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Use of principal component analysis for the study of the retention behaviour of anticancer drugs on a β-cyclodextrin polymer-coated silica column

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Abstract

The retention parameters of eighteen commercial anticancer drugs were determined on a β-cyclodextrin polymer-coated silica support (βCDP) using methanol–water mixtures as eluent and the relationship between the retention behaviour and physico-chemical parameters was elucidated by principal component analysis (PCA) followed by two-dimensional non-linear mapping. No significant linear correlation was found between the retention behaviour of drugs on octadecylsilica and βCDP silica columns, indicating that the retention capacity and selectivity of the columns are considerably different. The results of PCA indicated that hydrophobic and electronic interactions and steric conditions govern the retention of anticancer drugs on βCDP column, suggesting a mixed retention mechanism.

Keywords: Principal component analysis; Retention behaviour; Stationary phases, LC; Cyclodextrin polymer-coated columns; Cyclodextrins; Drugs

1. Introduction

The application of automated chromatographic equipment has enormously increased the number of retention data that can be produced in a given time interval. The evaluation of large retention data sets containing a considerable amount of information is practically impossible by traditional calculation methods. Computer-assisted multivariate mathematical statistical methods make possible the simultaneous evaluation of a virtually unlimited number of retention parameters, facilitating the solution of practical and theoretical problems in any field of chromatography. Principal component analysis (PCA) elucidates the similarities and dissimilarities between the members of a parameter set without one being the dependent variable [1,2]. PCA has been frequently used for data evaluation in gas chromatography (GC) [3], thin-layer chromatography (TLC) [4] and high-performance liquid chromatography (HPLC) [5–7].

Cyclodextrins are cyclic oligosaccharides which form inclusion complexes with a wide variety of organic and inorganic compounds [8]. As the formation of inclusion complexes considerably modifies the retention of solutes, cyclodextrins and cyclodextrin derivatives have frequently been used as stationary phases or mobile phase

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additives in many fields of chromatography such as TLC [9], GC [10] and HPLC [11].

Silica columns with β-cyclodextrin polymer (βCDP) coating have been prepared recently and their retention characteristics [12] and their capacity for enantiomer separation have been elucidated [13].

The objectives of this work were to study the retention characteristics of a β-cyclodextrin polymer-coated silica column using a non-homologous series of anticancer drugs as solutes, to find relationship between retention behaviour and physico-chemical parameters and to compare the applicability of various multivariate mathematical statistical methods for the evaluation of the retention behaviour of these solutes.

2. Experimental

The HPLC system consisted of a Model 307 pump (Gilson, Villiers-le-Bel, France), a CE-212 variable-wavelength UV detector (Cecil Instruments, Cambridge, UK), an injector (Valco, Houston, TX, USA) with a 20-μl sample loop and a Waters Model 740 integrator (Waters–Millipore, Milford, MA, USA). The β-CDP-coated silica support (patent pending) was prepared at the Cyclolab Research and Development Laboratory (Budapest, Hungary). A 25 cm × 4 mm I.D. column was filled in our laboratory with a Shandon (Pittsburgh, PA, USA) analytical HPLC packing pump by the procedure proposed for the filling of reversed-phase columns. The flow-rate was 1 ml/min and the detection wavelength was set at 215 nm. Mixtures of water–methanol were used as eluents, with methanol concentrations ranging from 0 to 50 vol.%.

The anticancer drugs are listed in Table 1. They were dissolved in methanol at a concentration of 0.05 mg/ml. The retention time of each compound in each eluent was determined with three consecutive determinations. Linear correlations was calculated between the log k' value of drugs and the methanol concentration in the eluent:

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

where k' = capacity factor, k'_0 = capacity factor extrapolated to zero methanol concentration in the mobile phase (intercept), b = change in log k' value caused by a unit change (1 vol.-%) in methanol concentration (slope) and C = methanol concentration (vol.-%). The intercept and slope values were considered to give the best estimate of the retention capacity and specific surface area of the drugs in contact with the stationary phase. The retention data of the anticancer drugs were evaluated by PCA combined with varimax rotation, cluster analysis [14] and non-linear mapping [15]. The parameters of Eq. 1 (slope and intercept values) and various physico-chemical parameters of drugs (a total of ten parameters) were considered as variables and the anticancer drugs were the observations. The limit of the variance explained was set to 99.9%. The physico-chemical parameters included in the calculation were the following: π = Hansch–Fujita substituent constant characterizing hydrophobicity [16,17]; *H-Ac* and *H-Do* = indicator variables for proton-acceptor and proton-donor properties, respectively [18]; *M-RE* = molar refractivity [19]; F and R = electronic parameters characterizing the inductive and resonance effect, respectively [20]; σ = Hammett's constant, characterizing the electron-withdrawing power of the substituent [21]; *Es* = Taft's constant, characterizing steric effects of the sustitutent [22]; and B_1 and B_4 = Sterimol width parameters determined by the distance of substituents at their maximum point perpendicular to attachment [23,24].

The physico-chemical parameters of the anticancer drugs were taken from Ref. [25]. Varimax rotation, two-dimensional non-linear mapping [15] and cluster analysis [26] were carried out on the principal component loadings and variables. To elucidate the influence of PCA, on the data evaluation cluster analysis was also applied to the original data matrix. Varimax rotation, cluster analysis and non-linear mapping techniques are theoretically similar: each method calculates and visualizes the relative distances between the members of data matrix (in our case physico-chemical and chromatographic parameters of the drugs). To compare their information content, linear correlations were calculated between the

Table 1
Commercial and IUPAC names of anticancer drugs studied

No.	Commercial name	IUPAC name	Provenance
1	Ftorafur	N-(2-Furanidyl)-5-fluorouracil	Medexport (Russia)
2	Leukeran	4-[*bis*(2-chloroethyl)amino]benzenebutanic acid	Wellcome Foundation (UK)
3	Vincristin	22-Oxo-(3α,14β,16α)-14,15-dihydro-14-hydroxyeburnamenine-14-carbocyclic acid methyl ester	Gedeon Richter (Hungary)
4	Vinblastine	(3α,14β,16α)-14,15-dihydro-14-hydroxyeburnamenine-14-carbocyclic acid methyl ester	Gedeon Richter (Hungary)
5	Vumon	5′-O-Demethyl-1-O-(4,6-O-2-thenylidene-β-D-glucopyranosyl)epipodophyllotoxin	Bristol-Arzneimettel (Germany)
6	Provera	17-α-Acetoxy-6-α-(methyl)progesterone	Upjohn (UK)
7	Bleogin	N′-[3-Diemthyl(sulfonio)propyl]bleomycinamide	Nippon Kagaku (Japan)
8	Paraplatin	9,11,15-Trihydroxy-15-methylprosta-5, 13-dienoic acid	Bristol-Arzneimettel (Germany)
9	Zitazonium	2-[4-(2-Chloro-1,2-diphenylethynyl)phenoxy]-N,N-diethylethaminecitrate	EGIS Pharm. Works (Hungary)
10	Adriblastine (Doxorubicine)	10-[3-(Amino-2,3,6-trideoxy-α-L-hexapyranosyl)oxy]-7,8,9-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5-12-naphthacenedione	Farmitalia (Italy)
11	Natulan	N-(1-Methylethyl)-4-[(2-methylhydrazino)methyl]benzamide	Roche (Switzerland)
12	Alexan	4-Amino-1-β-D-arabifuranosyl-2(14)-pyrimidine	Mack (Germany)
13	Mitomycin C Kyowa	[1-a*R*]-6-Amino-8-[(aminocarbonyl)oxymethyl]-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methylazirino-[2′,3′:3,4]pyrrolo[1,1a]indole-4,7-dione	Kyowa (Japan)
14	Cytoxan	2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide monohydrate	Bristol-Myers (Germany)
15	Estracyt	Estra-1,3,5(10)-triene-3,17-diol-3-(bischloroethyl)carbamate	Aktiebolaget (Sweden)
16	Deticene	5-(3,3-Dimethyl-1-triazenyl)-1*H*-imidazole-4-carboxamide	Rhone-Poulenc (France)
17	Metotrexate	2,4-Diamino-10-methylpteroylglutamic acid	Lachema (Czech Republic)
18	Elobromol	1,6-Dibromo-1,6-bis(desoxy)-D-dulcit	Chinoin (Hungary)

distances determined by non-linear mapping and by varimax rotation techniques:

$$Y_{1,2} = a + bX_{1,2} \quad (2)$$

where $Y_{1,2}$ = first and second coordinates of the non-linear map and $X_{1,2}$ = first and second coordinates of the varimax rotation.

To find the relationship between the retention characteristics of the traditional octadecylsilica and β-cyclodextrin polymer-coated columns, linear correlations were calculated between the corresponding retention parameters. The slope and intercept values of anticancer drugs on ODS column were taken from Ref. [27].

3. Results and discussion

The parameters of Eq. 1 are compiled in Table 2. The relationships between the logarithm of the capacity factor and the concentration of organic phase in the eluent was linear. In each instance the significance level of the correlation was over 95%, proving the applicability of Eq. 1. The results of PCA are summarized in Table 3. Five

Table 2
Parameters of linear correlations between the logarithm of capacity factors ($\log k' = \log k'_0 + bC$) of anticancer drugs and the methanol concentration [C, %(v/v)] in the eluent

Compound no.[a]	$\log k'_0 \cdot 10$	$-b \cdot 10^2$	$S_b \cdot 10^3$	r
1	−2.27	2.01	5.45	0.9050
2	19.26	4.13	8.90	0.9565
3	−0.82	3.12	2.17	0.9976
4	−0.25	3.00	5.02	0.9731
5	4.03	1.00	0.59	0.9965
6	20.37	3.61	8.40	0.9499
7	−0.70	2.57	4.05	0.9648
8	−0.18	2.64	3.20	0.9786
9	−0.50	2.60	5.49	0.9392
10	7.88	1.23	0.63	0.9974
11	8.49	2.38	1.71	0.9949
12	−2.26	2.84	4.89	0.9716
13	3.83	1.58	1.14	0.9949
14	−0.48	2.34	5.08	0.9560
15	2.11	0.90	1.00	0.9820
16	7.68	1.20	1.67	0.9811
17	2.17	1.98	1.05	0.9972
18	3.62	4.68	7.66	0.9621

[a] See Table 1.

principal components explain more than 90% of the total variance. This result indicates that the ten physico-chemical and chromatographic parameters can be substituted by five background variables without a substantial loss of information. Unfortunately, PCA does not prove the existence of such background variables as concrete physico-chemical entities but only indicates its mathematical possibility.

The two-dimensional non-linear map of PCA loadings (HPLC systems) is shown in Fig. 1. The retention parameters form a loose cluster with the hydrophobicity (π), resonance effect (R) and Taft's steric parameter (Es) of the substituents. This finding suggests that the retention of anticancer drugs on a βCDP column is of mixed character involving hydrophobic and electronic forces and steric interactions. We assume that the following interactions may influence the retention behaviour of solutes on βCDP column:

(1) Interactions of solutes with the CD cavity: these interactions are determined by the size of

Table 3
Similarities and dissimilarities between the physico-chemical parameters of the anticancer drugs and their retention on β-cyclodextrin polymer-coated silica column: results of PCA

No. of component	Eigenvalue	Variance explained (%)	Sum of variance explained (%)
1	5.31	48.35	48.35
2	1.57	14.29	62.64
3	1.37	12.45	75.09
4	1.12	10.14	85.23
5	0.67	6.08	91.31

Principal component loadings

Parameter	No. of principal component				
	1	2	3	4	5
π	−0.46	0.59	0.05	0.61	0.17
H-Do	0.13	0.65	−0.46	−0.35	0.19
M-RE	0.89	0.22	−0.07	0.24	0.09
F	0.67	−0.01	0.57	0.07	0.26
R	−0.88	0.28	0.06	0.33	0.10
σ	0.61	−0.07	0.65	−0.02	0.22
Es	−0.95	−0.15	0.16	0.00	0.01
B_1	0.92	0.05	−0.21	−0.07	−0.13
B_4	0.90	0.30	−0.17	0.23	−0.04
Log k'_0	−0.35	0.45	0.23	−0.62	0.28
b	−0.02	0.59	0.51	−0.11	−0.61

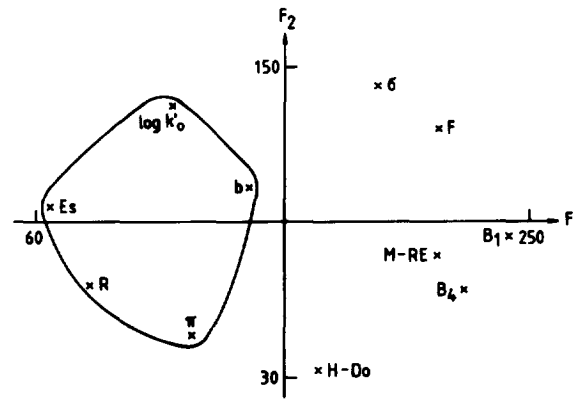

Fig. 1. Similarities and dissimilarities between the physico-chemical parameters and retention characteristics of the anticancer drugs on the β-cyclodextrin polymer-coated column. Two-dimensional non-linear map of principal component loadings. For symbols, see Experimental.

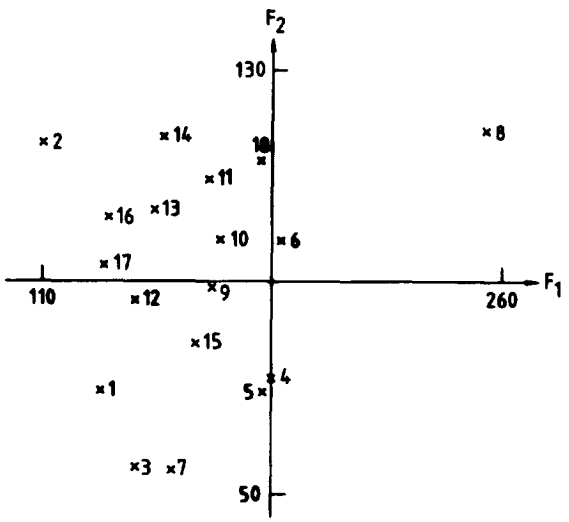

Fig. 2. Similarities and dissimilarities between the anticancer drugs on the β-cyclodextrin polymer-coated column. Two-dimensional non-linear map of principal component variables. Numbers refer to anticancer drugs in Table 1.

the guest molecules and their lipophilicity. The steric parameters define the capacity of the guest molecule to enter in the CD cavity and the lipophilicity of the guest molecule determines the strength of interactions with the hydrophobic inner surface of the CD cavity.

(2) Polar interactions between the solutes and surface of βCDP support: hydrophilic forces can bind the polar substructure of solutes to the hydroxyl groups on the βCDP surface or to the free silanol groups of silica support not covered by βCDP.

The retention of solutes is probably determined by the interplay of the various binding forces discussed above. We stress that the other physico-chemical parameters, not included in the calculations, may also have some influence on the retention and our conclusion refer only to parameters listed under Experimental. Anticancer drugs do not form distinct clusters on the two-dimensional non-linear map of principal component variables (Fig. 2), indicating the high structural diversity of these solutes.

The dendograms of cluster analysis calculated from the original data matrix and from the principal component loadings are shown in Fig. 3. The information contents of the clusters are different, indicating the influence of PCA on the visualization of the results. The dendograms of cluster analysis calculated from the original data matrix and from the principal component variables are shown in Fig. 4. These dendograms also show that the application of PCA modifies the distribution of variables. Good linear correlations were found between the first coordinates of

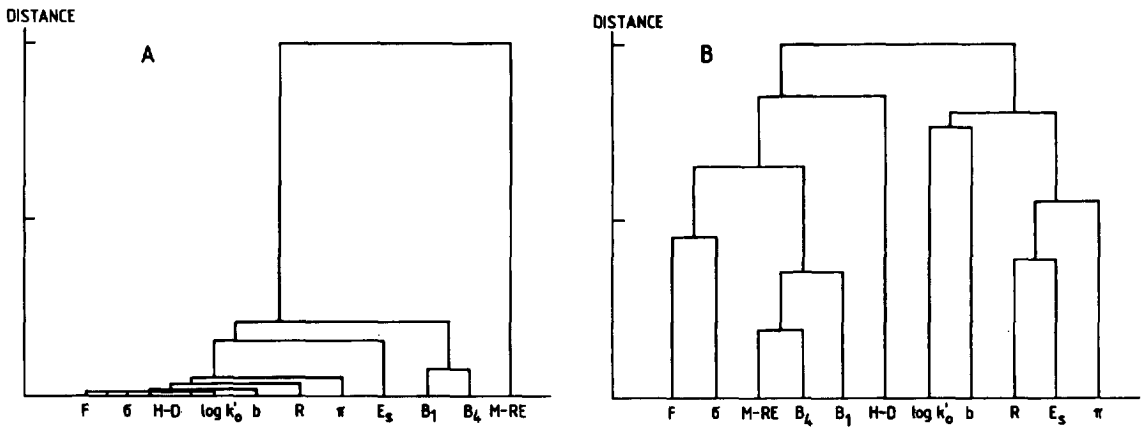

Fig. 3. Similarities and dissimilarities between the physico-chemical parameters and retention characteristics of the anticancer drugs on the β-cyclodextrin polymer-coated column. Cluster dendograms calculated (A) from the original data matrix and (B) from the principal component loadings. For symbols, see Experimental.

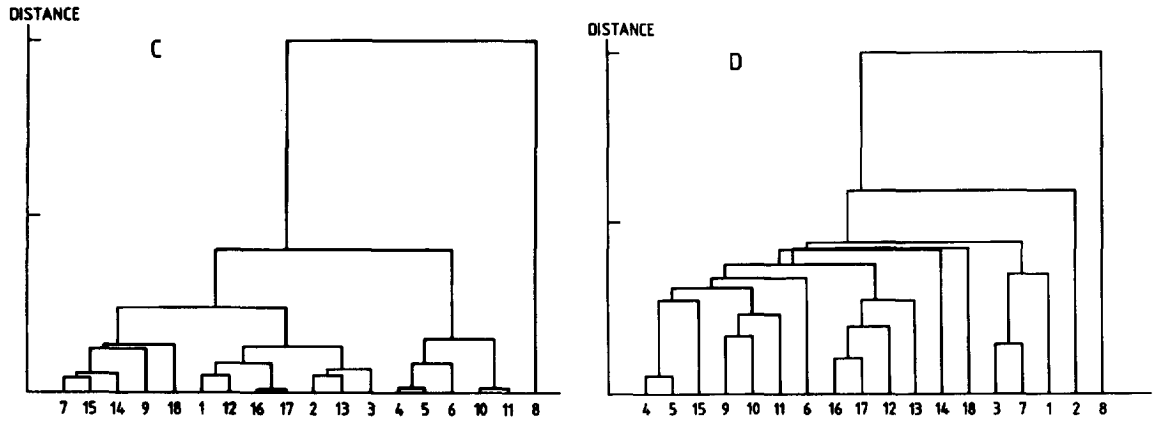

Fig. 4. Similarities and dissimilarities between the anticancer drugs on the β-cyclodextrin polymer-coated column. Cluster dendograms calculated (C) from the original data matrix and (D) from the principal component variables. Numbers refer to anticancer drugs in Table 1.

varimax rotation and two-dimensional non-linear map ($n = 11$):

$$\text{nlmap}_1 = 143.8 + (87.6 \pm 4.78) \cdot \text{varimax}_1$$
$$r_{\text{calc.}} = 0.9869; \quad r_{99.9\%} = 9.8471 \qquad (3)$$

The correlation between the second coordinates was not significant. These data indicate that the results obtained by the methods are similar but not identical. We stress that the conclusions discussed above are not the results of theoretical considerations and hence are valid only for this special data set. A generalization of these conclusion can lead to severe misinterpretation.

The correlations between the intercept (log k'_0) and the slope values (b) of Eq. 1 determined on βCDP and ODS columns were not significant, the correlation coefficients being 0.1886 and 0.1481, respectively. This result indicates that the retention mechanism of the βCDP column deviates significantly from that of the traditional reversed-phase column, but the eluents are typical reversed-phase eluents.

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