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Note

Effect of layer and eluent characteristics on the reversed-phase thin-layer chromatographic behaviour of some barbituric acid derivatives

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Reversed-phase thin-layer chromatography (RPTLC) has been applied extensively to the determination of partition coefficients[1–3]. In earlier RPTLC studies the sorbents (generally silica) were impregnated by paraffin or silicone oils[4]; later chemically bonded reversed-phases found increasing application[5] and their performances have been compared[6].

The objective of this study was to compare the RPTLC behaviour of some barbituric acid derivatives on various chemically bonded reversed phases with different eluents.

EXPERIMENTAL

The structures of the barbituric acid derivatives are shown in Fig. 1 and the reversed-phase layers and eluents applied are listed in Table I. The other chromatographic conditions have been published earlier[7,8]. The retention data (R_F values) were evaluated by principal component (PC) analysis[9,10] and by spectral mapping[11] and the results were displayed by the non-linear mapping technique[12].

RESULTS AND DISCUSSION

The 100 · R_F values of barbituric acid derivatives measured under different RPTLC conditions and the results of PC analysis are compiled in Tables II and III, respectively.

Only one component is needed to explain the majority (about 95%) of the total variance, that is, on the basis of only one hidden variable the retention behaviour of compounds can be predicted in all RPTLC systems. The map of PC loadings (Fig. 2) shows that the determinative factor in the grouping of compounds according to their RPTLC behaviour is the length of the alkyl chains, *i.e.*, the lipophilicity. Compounds 1 and 2 and compounds 4 and 6 behave very similarly in the 21 RPTLC systems, which means that the site of branching has a negligible influence on the retention, although the branching itself affects the retention (compound 5 differs from compounds 1 and 2 and compound 3 differs from compound 7).

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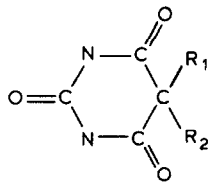

No. of compound	R_1	R_2
1	CH_3CH_2	$(CH_3)_2CHCH_2CH_2$
2	CH_3CH_2	$CH_3CH_2CH_2CH(CH_3)$
3	CH_3CH_2	$CH_3CH_2CH_2C(CH_3)_2CH_2CH_2$
4	$CH_3(CH_2)_3$	$CH_3CH_2CH_2CH(CH_3)$
5	CH_3CH_2	$CH_3(CH_2)_4$
6	$CH_3(CH_2)_3$	$(CH_3)_2CHCH_2CH_2$
7	CH_3CH_2	$CH_3(CH_2)_7$

Fig. 1. Structure of barbituric acid derivatives.

TABLE I

RPTLC CONDITIONS FOR THE DETERMINATION OF THE LIPOPHILICITY OF SOME BARBITURIC ACID DERIVATIVES

No. of system	Layer	Organic solvent		Eluent potency (P)
		Compound	Concentration in eluent (vol.-%)	
1	RP-8_{F254} (Merck)	Acetonitrile	60	172.35
2		Methanol	70	104.32
3	RP-18_{F254} (Merck)	Acetonitrile	50	113.01
4		Methanol	50	24.57
5		Methanol	55	41.95
6		Methanol	60	59.72
7		Methanol	65	74.08
8		Methanol	70	91.47
9	Nano SIL C18 50	Acetonitrile	55	103.94
10		Methanol	70	137.96
11	Nano SIL C18 75	Acetonitrile	50	93.74
12		Methanol	65	96.00
13	Nano SIL C18 100	Acetonitrile	50	55.94
14		Methanol	70	128.89
15	KC18 (Whatman)	Acetonitrile	50	113.77
16		Methanol	60	77.10
17	Kieselgel 60_{F254}, silanized (Merck)	Methanol	55	89.20
18		Acetonitrile	50	85.04
19		Isopropanol	40	75.59
20		Ethanol	50	102.81
21		*n*-Propanol	60	120.57

TABLE II

100 · R_F VALUES OF SOME BARBITURIC ACID DERIVATIVES MEASURED UNDER DIFFERENT RPTLC CONDITIONS

No. of RPTLC system	*No. of compound*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
1	75	75	57	61	72	62	54
2	54	53	29	34	50	34	22
3	57	56	31	38	53	38	26
4	14	14	6	7	13	7	4
5	27	26	8	10	23	10	7
6	37	36	11	16	33	16	9
7	42	42	16	21	40	22	13
8	48	48	23	29	46	29	19
9	48	48	32	36	46	36	29
10	64	62	44	49	60	49	37
11	45	44	28	32	42	32	25
12	48	47	26	32	46	33	22
13	31	30	14	17	28	17	11
14	60	60	39	44	60	45	33
15	52	52	37	39	50	40	31
16	42	42	18	24	41	25	12
17	46	47	23	30	44	28	18
18	39	39	27	28	38	29	25
19	36	35	24	25	35	23	22
20	49	49	30	35	47	34	28
21	48	47	40	48	48	49	39

TABLE III

RESULTS OF PRINCIPAL COMPONENT ANALYSIS

Eigenvalues	*Sum of total variance explained (%)*
6.66	95.16
0.31	99.52

No. of compound	*Principal component loadings*	
	I	*II*
1	0.97	0.24
2	0.97	0.25
3	0.98	−0.19
4	0.99	−0.11
5	0.98	0.20
6	0.99	−0.11
7	0.95	−0.28

As the PC analysis does not distinguish solvent strength from selectivity, the map of PC variables (Fig. 3) contains the clusters of RPTLC systems taking into consideration also the solvent strength. The first PC variables (PCV) measuring the

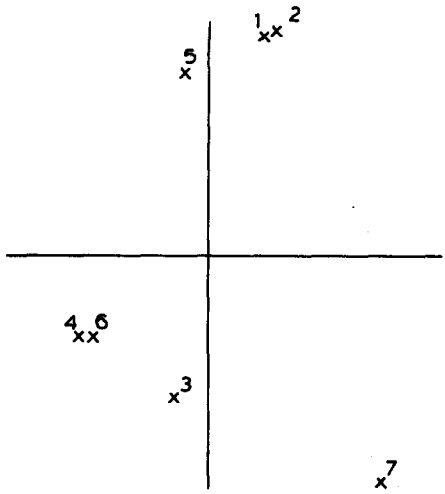

Fig. 2. Map of principal component loadings. Numbers indicate compounds in Fig. 1.

influence of individual RPTLC systems on the ratio of total variance explained by the first PC loading correlated well with the eluent potency (P) calculated by the spectral mapping technique[14]:

$$P = 93.43 + 12.94\text{PCV} \; (r = 0.99998; \; n = 21)$$

The correlation above confirms our previous statement.

In spite of the preponderant role of eluent potency, the systems with methanol or acetonitrile in the eluent form two distinct groups, that is, the RPTLC behaviour of the barbituric acid derivatives investigated is determined by the characteristics of the organic component of the eluent rather than by the differences in the layer.

The spectral mapping technique calculates first the solvent strength of eluents (potency data in Table I). The same eluent composition never had the same potency on different layers, which indicates that the RPTLC layers, although they are theo-

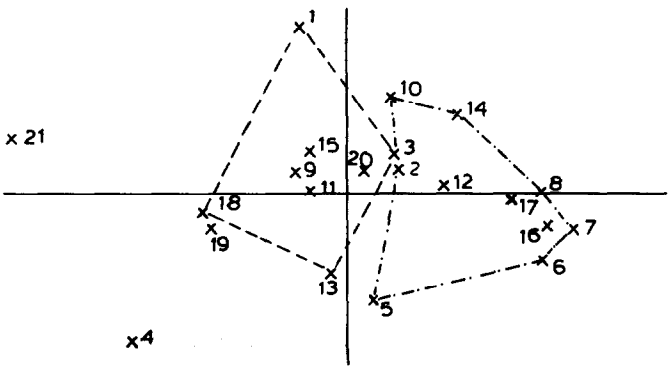

Fig. 3. Map of principal component variables. Left, eluents containing acetonitrile; right, eluents containing methanol. Numbers indicate RPTLC systems in Table I.

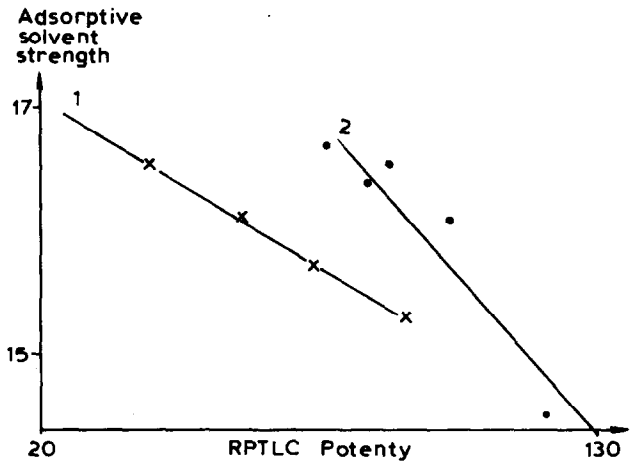

Fig. 4. Linear correlations between RPTLC potency and adsorptive solvent strength of eluents. 1, Water–methanol mixtures on RP-18_{F254} layer; 2, water–various organic phases on Kieselgel 60_{F254} silanized layer.

retically similar, have a strong influence on the retention, which makes questionable the value of comparisons of eluent potencies determined on different RPTLC layers. The order of eluent strength may also be reversed on different layers: on an RP-18_{F254} layer the eluent water–acetonitrile (1:1) has a higher potency (113.01) than water–methanol (3:7) (91.47); on a Nano SIL C18 100 layer the situation is totally different, water–methanol (3:7) being stronger (128.9) than water–acetonitrile (1:1) (55.94). For one organic phase applied in various concentrations a very good linear correlation was found between the RPTLC potency (P) calculated by us and the adsorptive solvent strength (ε) calculated from the data in ref. 13 (Fig. 4). For systems 4–8:

$$\varepsilon = 17.56 - 2.44 \cdot 10^{-2}P \; (r = 0.9995; r_{99.9\%} = 0.9912) \qquad (1)$$

An increasing adsorptive solvent strength means a lower eluent potency in RPTLC.

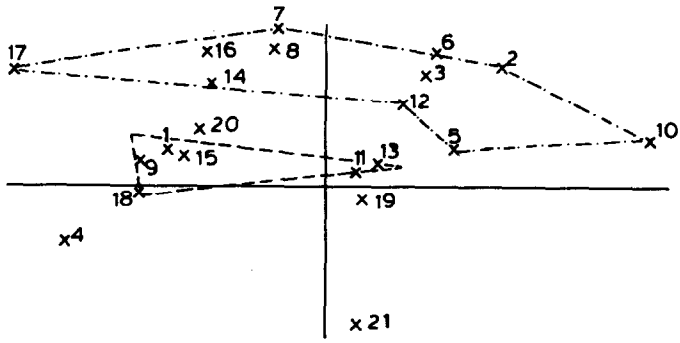

Fig. 5. Two-dimensional non-linear mapping of spectral mapping variables. Upper part, eluents containing methanol; lower part, eluents containing acetonitrile numbers indicate RPTLC systems in Table I. No. of iterations: 21. Error of mapping: $8.6 \cdot 10^{-3}$.

The same correlation also exists for various organic phases (Fig. 4, line 2), but the correlation is not so good, indicating that different side effects may play a considerable role. For RPTLC systems 17–21:

$$\varepsilon = 20.48 - 4.69 \cdot 10^{-2}P \; (r = 0.9291; r_{95\%} = 0.8783) \quad (2)$$

The conclusions drawn from the two-dimensional non-linear mapping of spectral mapping calculations (Fig. 5) are identical with those drawn from Fig. 3. In contrast to the PC analysis, this map clusters the RPTLC systems only on the bases of their selectivities, excluding the effect of solvent strength. However, the clustering of systems is the same, only the different organic phases (methanol or acetonitrile) and not the identical layers forming groups. This finding supports our previous supposition that the determinative factor in classifying RPTLC systems is the quality of the organic solvent and not the quality of the RPTLC layer.

We assume that the organic component of the eluent is bonded more or less strongly to the lipophilic surface of chemically bonded reversed-phase layers, and therefore the partition occurs between the unmobilized organic component and the bulk of the eluent and not between the lipophilic layer and the eluent. This assumption explains that in our case the various layers influence the retention strength by adsorbing different amounts of organic phase but not the selectivity, which is the result of the partition behaviour of barbituric acid derivatives between the adsorbed organic components of the eluent and the bulk of the eluent.

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