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Use of principal component analysis for studying the separation of pesticides on polyethylene-coated silica columns

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Abstract

The retention time of 27 pesticides was determined on an polyethylene-coated silica high-performance liquid chromatography column (PEE_{sil}) using water–methanol mixtures as eluents. Linear correlations were calculated between the logarithm of the capacity factor and the methanol concentration in the eluent (C) and the relationship between the slope and intercept values of the above correlation, the hydrophobicity, specific hydrophobic surface area of the pesticides and their retention parameters on porous graphitized carbon column was elucidated by using principal component analysis followed by two-dimensional nonlinear mapping. The most polar pesticides showed irregular retention behaviour of the PEE_{sil} column, their retention decreased with increasing concentration of methanol in the eluent. This irregular retention was tentatively explained by the silanophile effect: at higher methanol concentrations the polar substructures of pesticides have a higher probability of binding to the polar adsorption centers on the silica surface not covered by the hydrophobic ligand increasing in this manner the retention capacity of the support. Principal component analysis showed that PEE_{sil} support has retention characteristics different from those of traditional reversed-phase supports. © 1998 Elsevier Science B.V.

Keywords: Principal component analysis; Chemometrics; Polyethylene-coated silica; Stationary phases, LC; Pesticides

1. Introduction

In recent decades automated chromatographic equipment have found growing acceptance and application both in gas–liquid and high-performance liquid chromatography (HPLC). Automated instruments produce a huge amount of retention data in a relatively short time and the evaluation of these large retention data matrices by traditional regression models is either time-consuming or practically impossible. High-speed computers and multivariate mathematical–statistical methods such as principal component analysis (PCA) [1], factor analysis [2], canonical correlation analysis [3], cluster analysis [4] and spectral mapping techniques [5,6] have been developed and successfully used for the extraction of maximal information from any type of retention data matrices. PCA has been frequently applied in chromatography to select structural descriptors related to the chromatographic behaviour [7,8] to extract retention data correlated with the biological activity of solutes [9–11], and to compare the performance of various chromatographic systems [12,13].

Due to its high separation capacity and versatility HPLC became a method of preference for the analysis of a wide variety of organic and inorganic compounds. Many HPLC methods have been developed for the separation and quantitative determination of commercial pesticides in various matrices such as surface [14] and sewage waters [15], biological fluids [16], soils [17] and in food products such as eggs [18], milk [19], etc. The majority of

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separations have been carried out on reversed-phase supports based on silica. Due to its higher chemical stability polymer-coated silica became popular and used more and more frequently in HPLC practice. A great number of polymers such as poly(alkyl)aspartamide [20], alkyl polyxiloxanes [21], polyvinylpyrrolidone [22], polyethyleneimine [23], polyamine [24], etc. have been tested as coating agents and successfully used for the separation of peptides [25], proteins [26] and various alkaline compounds [27].

The objectives of our investigation were to study the retention behaviour of some commercial pesticides on a polyethylene-coated silica (PEE_{sil}) support, to determine the influence of hydrophobicity parameters of solutes on the retention behaviour and to compare the retention characteristics of the column with those of other chromatographic systems using multivariate mathematical–statistical methods.

2. Materials and methods

The HPLC equipment consisted of a Gilson gradient analytical system (Gilson Medical Electronics, Villiers-le-Bell, France) with two piston pumps (Model 302), detector (Model 116), Rheodyne injector with 20 μl sample loop (Cotita, CA, USA), and a Waters 740 integrator (Milford, MA, USA). The column was a PEE_{sil} column prepared in our laboratory (250×4 mm I.D.) [28]. The flow-rate was 1.0 ml/min and the detection wavelength was 230 nm. The column was not thermostated; each determination was run at room temperature. Mixtures of methanol–water were used as eluents, the concentration of methanol in the eluent varied between 5–60% (v/v) in steps of 5%. The use of this wide concentration range was motivated by the highly different retention of pesticides on the PEE surface. The commercial and chemical names of the pesticides and their biological activity are compiled in Table 1. The pesticides were dissolved in the eluent at a concentration of 0.5 mg/ml. The retention time of each compound was determined by three consecutive determinations. Linear correlations was calculated between the log k' values and the methanol concentration (C) in the eluent:

$$\log k' = \log k'_0 + bC \quad (1)$$

where k' = capacity factor, k'_0 = capacity factor extrapolated to zero concentration of organic component in mobile phase (related to the retention strength of pesticides on the PEE column); b = change of log k' caused by unit change of concentration of organic component (related to specific surface area of solutes in contact with the PEE surface [29]) and C = concentration of organic component (v/v).

In the case of an homologous series of solutes, the intercepts and slope values of Eq. (1) are intercorrelated [30]. To test whether the same is true for the non-homologous pesticides in this study, linear correlations were calculated between the corresponding parameters.

To find the similarities and dissimilarities between the chromatographic characteristics and hydrophobicity parameters of pesticides, PCA was applied. The parameters of Eq. (1), the same parameters determined on a porous graphitized carbon column (PGC) [31], and the hydrophobicity and specific hydrophobic surface area [32] were the variables and the pesticides were the observations. The inclusion of the retention parameters of pesticides on the PGC column was motivated by the finding that the PGC column shows different retention characteristics from those of traditional reversed-phase columns although the eluents used are typical reversed-phase eluents [33]. Pesticides 21–27 were omitted from the calculation because their parameters were not given in Refs. [31] and/or [32]. The limit of the variance explained was set to 99.9%. To decrease the dimensionality of the matrices of principal component loadings and variables, a two-dimensional nonlinear mapping technique was used [34]. The iteration was carried out to the point when the difference between the two last iterations was lower than 10^{-8}.

3. Results and discussion

The parameters of Eq. (1) are listed in Table 2. S_b and r values are the standard deviation of the slope "b" and the coefficient of correlation indicating the fitness of the equation to the experimental data. The relationship between the log k' and organic phase concentration was significantly linear in each instance; however, the coefficients of regression were

Table 1
Chemical name and biological activity of commercial pesticides

No.	Common name	Chemical name	Biological activity
1	Terbutryn	N^2-*tert.*-Butyl-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine	Herbicide
2	Oxabetrinil	(*Z*)-1,3-Dioxolan-2-ylmethoxy-imino(phenyl)-acetonitrile	Herbicide
3	Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea	Herbicide
4	Isoproturon	3-(4-Isopropylphenyl)-1,1-dimethylurea	Herbicide
5	Chlorbromuron	3-(4-Bromo-3-chlorophenyl)-1-methoxy-1-methylurea	Herbicide
6	Terbutylazine	N^2-*tert.*-Butyl-6-chloro-N^4-ethyl-1,3,5-triazine-2,4-diamine	Herbicide
7	Atrazine	6-Chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine	Herbicide
8	Terbacil	3-*tert.*-Butyl-5-chloro-6-methyl-uracyl	Herbicide
9	Carboxin	5,6-Dihydro-2-methyl-1,4-oxa-thiine-3-carbox-anilide	Fungicide
10	Oxadiazon	5-*tert.*-Butyl-3-(2,4-dichloro-5-isopopoxyphenyl-0-1,3,4-oxa-diazol-2(3H)-one	Herbicide
11	Prochloraz	N-Propyl-N-[2-(2,4,6-tri-chlorophenoxy)-ethyl]imidazole-1-carboxamide	Fungicide
12	Iprodione	3-(3,5-Dichlorophenyl-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide	Fungicide
13	Buprofezin	2-*tert.*-Butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one	Insecticide
14	Flutriafol	(*RS*)-2,4′-Difluoro-α-(1H-1,2,4-triazol-1-ylmethyl)benzhydryl alcohol	Fungicide
15	Chlorotoluron	3-(3-Chloro-*p*-tolyl)-1,1-dimethylurea	Herbicide
16	Iodofenphos	O-2,5-Dichloro-4-iodophenyl O,O-dimethyl phosphorothioate	Insecticide, acaricide
17	Binapacryl	2-*sec*.-Butyl-4,6-dinitrophenyl-3-methylcrotonate	Fungicide, acaricide
18	Fuberidazole	2-(2-Furyl)benzimidazole	Fungicide
19	Lenacil	3-Cyclohexyl-1,5,6,7-tetrahydrocyclopenta-pyrimidine-2,4(3H)-dione	Fungicide
20	Diphenamid	N,N-Dimethyldiphenylacetamide	Herbicide
21	Triasulfuron	1-[2-(2-Chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea	Herbicide
22	Oxadixyl	2-Methoxy-N-(2-oxo-1,3-oxazolidin-3-yl)acet-2′,6′-xylidide	Fungicide
23	Ethofumasate	(±)-2-Ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate	Herbicide
24	Thiram	Bis(dimethylthiocarbamoyl)disulfide	Fungicide
25	Chlorfenson	4-Chlorobenzenesulfonic acid 4-chlorophenylester	Miticide
26	Cymoxanyl	1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea	Fungicide
27	Aziprotrin	4-Azido-N-isopropyl-6-methylthio-1,3,5-triazine-2-ylamine	Herbicide

Table 2
Parameters of linear correlations between log k' and methanol concentration (C) in the eluent: $\log k' = \log k'_0 + bC$

No. of pesticide	$\log k'_0$	$-b \cdot 10^{-2}$	$S_b \cdot 10^{-3}$	r
1	0.89	1.59	4.44	0.9296
2	0.91	1.98	3.84	0.9644
3	0.86	1.49	2.80	0.9665
4	0.86	2.13	4.88	0.9514
5	1.20	2.05	1.09	0.9972
6	0.78	1.26	1.13	0.9960
7	0.98	2.51	7.56	0.9201
8	1.32	0.50	1.35	0.9993
9	1.22	1.10	1.47	0.9827
10	1.22	1.22	1.68	0.9814
11	1.58	1.79	3.64	0.9801
12	1.04	1.41	2.08	0.9789
13	1.44	1.64	5.01	0.9566
14	0.93	2.26	1.39	0.9981
15	0.91	1.86	4.42	0.9481
16	1.21	0.58	0.57	0.9952
17	1.42	1.20	2.77	0.9505
18	0.96	2.29	1.83	0.9968
19	1.47	2.97	11.52	0.9324
20	−0.98	−0.31	14.49	0.8334
21	0.96	2.32	1.36	0.9966
22	−0.35	−1.83	6.61	0.8902
23	−0.51	−2.45	9.47	0.8774
24	1.04	3.58	3.01	0.9930
25	1.16	0.22	0.14	0.9979
26	1.12	0.87	2.55	0.8917
27	0.85	1.73	5.93	0.9988

sometimes lower than those generally accepted in up-to-date HPLC practice. We assume that this discrepancy may be due to the fact that the column was not thermostated and the changing ambient temperature slightly modified the retention resulting in the decrease of the coefficient of regression. The slope and intercept values differ considerably from each other indicating that the pesticides can be separated on the PEE column in a methanol–water eluent system. The parameters in Table 2 make the calculation of retention time differences for each pair of pesticides at each eluent composition possible:

$$t_1 - t_2 = t_0(10^{a_1+b_1 C} - 10^{a_2+b_2 C}) \quad (2)$$

where a and b = intercept ($\log k'_0$) and slope values for compounds 1 and 2 at C organic phase concentration. The eluent composition corresponding to the maximum retention time difference can also be calculated: the first derivative of Eq. (2), must be zero and the organic phase concentration expressed accordingly:

$$C = (a_1 - a_2 + \log b_1/b_2)/(b_2 - b_1) \quad (3)$$

The most polar pesticides showed irregular retention behaviour on the PEE column; their retention decreased with increasing concentration of methanol in the eluent. This irregular retention was tentatively explained by the silanophile effect: at higher methanol concentrations the polar substructures of pesticides have a higher probability of binding to the polar adsorption centers on the silica surface not covered by the hydrophobic ligand increasing in this manner the retention capacity of the support.

Significant linear correlation was found between the intercept ($\log k'_0$) and slope values (b) of Eq. (1) (Fig. 1). This result indicates that the general relationship developed for alkyl-bonded reversed-phase columns is also valid for PEE, and the pesticides behave as a homologous series of compounds, however, their chemical structure is highly different. Although the relationship is highly significant (significance level being over 99.9%), the variance explained is relatively low (about 40%). This finding indicates that the separate inclusion of both retention parameters in chemical structure–retention behaviour calculation is justified. We have to emphasize that the

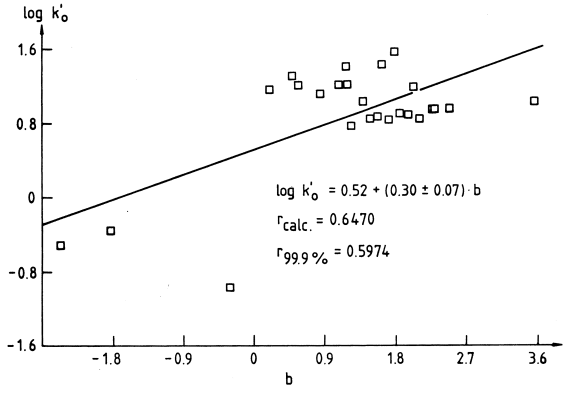

Fig. 1. Relationship between the $\log k'_0$ and b values of Eq. (1).

correlation is only significant when the barbituric acid derivatives showing irregular retention behavior are included in the calculation. As Eq. (1) can also be successfully used for the calculation of the relationship between retention of these solutes and the composition of the mobile phase we assumed that the inclusion of these data in the calculation of the correlation between $\log k'_0$ and b is justified.

The results of the PCA are compiled in Table 3. An overwhelming majority of the information about the retention behaviour of pesticides can be described by three background variables. In other words, three theoretical chromatographic parameters are sufficient to describe the retention behaviour of pesticides in the chromatographic systems investigated. Unfortunately, PCA does not define these three parameters as concrete physical or physicochemical entities, only indicates their mathematical possibility. The data clearly indicate that the retention characteristics of PEE columns have a high loading in the second principal component whereas the other parameters have high loadings in the first principal component. This result suggests that the retention behaviour of pesticides on PEE supports can be governed other than by the hydrophobicity parameters.

Each chromatographic system forms a distinct cluster on the two-dimensional nonlinear map of principal component loadings indicating the considerable differences between their retention characteristics (Fig. 2). The data support our previous conclusions that parameters other than hydrophobicity may have a considerable impact on the retention of pesticides on the PEE support. It was assumed that the apolar polyethylene chain lies parallel to the surface of the silica support and the hydrophobic substructures of pesticides have only limited access to this surface layer. As the irregular retention behaviour of more polar pesticides indicated the adsorptive centers of silica not covered by the hydrophobic ligand can also bind the pesticides. It can be assumed that the retention of pesticides on the PEE support is governed by the interplay of hydrophobic and hydrophilic forces occurring between the support surface and the pesticide solutes.

The distribution of pesticides according to their

Table 3
Similarities and dissimilarities between the retention characteristics of various chromatographic systems: results of principal component analysis

No. of principal component	Eigenvalue	Variance explained (%)	Total variance explained (%)
1	3.12	52.00	52.00
2	1.45	24.20	76.20
3	0.74	12.37	88.57

Parameters	Principal component loadings No. of principal component		
	1	2	3
$\log k'_{0(\mathrm{PEE})}$	0.17	0.90	0.10
$b_{(\mathrm{PEE})}$	−0.43	0.77	−0.02
$\log k'_{0(\mathrm{PGC})}$	0.90	0.13	0.30
$b_{(\mathrm{PGC})}$	0.77	−0.11	0.53
R_{M0}	0.90	0.17	−0.33
b_{RPTLC}	0.83	−0.07	−0.50

$\log k'_{0(\mathrm{PEE})}$ = retention capacity of pesticides on polyethylene-coated silica column.
$b_{(\mathrm{PEE})}$ = surface area of pesticides in contact with the support.
$\log k'_{0(\mathrm{PGC})}$ = retention capacity of pesticides on porous graphitized carbon column.
$b_{(\mathrm{PGC})}$ = surface area of pesticides in contact with the porous graphitized carbon support.
R_{M0} and b_{RPTLC} = hydrophobicity and specific hydrophobic surface area of pesticides determined by reversed-phase thin-layer chromatography, respectively.

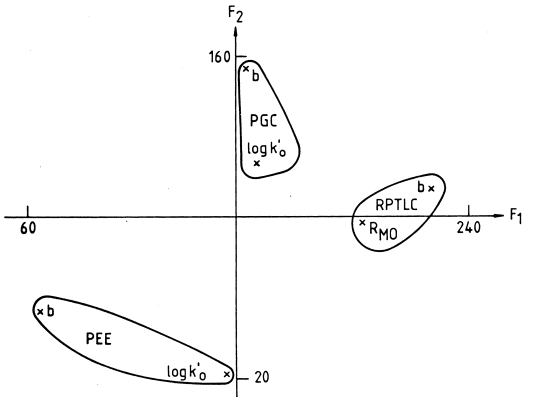

Fig. 2. Similarities and dissimilarities between the retention characteristics and hydrophobicity parameters of pesticides. Two-dimensional nonlinear map of principal component loadings. No. of iterations: 71; maximum error: $1.67 \cdot 10^{-2}$.

chromatographic characteristics and hydrophobicity parameters (two-dimensional nonlinear map of principal component variables) is shown in Fig. 3. Pesticides do not form separate clusters either according to the chemical structure or according to their biological activity. This result indicates that the biological activity of pesticides cannot be predicted by their retention behaviour and hydrophobicity parameters.

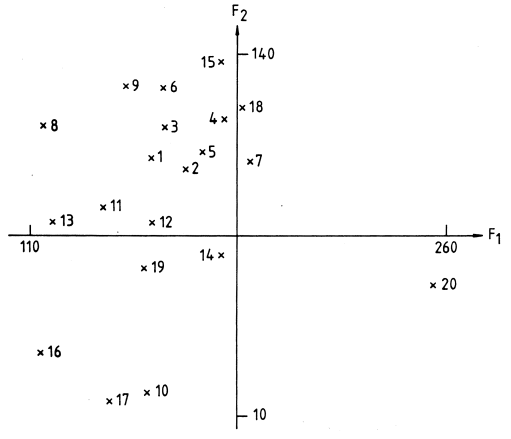

Fig. 3. Similarities and dissimilarities between pesticides according to their retention behaviour and hydrophobicity parameters. Two-dimensional nonlinear map of principal component variables. No. of iterations: 283; maximum error: $2.36 \cdot 10^{-2}$. Numbers refer to pesticides in Table 1.

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