ELSEVIER

Journal of Chromatography A, 668 (1994) 495–500

JOURNAL OF
CHROMATOGRAPHY A

Relationship between the high-performance liquid and thin-layer chromatographic retention of non-homologous series of pesticides on an alumina support

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Abstract

The retentions of 26 commercial pesticides were determined on an alumina HPLC column and in TLC carried out on alumina layers using *n*-hexane–dioxane mixtures as eluents. Both the log k'_0 and R_{M0} values of the pesticides decreased linearly with increasing concentration of dioxane in the eluent and they were strongly intercorrelated. The prediction power of TLC for HPLC was low, probably owing to the different pH of the alumina surface. The hydrophilic retention parameters of pesticides determined on alumina supports have a negligible effect on the type of their biological activity (herbicidal, fungicidal, acaricidal or insecticidal).

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1. Introduction

In the last decade, alumina supports have gained growing acceptance and application in high-performance liquid chromatography (HPLC) [1,2]. A possible advantage for the use of alumina instead of silica supports is the greater pH stability of the former [3,4]. The characteristics and applications of alumina [5] and modified alumina supports [6] have recently been discussed. Alumina supports have been used successfully for the separation of transition metal ions [7] and alkyl- and phenylnaphthalenes [8] and for the preconcentration of sulphate from complex matrices [9]. Many efforts have been devoted to the development of modified alumina support coated with hydrophobic ligands [10,11]. Polybutadiene-coated alumina has been used for the determination of the lipophilicity of organic bases [12] and imidazol(in)e drugs [13], and for the separation of proteins [14]. However, octadecylsilica was better than octyl-coated alumina for peptide separations [15].

Thin-layer chromatography (TLC) is a rapid and inexpensive method suitable for the separation of many organic and inorganic compounds. As many HPLC sorbents are also applied in TLC [16–18], the use of TLC as a pilot method for the development of HPLC separation procedures offers considerable advantages. The predictive power of TLC for HPLC depends strongly on the type of solutes [19] and on the experimental conditions [20].

The objectives of this work were the determination of the retention of some pesticides frequently used in agricultural practice on both

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0021-9673/94/$07.00
SSDI 0021-9673(93)E1131-I

HPLC and TLC alumina supports, the evaluation of the predictive power of TLC for HPLC and the elucidation of the relationship between the biological activity and retention characteristics of pesticides [21].

2. Experimental

2.1. High-performance liquid chromatography

A 25 cm × 4 mm I.D. alumina column was used in each experiment. The alumina support was the experimental product of the research team of Dr. L. Zsembery (Hungarian Alumina Trust, Research and Development Laboratory, Budapest, Hungary). The retention characteristics of the column have been reported previously [22]. The HPLC equipment consisted of a Liquopump Type 312 (Labor MIM, Budapest, Hungary), a Cecil (Cambridge, UK) CE-212 spectrophotometer used as the detector, a 20-μl injector (Valco, Houston, TX, USA) and a Waters (Milford, MA, USA) Model 740 integrator. The flow-rate was 1 ml/min and the detection wavelength 240 nm. The column was not thermostated. Each HPLC measurement was run in triplicate.

2.2. Thin-layer chromatography

DC-Alufolien F_{254} precoated plates (Merck, Darmstadt, Germany) were used without any pretreatment. The developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room temperature and the running distance was *ca.* 15 cm. The chambers were not presaturated. After development the plates were dried at 105°C and the spots were detected under UV light or with iodine vapour. Each determination was run in quadruplicate. When the relative standard deviation (R.S.D.) between parallel determinations was higher than 5%, the data were omitted from the calculations.

The pesticides studied are listed in Table 1. The pesticides were dissolved in dioxane at concentrations of 5 and 0.1 mg/ml for TLC and HPLC investigations, respectively. The eluents were *n*-hexane–dioxane mixtures (10–60% (v/v) dioxane in steps of 5% (v/v) for HPLC and 5–60% (v/v) dioxane in steps of 5% (v/v) for TLC).

Linear correlations were calculated between the logarithm of the capacity factor and the dioxane concentration (C) in the eluent (Eq. 1) and between the R_M value and the dioxane concentration in the eluent (Eq. 2) separately for each pesticide:

$$\log k' = \log k'_0 + b_1 C \qquad (1)$$

where k' is the actual retention value of a pesticide at C% (v/v) dioxane concentration and k'_0 is the theoretical retention value of a pesticide at 0% (v/v) dioxane concentration (pure *n*-hexane),

$$R_M = R_{M0} + b_2 C \qquad (2)$$

where R_M is the actual R_M value of a pesticide determined at C% (v/v) dioxane concentration and R_{M0} is the theoretical R_M value extrapolated to zero dioxane concentration.

To elucidate the validity of the hypothesis that for homologous series of solutes the slope and intercept values are strongly intercorrelated [23,24], the homologous or inhomogeneous character of pesticides as solutes in adsorption chromatography was assessed by calculating linear correlations between the slope (b_1 and b_2) and intercept value ($\log k'_0$ and R_{M0}) of Eqs. 1 and 2. To find the relationship between the HPLC and TLC retention data the slope and intercept values of Eq. 1 were correlated with the corresponding value of Eq. 2.

To assess the similarities and dissimilarities between the chromatographic parameters and biological activites of pesticides, principal component analysis (PCA) was applied [25]. The adsorption capacities (intercept values of Eqs. 1 and 2) and the specific hydrophilic surface areas (slope values of Eqs. 1 and 2) of pesticides were taken as variables and the pesticides were the observations. Only pesticides with each physicochemical parameter determined were included in the calculations. The two-dimensional non-linear map of PC loadings and variables was also calculated [26]. Iteration was carried out to the point when the differences between the two last iterations was smaller than 10^{-8}.

3. Results and discussion

Pesticides separate well on an alumina column and they give symmetrical peaks with each eluent system. Their retention times differ considerably, hence an alumina column can be used successfully for the separation of pesticides.

The parameters of Eq. 1 are given in Table 1. The relationship between log k' and dioxane concentration is linear and the correlation coefficient in most instances is higher than 0.99, confirming the applicability of Eq. 1. The slope and intercept values differ considerably from each other, supporting the previous qualitative conclusion that an alumina column is suitable for the separation of commercial pesticides. The parameters in Table 1 make possible the calculation of retention time differences for each pair of pesticides at each eluent composition:

$$t_1 - t_2 = t_0(10^{a_1+b_2C} - 10^{a_2+b_2C}) \quad (3)$$

where a and b are intercept and slope values for pesticides 1 and 2 at dioxane concentration C. The eluent composition corresponding to the maximum retention time difference can also be calculated: the first derivative of Eq. 3 must be zero and the dioxane concentration expressed accordingly:

$$C = [a_1 - a_2 + \log(b_1/b_2)]/(b_2 - b_1) \quad (4)$$

As in HPLC, the TLC retention of pesticides decreases linearly with increasing concentration of dioxane in the eluent, and no anomalous retention behaviour was observed. However, the correlation coefficients were markedly lower,

Table 1
Parameters of linear correlations between log k' and dioxane concentration (C) in the eluent: $\log k' = \log k'_0 + b_1C$

No.	Pesticide	Log k'_0	$-b_1 \cdot 10^{-2}$	$s_{b_1} \cdot 10^{-3}$	r
1	Bromoxynil	0.78	2.47	1.80	0.9947
2	Isoproturon	1.30	3.62	2.48	0.9930
3	Chlorotoluron	1.30	3.61	2.39	0.9935
4	Methiocarb	0.98	3.35	2.94	0.9924
5	Chlorbromuron	0.72	3.09	0.88	0.9992
6	Linuron	0.75	3.32	2.37	0.9949
7	Bromopropylate	1.06	4.59	3.41	0.9978
8	*p,p'*-DDT	−0.22	2.14	1.81	0.9892
9	Diphenamid	0.97	3.61	2.41	0.9956
10	Carboxin	0.97	3.46	3.50	0.9850
11	Buprofezin	0.07	3.12	2.31	0.9919
12	Binacapryl	0.36	2.87	1.50	0.9959
13	Iodphenphos	0.06	2.34	1.21	0.9960
14	Oxadixyl	1.56	1.84	1.60	0.9925
15	Fuberidazol	1.46	3.06	3.79	0.9850
16	Iprodione	1.16	3.57	4.60	0.9838
17	Ethofumasate	0.93	3.28	1.06	0.9990
18	Oxabetrinil	0.53	3.00	2.42	0.9904
19	Oxadiazon	0.04	2.94	2.40	0.9901
20	Lenacil	1.71	3.37	0.88	0.9997
21	Terbacil	1.72	3.39	0.94	0.9996
22	Atrazine	0.79	3.03	4.18	0.9815
23	Terbutylazine	0.97	4.31	8.30	0.9486
24	Aziprotryne	0.32	2.84	3.08	0.9828
25	Terbutryn	0.33	2.91	2.95	0.9849
26	Clofentezine	0.46	2.88	1.07	0.9979

indicating the inherent lower reproducibility of TLC.

The correlation between the slope and intercept values of Eq. 1 was fairly weak:

$$\log k'_0 = -0.29 + 0.35b_1$$
$$n = 26,\ r_{\text{calc.}} = 0.3907,\ r_{95\%} = 0.3889 \qquad (5)$$

and no significant correlation was found between the corresponding parameters of Eq. 2. This finding indicates that from the chromatographic point of view pesticides cannot be regarded as a homologous series of solutes.

A good significant correlation was found between the log k'_0 and R_{M0} values:

$$\log k'_0 = -0.37 + 0.90R_{M0}$$
$$n = 25,\ r_{\text{calc.}} = 0.8060,\ r_{99.9\%} = 0.6177 \qquad (6)$$

The result indicates that in this instance TLC is suitable for the prediction of the retention behaviour of pesticides in HPLC. However, it must be emphasized that both the log k'_0 and R_{M0} values are theoretical constructions describing the retention of pesticides in pure n-hexane, in which they hardly elute.

No significant linear correlation was found between the slope values of Eqs. 1 and 2. The discrepancy can be tentatively explained by the supposition that the surface pH and the adsorption capacity of active centres on the alumina surface may be different, modifying the contact surface between solute and support. This finding also indicates that the predictive power of TLC is fairly low. Although TLC results may predict the theoretical retention behaviour of pesticides in n-hexane in HPLC, owing to the absence of a correlation between the slope values of Eqs. 1 and 2 they are inadequate for the prediction of the retention behaviour in n-hexane–dioxane mixtures in which the pesticides really elute.

The results of PC analysis are given in Table 2. The first two components account for about 80% of the total variance. This means that two background variables include most of the information content of the four chromatographic parameters. It must be emphasized that the two hypothetical variables need not to have any concrete physical (or chromatographic) meaning. PC analysis only proves the mathematical possibility. The adsorption capacities of HPLC (log k'_0) and TLC (R_{M0}) aluminas have high loadings in the first PC. This result indicates that the first PC can be regarded as a parameter related to the adsorption strength of the supports. The second PC contains the corresponding slope values b_1 and b_2. As the slope values can be regarded as quantities related to the surface of solutes in

Table 2
Relationship between the HPLC and TLC retention behaviours of pesticides: results of principal component analysis

No. of PC component	Eigenvalue	Variance explained (%)	Total variance explained (%)
1	1.99	49.76	49.76
2	1.23	30.48	80.24
3	0.69	17.28	97.53

Retention parameters[a]	Principal component loadings		
	1	2	3
Log k'_0 (adsorption capacity in HPLC)	0.96	−0.16	−0.03
b_1 (specific hydrophilic surface area in HPLC)	0.53	0.63	−0.57
R_{M0} (adsorption capacity in TLC)	0.88	−0.08	0.43
b_2 (specific hydrophilic surface area in TLC)	−0.12	0.89	0.43

[a] See Eqs. 1 and 2.

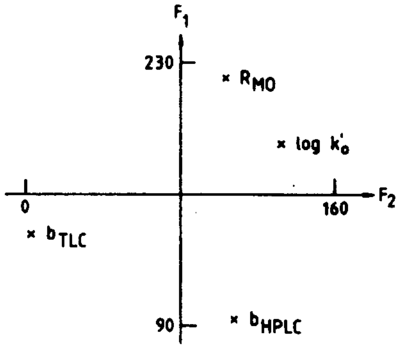

Fig. 1. Two-dimensional non-linear map of principal component loadings. No. of iterations, 29; maximum error, $9.89 \cdot 10^{-4}$.

contact with the support [27], the second PC characterizes the specific hydrophilic surface area of pesticides.

The capacity values of alumina supports (log k'_0 and R_{M0}) are nearer to each other on the two-dimensional non-linear map of PC loadings than the corresponding slope values (Fig. 1). This finding entirely supports the results of Eq. 6 that the adsorption capacities of HPLC and TLC alumina supports are similar.

Pesticides do not form separate clusters according to the type of their biological activity (herbicidal, fungicidal, acaricidal or insesticidal) on the two-dimensional non-linear map of PC variables (Fig. 2). This finding indicates that the hydrophilic retention parameters determined on an alumina support have a negligible impact on the type of biological activity of pesticides.

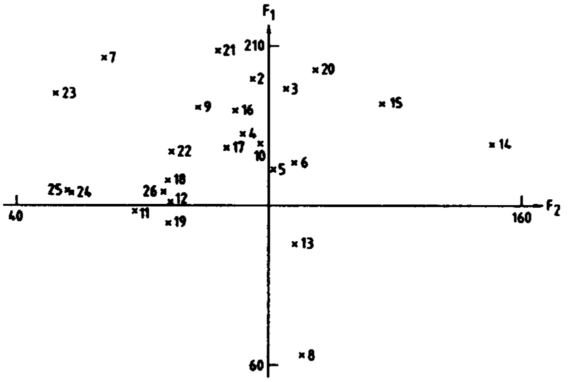

Fig. 2. Two-dimensional non-linear map of principal component variables. No. of iterations, 91; maximum error, $1.15 \cdot 10^{-2}$. Numbers refer to pesticides in Table 1.

It can be concluded that a wide variety of commercial pesticides can be successfully separated on alumina supports both in HPLC and TLC using *n*-hexane–dioxane eluents. However, the predictive power of TLC for HPLC is relatively low.

4. Acknowledgement

This work was supported by Grant OTKA 2670 of the Hungarian Academy of Sciences.

5. References

[1] J.E. Haky, S. Vemulapalli and L.F. Wieserman, *J. Chromatogr.*, 505 (1990) 307.
[2] J.E. Haky and S. Vemulapalli, *J. Liq. Chromatogr.*, 15 (1990) 3111.
[3] R. Kaliszan, J. Petrusewitz, R.W. Blain and R. Hartwick, *J. Chromatogr.*, 458 (1988) 395.
[4] A. Berthod, *J. Chromatogr.*, 549 (1991) 1.
[5] K. Cabdera, D. Lubda and G. Jung, *Kontakte (Darmstadt)*, (1992) 12.
[6] K. Cabdera, D. Lubda and G. Jung, *Kontakte (Darmstadt)*, (1992) 32.
[7] D.K. Singh and P. Mehrotra, *Chromatographia*, 23 (1987) 747.
[8] J. Puncochárová, J. Vareka, L. Vodicka and J. Kriz, *J. Chromatogr.*, 498 (1989) 248.
[9] W. Buchberger and K. Winsauer, *J. Chromatogr.*, 482 (1989) 505.
[10] J.J. Pesek and H.D. Lin, *Chromatographia*, 28 (1989) 565.
[11] J.E. Haky, N.D. Ramdial, A.R. Raghani and L.F. Wieserman, *J. Liq. Chromatogr.*, 14 (1991) 2859.
[12] G. Yilinkou and R. Kaliszan, *Chromatographia*, 30 (1990) 277.
[13] R.-G. Yilinkou and R. Kaliszan, *J. Chromatogr.*, 550 (1991) 573.
[14] J.E. Haky, A. Raghani and B.M. Dunn, *J. Chromatogr.*, 541 (1991) 303.
[15] J.E. Haky, N.D. Ramdial, B.M. Dunn and L.F. Wieserman, *J. Liq. Chromatogr.*, 15 (1992) 1831.
[16] L. Witherow, R.J. Thorp, I.D. Wilson and A. Warrander, *J. Planar Chromatogr.*, 3 (1990) 169.
[17] M. Mack, H.E. Hauck and H., Herbert, *GIT Fachz. Lab.*, 34 (1990) 276.

[18] W. Fischer, H.E. Hauck and W. Jost, in F.A.A. Dallas (Editor), *Recent Advances in Thin-Layer Chromatography (Proceedings of Chromatographic Society International Symposium, 1987)*, Plenum Press, New York, 1988, p. 139.
[19] T. Cserháti and T. Bellay, *Acta Phytopathol. Entomol. Hung.*, 23 (1988) 257.
[20] J.K. Rozylo and M. Janicka, *J. Planar Chromatogr.*, 4 (1991) 241.
[21] R. Kaliszan, *Quantitative Structure–Chromatographic Retention Relationships*, Wiley, New York, 1987.
[22] T. Cserháti, *Chromatographia*, 29 (1990) 593.
[23] T. Cserháti, *Chromatographia*, 18 (1984) 18.
[24] K. Valkó, *J. Liq. Chromatogr.*, 7 (1984) 1405.
[25] A.R. Dillon and M. Goldstein, *Multivariate Analysis*, Wiley, New York, 1984, p. 23.
[26] J.W. Sammon, Jr., *IEEE Trans. Comput.*, C18 (1969) 401.
[27] C. Horvath, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.