also be borne in mind that the first-eluting compound in HPLC, which is usually the sharpest, is the compound with the longest elution in TLC (broadest). This is the case for the space between two compounds as well, *e.g.* the distance between compounds eluting furthest on the TLC plate is large, whereas in HPLC it is smaller and *vice versa*. The range of the α values is kept rather constant in the methods.

To conclude, using a multicomponent elutent results in the same kind of behaviour with regard to the capacity factors of these fourteen coumarins. In the two- and three-dimensional evaluations of the capacity factors in TLC and HPLC, rather similar behaviour of the coumarins can be observed when the mobile phase selectivity is changed. In TLC and HPLC, retention of the compounds is similarly dependent the on mobile phase composition. It should be noted that a change in S_T in TLC has a different effect on the retention behaviour of the compounds than a change in S_T in HPLC. The range of the α values is kept rather constant in TLC and HPLC. A similar, three-dimensional figure for the α values is obtained with the methods.

The results showed that in normal-phase chromatography TLC can be used as an HPLC preassay method in the PRISMA system. The use of TLC

has the advantage that a great number of solvents can be screened for optimization of the mobile phase. It also gives preliminary information about the retention behaviour in HPLC (k' and α). The selectivity in HPLC can be described by experiments in TLC. However, the following must be taken into account when transferring the mobile phase: the elution process with regard to solvent strength is different in TLC and HPLC.

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