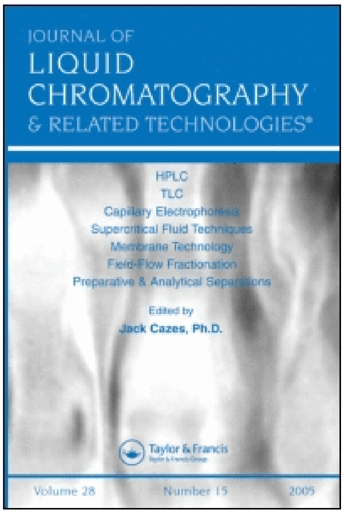

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Gullner, Gábor , Cserháti, Tibor , Bordás, Barna and Valkó, Klára(1989) 'Multivariate Methods to Evaluate the Role of Mixed Supports in Reversed-Phase Thin-Layer Chromatography', Journal of Liquid Chromatography & Related Technologies, 12: 6, 957 — 978

To link to this Article: DOI: 10.1080/01483918908051773

URL: http://dx.doi.org/10.1080/01483918908051773

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(6), 957-978 (1989)

MULTIVARIATE METHODS TO EVALUATE THE ROLE OF MIXED SUPPORTS IN REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Hydrophobic properties of 17 aniline and phenol derivatives were characterized by reversed-phase thin-layer chromatographic and high performance liquid chromatographic retention data.

In order to elucidate the role of thin-layer chromatographic supports in the hydrophobicity determination paraffin coated silica, aluminium oxide, cellulose, diatomaceous earth and their mixtures were used. Water, water-methanol 7:3 and 1 M NaCl served as mobile phases. The retention data were analyzed by spectral mapping technique.

The potency values differed from support to support proving that the composition of support has a deciding role in the hydrophobicity determination of aniline and phenol derivatives. The eluents did not influence considerably the potency order of supports.

The two dimensional non linear mapping of spectral data clearly showed that each sorbent influenced considerably the selectivity.

Good linear correlations were found between the reversed-phase high performance liquid chromatographic parameters of the compounds in partially dissociated state and the R values measured on supports containing high percentage of silica and diatomaceous earth.

INTRODUCTION

Hydrophobic properties of compounds are one of the most important physico-chemical parameters used in quantitative structure-activity relationship studies /1-3/. Lipophilic properties of compounds can be characterized by the logarithm of octanol-water partition coefficients (log P) measured by shake-flask method /4/. Correlation was found between reversed-phase thin-layer chromatographic (RP-TLC) retention data /5-7/, high performance liquid chromatographic (HPLC) retention data /8-10/ and gas-liquid chromatographic (GLC) retention data /11-13/ and log P values. All of these correlations depend on the similarity of the chromatographic partition systems to the octanol/water partition system used as reference.

Recent results indicate that in RP-TLC the adsorption characteristics of the impregnated supports also influence considerably the retention /14, 15/. In the case of 5-nitroimidazole derivatives it was shown that the quality of the impregnating agent influenced considerably the R_M values, however, the correlations between the R_M values determined on silica plates impregnated with various lipophilic agents were very good /16/. These lipophilicity values have been successfully correlated with the biological activity data /17/.

It was also revealed that for polar compounds not only the adsorptive strength but also the surface pH values of supports has a considerable influence on the lipophilicity determination /18/.

The aim of our present study was to evaluate the role of thin-layer supports in the traditional RP-TLC retention behaviour of basic (aniline) and acidic (phenol) compounds. Silica and aluminium oxide were applied as strong, cellulose and diatomaceous earth as weak sorbents. Silica represented a sorbent with acidic, aluminium oxide with alkaline surface pH values. Their mixtures served as to show the continuity of the adsorptive properties of them. The new covalently bonded silica supports were ommitted from our investigations, as their applications were reviewed in details by Brinkman and De Vries /19/.

Reversed-phase high performance liquid chromatographic retention data of the 17 model compounds were used as a reference measure of their lipophilicity.

EXPERIMENTAL

The following chromatographic sorbents were applied: Kieselgel 60 G (Merck Inc., Darmstadt, F. R.

G.), MN Aluminium oxide G (Macherey - Nagel Inc., Düren, F. R. G.), Cellulosepulver MN 300 (Macherey-Nagel) and Kieselgur G (Merck). The compositions of mixed supports used for the experiments are listed in Table 1.

The supports were mixed in the listed compositions and then a solution of 5% paraffin oil in n-hexane was added to each mixture to obtain support : paraffin oil 95:5 weight ratio. The suspensions were shaken overnight at room temperature then the n-hexane was evaporated in a rotary vacuum evaporator. Layers of 0.25 mm thickness were prepared on 20 x 20 cm glass plates from each sorbent.

Ortho-, meta-, and para-nitroaniline, 2,4-, 2,6-dinitroaniline, 2,4,6-trinitro-aniline, 2-chloro-4-nitroaniline, 4-chloro-3-nitroaniline, 2,6-dichloro-4-nitroaniline as well as para-nitrophenol, 2,4- and 2,6-dinitrophenol, 2,4,6-trinitrophenol, 3,5-dinitro-4-cyanophenol, 3-nitro-4-cyano-5-chlorophenol, 3-nitro-4-cyano-5-bromophenol and 3-nitro-4-cyano-5-iodophenol served as alkaline and acidic model compounds.

The compounds were dissolved in acetone at a concentration of 2 mg/ml, and 5 μl of each solution was spotted on the plates.

Water /I/, water-methanol 7:3 (v/v) /II/ and 1 M NaCl /III/ were applied as mobile phases.

Table 1. Compositions of mixed supports.

No. of support	Kieselgel %	Aluminium oxide %	Cellulose %	Kieselgur %
1	100	-	-	-
2	-	-	100	-
3	-	100	-	-
4	-	-	-	100
5	80	-	20	-
6	80	20	-	-
7	80	-	-	20
8	60	-	40	-
9	60	40	-	-
10	60	-	-	40
11	40	-	60	-
12	40	60	-	-
13	40	-	-	60
14	20	-	80	-
15	20	80	-	-
16	20	-	-	80
17	-	20	80	-
18	-	-	80	20
19	-	40	60	-
20	-	-	60	40
21	-	60	40	-
22	-	-	40	60
23	-	80	20	-
24	-	-	20	80
25	-	80	-	20
26	-	60	-	40
27	-	40	-	60
28	-	20	-	80
29	60	20	20	-
30	60	-	20	20
31	60	20	-	20
32	40	20	40	-
33	40	-	40	20
34	40	40	-	20
35	40	40	20	-
36	40	-	20	40
37	40	20	-	40
38	20	40	40	-
39	20	60	20	-
40	20	20	60	-
41	20	-	40	40
42	20	-	20	60
43	20	-	60	20
44	20	40	-	40
45	20	60	-	20

(continued)

Table 1. /Continued/

No. of support	Kieselgel %	Aluminium oxide %	Cellulose %	Kieselgur %
46	20	20	-	60
47	-	20	60	20
48	-	40	40	20
49	-	20	40	40
50	-	40	20	40
51	-	20	20	60
52	-	60	20	20
53	40	20	20	20
54	20	20	40	20
55	20	40	20	20
56	20	20	20	40

After development, the compounds were detected visually. For each experiment five independent parallel determinations were carried out.

The measured retention data were analyzed separately for each eluent by spectral mapping technique (20) in order to separate the retention strength (potency =P) and selectivity of supports. To facilitate calculations the behaviour of compounds was characterized by 100 * R_f instead of R_f value. This method has been successfully adapted to classify eluents /21/ and covalently bonded sorbents /22/ in RP-TLC. The two dimensional non-linear mapping of selectivity spectra was carried out by the method published in ref. 23.

To assess the role of the eluents in the selectivity in RP-TLC separation, linear correlations were calculated between the potency orders determined for each eluent. The significance of the differences between the *r* values was checked by the *z*-test. To evaluate the individual contribution of pure supports to the overall retention multivariate linear regressions were calculated between the separate potency values (dependent variables) and the data of Table 1 (independent variables) according to the equation bellow:

$$y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_4$$

where y= potency values of eluents I, II, and III;

x_1 = ratio of Kieselgel in the mixed support /%/,

x_2 = ratio of aluminium oxide in the mixed support /%/,

x_3 = ratio of cellulose in the mixed support /%/,

x_4 = ratio of Kieselgur in the mixed support /%/

The RP-HPLC system consisted of a Liquopump Model 312 (LaborMIM, Budapest, Hungary) with modified electronics and Altex (Cotati, Calif., U.S.A.) check valves, a Rheodyne (Cotati, Calif., U.S.A.) Model 7010 sample injection valve with 20 µl loop, a 250 x 4.6 mm column with Dimesil C-18 (10 µm) (Chromatronix Inc., Calif., U. S. A.) stationary phase and an ISCO (Lincoln, Nebraska, U. S. A.) Model 226 absorbance monitor with 254 nm source screen and an Endim Model 621.01 (Berlin, GDR) recorder. Retention time measurements and data processing were carried out on an Apple II.

microcomputer (Apple Computer Inc., Cupertino, Calif., U.S.A.).

To determine the log k' values of the undissociated compounds the eluents were mixtures of methanol and 0.025 M phosphate buffers, pH =1.70 for phenols and pH=7.99 for the anilines, ranging from 65 to 80 % (v/v) methanol (RP-HPLC system A). The log k' values were also determined using the same pH for both type of compounds, i. e. 0.025 M fosphate buffer pH= 4.35 (RP-HPLC system B). In both cases the flow rate was 1.00 ml/min; 0.1 mg of the compound tested and 2 mg $NaNO_3$ were dissolved in 5 ml eluent and 20 µl of the solutions was injected onto the column. The retention time of $NaNO_3$ was regarded as the dead time (t_0). Both retention time (t_R) and the dead time were measured three times. The average log k' values were calculated. The log k' values obtained using eluents with various methanol concentrations were plotted against the methanol concentration. As both the intercepts (log k'_0) and the slopes (b) of the obtained straight lines may correlate to the log P values /24, 25/, linear correlations were separately calculated between the 4 types of RP-HPLC parameters (log k'_0, b referring to systems A, and B, respectively) and the 168 series of R_M values determined on 56 support mixtures using 3 types of eluent system.

RESULTS AND DISCUSSION

The potency values calculated from the 100xR_f values are compiled in Table 2. These values show the retention strengths on the given support. The considerable difference between the retention strength of support impregnated exactly the same way proved again that the supports retain their original adsorptive characteristics that is the R_f values of compounds depend considerably on the type of support.

Table 2. The potency (P values) calculated from R_f values for the various mixed supports.

No. of support	Type of the eluent I	II	III
1	144	298	94
2	232	307	160
3	54	252	48
4	388	412	388
5	158	279	103
6	136	272	83
7	172	310	111
8	162	266	105
9	121	254	78
10	171	307	106
11	161	261	109
12	93	243	76
13	193	320	140
14	165	264	122
15	73	223	67
16	221	348	170
17	157	237	128
18	256	300	191
19	131	231	110
20	268	310	215
21	117	214	106
22	296	326	230

(continued)

Table 2. /Continued/

No. of support	Type of the eluent I	 II	 III
23	114	217	97
24	321	351	288
25	99	258	82
26	135	298	114
27	166	329	162
28	241	355	209
29	148	255	103
30	176	289	123
31	153	281	97
32	155	262	110
33	177	272	125
34	138	281	91
35	125	240	98
36	179	286	134
37	152	289	104
38	128	244	100
39	113	243	92
40	160	255	122
41	195	289	150
42	204	307	156
43	172	276	129
44	135	303	108
45	99	258	75
46	186	316	150
47	193	277	146
48	155	251	134
49	187	285	169
50	131	254	114
51	205	313	178
52	128	255	114
53	154	277	113
54	156	260	114
55	128	259	98
56	169	290	134

The P values obtained for the three types of mobile phases correlated with each other fairly well as it is shown by equ. 1 - 3.

$$P_{II} = 192.9\ (\pm 8.8) + 0.525\ (\pm 0.050) P_{I} \qquad (1)$$

n= 56 r= 0.821

$$P_{III} = -20.9\ (\pm 6.9) + 0.903(\pm 0.039)P_{I} \qquad (2)$$

$n=56 \qquad r=0.953$

$$P_{III} = -198.8\ (\pm 35.1) + 1.172\ (\pm 0.124)P_{II} \qquad (3)$$

$n=56 \qquad r=0.790$

Equations 1-3 suggests that the eluent composition did not influence considerably the retention order of the compounds investigated on the mixed supports. Eluent II was stronger than eluent I, according to the general rule, however eluent III exposed a lower eluent strength than eluent I. This observation was somewhat unexpected because the ions present in the eluents are bond to the available silanol groups non covered by paraffin oil. This phenomenon could have reduced the retention capacity of supports. However, an increase of retention was found in the presence of ions. It is assumed that the ions supress the dissociation of polar amino and hydroxyl groups increasing in this way the R_M values of compounds /26/.

The z-test showed that the correlation coefficient of $P_{III} - P_{I}$ relationship was significantly higher than the other two correlation coefficients (significance level was over 99.9%), but between the fitness of $P_{II} - P_{I}$ and $P_{III} - P_{II}$ relationships no significant difference was found (t=0.46). This finding suggests that eluent pairs I - III showed a higher similarity than the eluent pairs I - II and II - III.

The potency values of supports did not show linear correlations to the percentage of their compositions (F values were 1.45, 0.75 and 2.89 for eluent I, II and III, respectively). The leak of correlations can be explained in two ways, namely, either the correlation is non linear or the supports interact with each other. The two dimensional non linear maps of selectivity data with water as eluent (Fig. 1.) shows, that the support mixtures containing silica or alumina form distinct clusters. It means that the supports retain their original selectivity character also after impregnation. Of course, the support mixtures containing the both sorbents overlap in the map.

The situation is similar for the other two eluents (Fig. 2 and 3.). The cluster formation prevails independently of the eluent composition. These observations indicates that the composition of support has a predominant role in the selectivity even in RP-TLC separation of polar compounds and the eluent composition is of minor importnace.

The parameters of the linear relationships between the log k' values and the methanol concentrations of the eluent in the RP-HPLC measurements are shown in Table 3. The correlation coefficients were over 99% significance level in each case. The values of log k'_0 and the slope (b) of the aniline derivatives are the

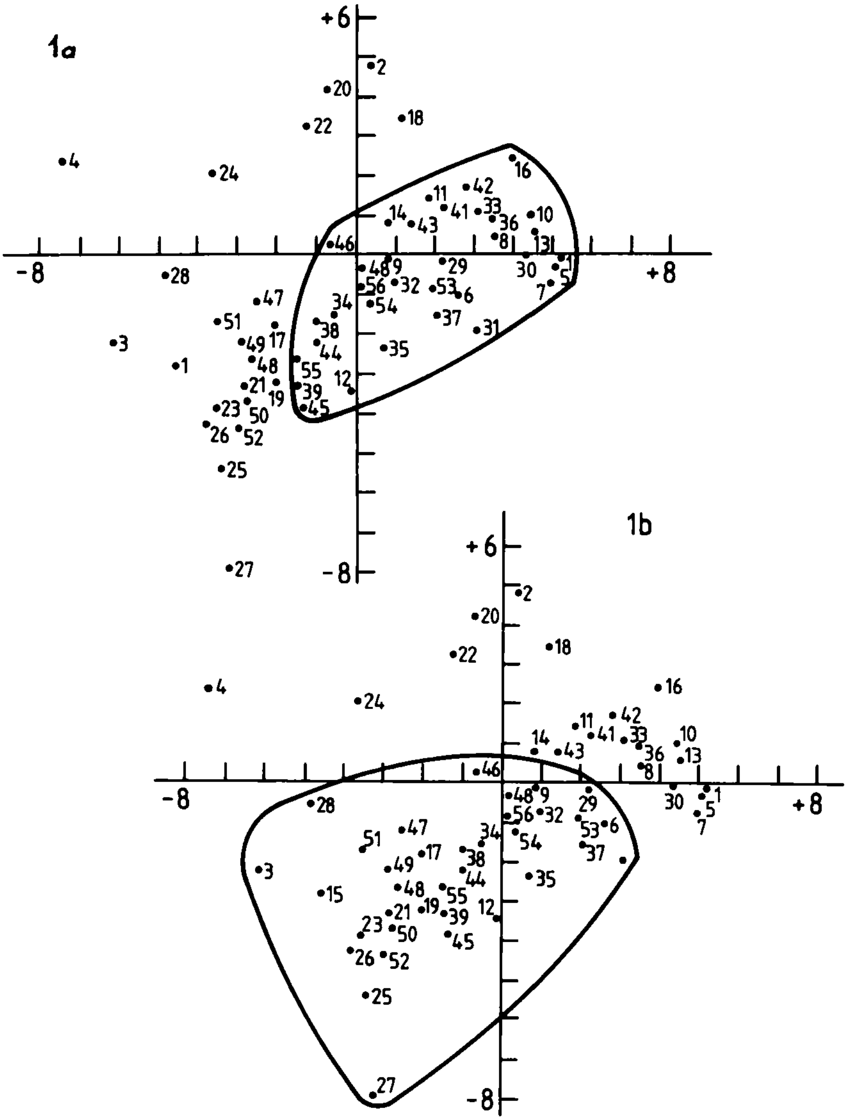

Figure 1.
Two dimensional non linear maps of selectivity data obtained by using water as eluent. Number of iterations: 10. Error of mapping: 0.0238. Numbers indicate supports listed in Table 1.
a: supports containing silica encircled
b: supports containing aluminium oxide encircled

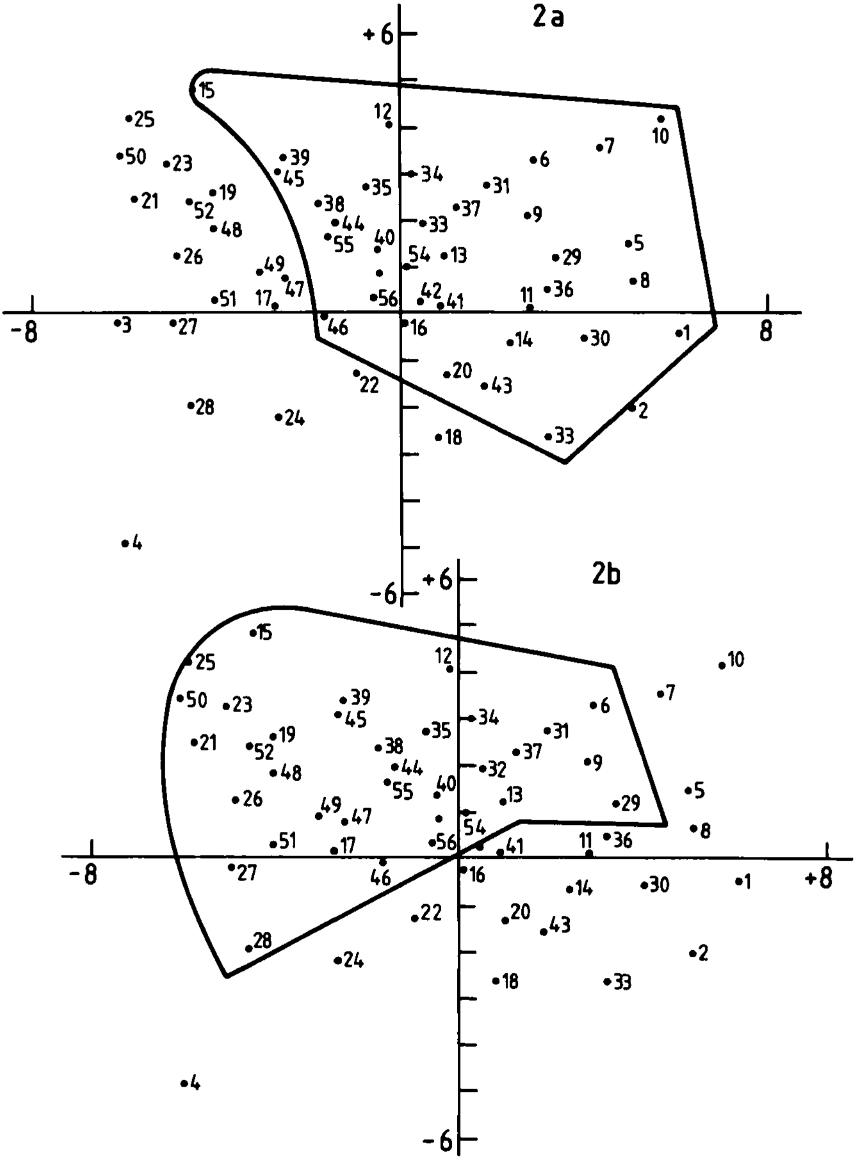

Figure 2.
Two dimensional non linear maps of selectivity data obtained by using water - methanol (7:3) as eluent. Number of iterations: 8. Error of mapping: 0.025. Numbers indicates supports listed in Table 1.
a: supports containing silica encircled
b: supports containing aluminium oxide encircled

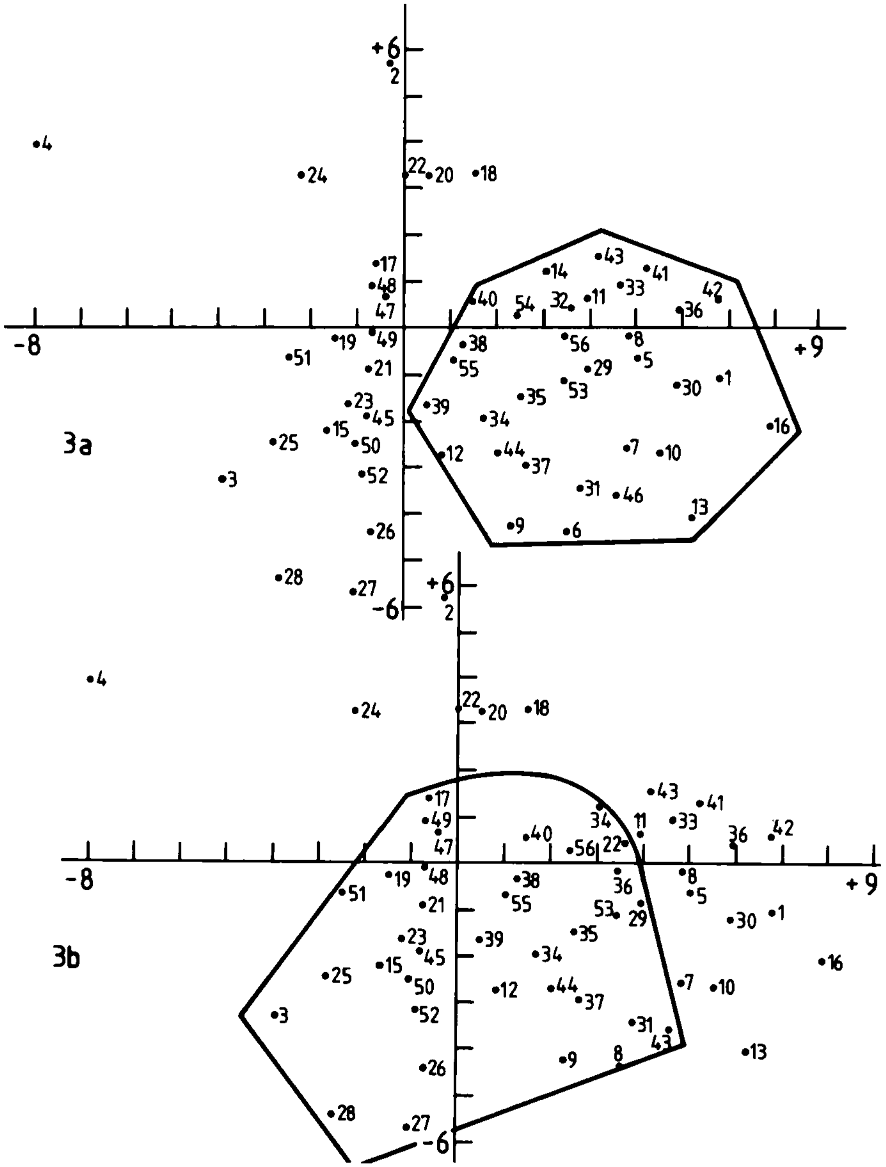

Figure 3.
Two dimensional non linear maps of selectivity data obtained by using 1M NaCl as eluent. Number of iterations: 10. Error of mapping: 0.0246. Numbers indicate supports listed in Table 1.
a: supports containing silica encircled
b: supports containing aluminium oxide encircled

Table 3.
Parameters of the linear relationships between the log k' values and the methanol concentration of the eluent (C%) determined by RP-HPLC. ($\log k' = \log k'_0 + bC\%$)

Compounds	System A* (undissociated form)		System B* (partially dissociated form)	
	100b	log k'_0	100b	log k'_0
o-NO_2-aniline	-2.48	1.507	-2.48	1.507
m-NO_2-aniline	-2.37	1.253	-2.37	1.253
p-NO_2-aniline	-2.14	0.980	-2.14	0.980
2,4-$(NO_2)_2$-aniline	-2.67	1.715	-2.67	1.715
2,4,6-$(NO_2)_3$-aniline	-2.64	1.676	-2.64	1.676
2-Cl-4-NO_2-aniline	-2.80	1.879	-2.80	1.879
4-Cl-3-NO_2-aniline	-2.89	1.814	-2.89	1.814
2,6-$(Cl)_2$-4-NO_2-an.	-3.29	2.568	-3.29	2.568
p-NO_2-phenol	-2.58	1.486	-8.83	5.044
2,4-$(NO_2)_2$-phenol	-2.90	1.789	-12.24	5.699
2,6-$(NO_2)_2$-phenol	-5.17	2.984	-8.85	3.649
2,4,6-$(NO_2)_3$-phenol	-4.90	2.487	-12.10	6.325
3,5$(NO_2)_2$ 4CN-phenol	-4.21	2.417	-11.60	5.305
3NO_2,4CN,5Cl-phenol	-3.25	2.332	-9.96	4.620
3NO_2,4CN,5Br-phenol	-4.24	2.684	-12.80	7.009
3NO_2,4CN,5I-phenol	-2.85	1.592	-11.80	5.452

* see Experimental

same using the two different eluent systems that is the dissociation state of these compounds does not change in this pH region significantly (from pH=4.35 to pH=7.99), resulting in unchanged chromatographic retention.

The linear relation of the R_M values to the RP-HPLC retention parameters (log k'_0 and b) was characterized by correlation coefficient. The correlation coefficients were calculated for the 3 times 56 R_M values and for the 4 types of RP-HPLC retention data, and their significance level was also investigated. The results are summarized in Table 4.

The results show that higher correlation coefficients were obtained when R_M values were correlated to the RP-HPLC retention data obtained in eluent system B in which the pH was 4.35 and the compounds were partially dissociated form. As the R_M values were obtained without using buffer in that case the compounds were also partially dissociated form.

It is also noticable that R_M values obtained by using eluent III (i.e. 1 M NaCl) do not show as many high correlation coefficients with the RP-HPLC retention data obtained with mobile phase B as in case of the other two eluent systems. Salts in the mobile phase can modify the retention behaviour of polarizable compounds in several ways. They can adsorb on the free silanol groups of silica surface not covered by paraffin oil, they can lessen the degree of dissociation of polar

Table 4.
The significance of the correlation coefficients (r) for the relation of 4 RP-HPLC paramers to the 168 series of R_M values (obtained on 56 support mixtures using 3 eluent systems).

RP-HPLC data	RP-TLC R_M obtained by	Significance level of r (%)			
		under 98	98-99	99-99.9	over 99.9
		Number of r values			
log k'_0 (A)	eluent I	37	5	13	1
b (A)	eluent I	52	-	4	-
log k'_0 (B)	eluent I	12	2	7	35
b (B)	eluent I	11	3	7	35
log k'_0 (A)	eluent II	42	5	8	1
b (A)	eluent II	54	2	-	-
log k'_0 (B)	eluent II	8	2	8	38
b (B)	eluent II	12	4	8	32
log k'_0 (A)	eluent III	51	4	1	-
b (A)	eluent III	56	-	-	-
log k'_0 (B)	eluent III	17	2	13	24
b (B)	eluent III	20	2	23	11

compounds, they increase the dielectric constant and surface tension of water, which also influence the retention and they can modify the intramolecular forces between polar substituents. The influence of salts on the retention of polar compounds depends on the processes mentioned above making undpredictable their

Table 5.
Parameters of the five best correlations between the 4 RP-HPLC retention data (Y) and the R_M values (X).(r stands for the correlation coefficient, s is the standard deviation.)

$$Y = a + b*X$$

RP-HPLC data (Y)	RP-TLC data(X) Support	Eluent	Parameters of correlation a	b	s	r
log k'_o(A)	18	I	1.62	-0.68	0.196	0.681
log k'_o(A)	22	I	1.45	-0.66	0.201	0.661
log k'_o(A)	20	I	1.58	-0.67	0.223	0.629
log k'_o(A)	2	I	1.72	-0.74	0.250	0.625
log k'_o(A)	2	II	1.65	-0.16	0.059	0.583
b (A)	18	I	-0.026	0.013	0.003	0.753
b (A)	22	I	-0.023	0.012	0.003	0.722
b (A)	2	I	-0.028	0.015	0.004	0.718
b (A)	2	II	-0.026	0.003	0.001	0.709
b (A)	20	I	-0.026	0.013	0.003	0.704
log k'_o(B)	22	I	1.32	-3.33	0.469	0.885
log k'_o(B)	5	I	4.24	-2.55	0.367	0.881
log k'_o(B)	30	I	3.92	-2.87	0.428	0.874
log k'_o(B)	42	I	3.37	-3.29	0.493	0.872
log k'_o(B)	5	II	2.09	-2.69	0.390	0.872
b (B)	7	II	-0.028	0.021	0.002	0.946
b (B)	22	I	-0.016	0.078	0.007	0.943
b (B)	1	II	-0.031	0.021	0.002	0.938
b (B)	30	II	-0.029	0.072	0.007	0.935
b (B)	5	II	-0.035	0.062	0.006	0.932

effects and decreasing the correlation between the R_M values and RP-HPLC parameters.

From Table 4. it also can be seen, that about the same number of good correlation was found when log k'_0 or the b values were used. It means that the slope and the intercept values correlate with each other.

The parameters of the 5 best correlations for each RP-HPLC parameter are compiled in Table 5. It is noticable that the best correlations were obtained in the case of supports containing high percentage of silica and diatomaceous earth.

These results proved that the multivariate methods are suitable to elucidate adequately the role of supports in RP-TLC and they promote the better understanding of physico-chemical parameters influencing the retention in RP-TLC.

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