Journal of Chromatography, 286 (1984) 131–137
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 202

CLASSIFYING ELUENTS IN REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY BY SPECTRAL MAPPING

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SUMMARY

The retentions of 17 substituted symmetrical triazine derivatives were determined in reversed-phase thin-layer chromatography using 27 organic solvents miscible with water. The data matrix was evaluated by multivariate techniques. It was established that a higher solvent lipophilicity means a higher solvent strength, but the lipophilicity explains only about 67% of total variance. The influence of lipophilicity was the lowest with acetonitrile, tetrahydrofuran, methyl ethyl ketone, dimethylformamide and ethylene glycol. $-CH_3$ and $-CH_2-$ groups increased significantly and primary –OH, –C–O–C– and –N– groups decreased the solvent strength. The presence and position of nitrogen and oxygen atoms and free hydroxyl groups govern the solvent selectivity in reversed-phase thin-layer chromatography.

INTRODUCTION

In the literature, the concepts of the eluent strength of binary[1] and ternary[2] solvent mixtures in adsorption and reversed-phase partition chromatography[3] have been extensively discussed. The theoretical basis for the systematic optimization of the mobile phase in adsorptive[4] and reversed-phase partition chromatography[5] have also been described. Recent research emphasizes the growing importance of solvent selectivity in practical separation problems[6] and in theoretical optimization[7,8].

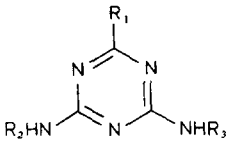
It is known that the solvent strength and selectivity determined experimentally depend considerably on the experimental conditions and on the solutes investigated[9]. With large numbers of solvents and solutes, computer-assisted multivariate techniques offer the unique possibility of evaluating all retention data simultaneously[10,11].

The solvent strength can be expressed by the retention of a number of solutes. The use of retention spectra is aimed at grouping of those solvents which share some (possibly unknown) mechanism of retention. Usually the information on the spectral properties is disturbed by the relative strength of the solvents, and for this reason any classification procedure has to be preceded by the separation of the relative solvent strength from the retention mechanism. The technique of spectral mapping complies with these requirements[12]. With the help of this technique, it is possible to calculate first the order of potency of eluents and than the spectra of eluents, inde-

0021-9673/84/$03.00

TABLE I

STRUCTURES OF SUBSTITUTED SYMMETRICAL TRIAZINE DERIVATIVES



No.	*Common name*	R_1	R_2	R_3
1	Simazine	Cl	Ethyl	Ethyl
2	Atrazine	Cl	Ethyl	Isopropyl
3	Propazine	Cl	Isopropyl	Isopropyl
4	Terbutylazine	Cl	Ethyl	*tert.*-Butyl
5	Trietazine	Cl	Ethyl	Triethylmethyl
6		Cl	*tert*-Butyl	*tert.*-Butyl
7		Cl	Cl	*tert.*-Butyl
8		Cl	Cl	Ethyl
9		Cl	H	H
10		Cl	H	Ethyl
11	Atraton	OCH_3	Ethyl	Isopropyl
12	Prometon	OCH_3	Isopropyl	Isopropyl
13	Terbumeton	OCH_3	Ethyl	*tert.*-Butyl
14	Etazine	OCH_3	Ethyl	Isobutyl
15	Ametryn	SCH_3	Ethyl	Isopropyl
16	Prometryn	SCH_3	Isopropyl	Isopropyl
17	Terbutryn	SCH_3	Ethyl	*tert.*-Butyl

pendently of the solvent strength. From the potency and composition of the eluent in binary mixtures, the strength of organic solvents miscible with water (solvent strength of water = 21) can be calculated in reversed-phase thin-layer chromatography (RP-TLC).

In the spectra, organic solvents that exhibit similar retention mechanisms form clusters. To our knowledge the spectral mapping technique has never been applied to the evaluation of chromatographic data.

EXPERIMENTAL

The RP-TLC behaviour of 17 substituted symmetric triazine derivatives (Table I) was studied using eluents containing 27 organic solvents miscible with water at various concentrations (Table II).

DC-Alufolien Kieselgel 60 F_{254} (Merck) plates were impregnated with 5% paraffin oil in *n*-hexane overnight. After evaporating the *n*-hexane at room temperature, 2 μl of solutions of triazine in chloroform (5 mg/cm^3) were spotted on the plates and developed with the eluents listed in Table II. The triazine derivatives were detected by UV adsorption. All experiments were run with five independent parallel determinations. The resulting data matrix was treated by the spectral mapping technique.

To elucidate the effect of lipophilicity on the solvent strength, a linear correlation was calculated between the log *P* value[13] and solvent strength (log *P* values

TABLE II

COMPOSITION AND POTENCY OF RP-TLC ELUENTS

No.	*Organic solvent*		*Potency of eluent*	*Calculated solvent strength of organic phase*
	Name	*% in eluent*		
1	Methanol	50	144.79	2.69
2	Ethanol	35	138.49	3.57
3	*n*-Propanol	15	105.50	5.84
4	Isopropanol	20	109.87	4.65
5	*n*-Butanol	7.9	80.52	7.74
6	*tert*-Butanol	15	88.28	4.70
7	Pentan-2-ol	5.3	63.30	8.19
8	Diethylene glycol	50	107.69	1.94
9	1,2-Propylene glycol	50	134.36	2.48
10	Tetraethylene glycol	30	68.88	1.81
11	Ethylene glycol	50	69.64	1.18
12	Glycerol	80	42.20	0.48
13	Methanol–glycerol (1:1)	33:33	114.96	1.62
14	Methoxy ethanol	40	125.63	2.83
15	Ethoxy ethanol	30	115.20	3.35
16	Butoxy ethanol	15	248.60	15.38
17	Butoxyethanol	8	76.64	7.17
18	Diethylene glycol dimethyl ether	30	103.08	2.95
19	Dioxan	30	132.42	3.92
20	Tetrahydrofuran	30	127.33	3.75
21	Acetone	50	232.35	4.44
22	Methyl ethyl ketone	20	195.48	8.93
23	Triethanolamine	30	50.45	1.19
24	Ethyl acetate	8.6	84.16	7.55
25	Acetonitrile	30	213.67	6.63
26	Pyridine	15	118.60	6.72
27	Dimethylformamide	30	120.54	3.53
28	Ethylene diamine	40	59.42	1.17
29	Trimethylamine	24	123.69	4.49

of solvents 18, 21, 22, 23, 24, 26 and 27 were not found). As the solvents contained a limited number of structural parameters, their effect on the solvent strength was computed by stepwise regression analysis. The number of the following substructures in the solvent molecules were taken as independent variables: $x_1 = -CH_3$; $x_2 = -\overset{|}{C}H_2$; $x_3 = -\overset{|}{\underset{|}{C}}H$; $x_4 = -\overset{|}{\underset{|}{C}}-$; x_5 = primary –OH; x_6 = secondary –OH; x_7 = tertiary –OH; $x_8 = C-O-C$; $x_9 = C=O$; $x_{10} = -\overset{|}{\underset{|}{N}}$; $x_{11} = -C\equiv N$; $x_{12} = -NH_2$.

The number of accepted independent variables was not restricted and the partial F value was set at 0.8.

TABLE III

R_F × 100 VALUES OF 17 SUBSTITUTED SYMMETRICAL TRIAZINE DERIVATIVES DETERMINED IN 29 RP-TLC SYSTEMS

No. of compound	*No. of eluent*																												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*
1	47	43	37	38	29	32	23	38	38	27	19	10	35	43	37	65	27	32	43	46	66	63	19	26	61	33	40	13	16
2	44	42	27	30	18	25	10	28	37	18	13	5	31	36	30	63	19	27	37	32	62	53	6	27	56	28	32	9	12
3	32	30	15	19	10	15	4	13	19	8	5	0	19	23	21	57	11	14	26	22	54	42	0	3	47	18	18	3	6
4	28	25	10	12	6	9	2	13	23	5	5	0	18	19	17	58	8	12	20	20	52	37	0	7	44	16	15	2	7
5	22	22	10	11	6	5	3	10	16	5	5	0	13	17	15	53	5	11	18	19	45	31	2	6	38	17	12	2	10
6	13	11	3	3	0	0	0	3	8	0	1	0	5	5	4	48	0	3	7	10	34	11	0	3	20	6	4	0	2
7	28	22	13	18	9	9	6	19	24	7	10	7	16	17	13	51	6	9	18	10	37	31	5	5	41	19	13	5	22
8	54	48	50	43	43	40	41	34	54	26	36	25	44	44	41	68	33	30	46	26	67	57	43	34	67	51	40	47	100
9	76	69	76	72	68	71	66	67	78	50	71	71	83	67	63	76	57	49	66	48	79	79	44	59	85	57	71	54	100
10	73	70	70	69	59	62	55	71	74	55	56	44	71	74	69	78	59	66	68	67	85	79	35	61	83	54	78	60	25
11	36	39	33	34	25	27	15	34	40	22	17	6	30	40	40	67	26	42	43	49	68	62	16	20	59	41	45	15	54
12	28	30	22	24	15	17	10	24	29	14	11	0	21	28	26	64	17	30	32	40	59	52	9	25	52	33	29	8	39
13	25	25	14	17	8	9	5	19	25	11	9	0	20	24	21	59	10	23	27	34	55	46	7	16	49	27	23	6	30
14	27	30	21	24	15	16	9	21	27	13	9	0	20	26	27	59	16	29	32	42	58	52	7	28	52	33	28	7	38
15	27	28	19	20	12	14	7	23	28	12	10	6	22	25	25	57	12	22	28	26	54	44	7	15	50	24	24	7	25
16	19	19	9	12	6	8	3	15	18	6	5	0	14	17	15	51	6	13	18	17	44	35	4	7	41	18	14	3	15
17	16	16	6	7	3	5	2	12	16	5	5	0	12	13	11	51	4	11	16	17	43	32	3	5	36	14	11	3	11

TABLE IV

SOLVENT STRENGTHS IN RP-TLC

Compound	*Solvent strength*	*Compound*	*Solvent strength*
Methyl ethyl ketone	8.93	Ethanol	3.57
Pentan-2-ol	8.19	Dimethylformamide	3.53
n-Butanol	7.74	Ethoxyethanol	3.35
Ethyl acetate	7.55	Diethylene glycol dimethyl ether	2.95
Buthoxyethanol	7.17		
Pyridine	6.72	Methoxyethanol	2.83
Acetonitrile	6.63	Methanol	2.69
n-Propanol	5.84	1,2-Propylene glycol	2.48
tert.-Butanol	4.70	Diethylene glycol	1.94
Isopropanol	4.65	Tetraethylene glycol	1.81
Trimethylamine	4.49	Triethanolamine	1.19
Acetone	4.44	Ethylene glycol	1.18
Dioxan	3.92	Ethylene diamine	1.17
Tetrahydrofuran	3.75	Glycerol	0.48

RESULTS AND DISCUSSION

The potency of the eluents, the calculated solvent strength of the organic phase and the mean R_F values are given in Tables II and III. The composition of eluent 16 was not adequately chosen (all triazine derivatives have high R_F values) and therefore the data with this eluent have been excluded.

In all instances the solvent strength increases with increasing length of the alkyl chain (solvents 1–2–3–5, 14–15–17, 21–22 and 8–18 in Table II) and decreases considerably with increasing number of hydroxyl groups on the alkyl chain (solvents 3–9–12 and 2–11). Branching of the alkyl chain also decreases the solvent strength (solvents 3–4 and 5–6). Our calculated solvent strength order correlates reversedly with the solvent strength orders in adsorptive chromatography[9] (Table IV). This finding can be easily understood by considering the opposite retention mechanism of these two chromatographic techniques. A highly significant correlation was found between log P and solvent strength:

$$\text{Solvent strength} = 5.14 + (2.29 \pm 0.38)\log P \; (n = 20)$$
$$r = 0.8206;\; r^2 = 0.6733;\; r_{99.9\%} = 0.6787$$

This means that the change is lipophilicity explains about 67% of the change of solvent strength. The measured strengths of the solvents methyl ethyl ketone, tetrahydrofuran, acetonitrile, dimethylformamide and ethylene glycol deviate the most strongly from the calculated values, *i.e.*, with these solvents molecular parameters other than lipophilicity play a considerable role in the determination of solvent strength (Fig. 7).

As the substructures x_4 and x_7 occurred only in *tert.*-butanol (solvent 6), resulting in a singular matrix that cannot be solved by the stepwise analysis program, they were excluded from the calculation. The results of stepwise regression analysis are given in Table V.

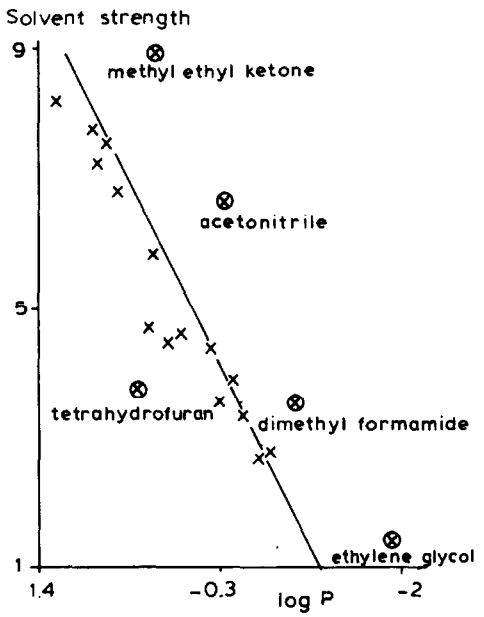


Fig. 1. Correlation between the solvent strengths and log P values of solvents.

Only five substructures influence the solvent strength significantly: $-CH_3$ and $-CH_2-$ groups increase and primary –OH, –C–O–C– and –N– groups significantly decrease the solvent strength. The insignificant contributions of –CH– and secondary –OH groups reveal that the position of the same group even in relatively small molecules may change drastically their effect on the solvent strength. The two-dimensional spectral map of solvents (Fig. 2) indicates that the solvents commonly applied in RP-TLC do not show great differences in their retention mechanism. However, acetonitrile and trimethylamine (points 25 and 29) differ strongly and the other solvents containing nitrogen atoms in their molecules, such as triethanolamine, pyridine

TABLE V

RESULTS OF STEPWISE REGRESSION ANALYSIS

$n = 25$; $F_{99\%} = 3.79$; $t_{95\%} = 2.11$; $t_{99\%} = 2.90$; $a = 2.56$; $r = 0.8566$; $s = 1.55$; $F = 5.41$.

Number of variable	*b*	s_b	*t-Test*	*Path coefficient*
1	1.74	0.69	2.52	0.64
2	1.32	0.36	3.71	1.13
5	−1.79	0.69	2.61	−0.65
6	−1.42	0.93	1.52	−0.22
8	−2.66	0.75	3.55	−0.90
10	−3.60	1.48	2.44	−0.41
11	2.33	1.75	1.33	0.19
12	−2.01	1.01	1.99	−0.33

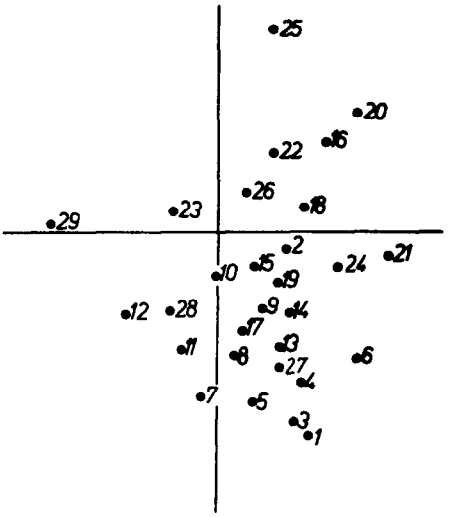


Fig. 2. Two-dimensional non-linear mapping of solvents. For numbers see Table II.

and ethylenediamine (points 23, 26 and 28), differ only slightly from the rest of the solvents.

Most solvents form a compact cluster, the lower part containing the monovalent alcohols (points 1, 3, 4, 5, 6 and 7). On the upper part the divalent alcohols are positioned on the left (points 8, 9, 10 and 11) and their monoethers on the right (points 14, 15 and 17). Glycerol (point 12) is at the farthest left of the cluster. Under the given conditions, dioxan is very similar to methoxy- and ethoxyethanol and to 1,2-propyleneglycol (points 19, 14, 15 and 9). The solvents containing oxygen atom but having no free hydroxyl group form a loose group (points 18, 20, 21, 22 and 24).

Owing to their opposite positions on the map, complementary selectivity can be expected from methanol, acetonitrile, acetone and trimethylamine in RP-TLC (points 1, 25, 21 and 29).

To summarize, we conclude that in RP-TLC the solvent selectivity is determined mainly by the presence and number of free hydroxyl groups and by the presence and position of nitrogen and oxygen atoms in the solvent molecule.

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