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Use of principal component analysis for the evaluation of the retention behaviour of monoamine oxidase inhibitory drugs on β-cyclodextrin column*

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Abstract: The retention of 17 monoamine oxidase inhibitory drugs (proparlgylamine derivatives) were determined on a β-cyclodextrin polymer (βCDP)-coated silica column using ethanol–0.05 M K_2HPO_4 (6:4 v/v) as the eluent. The relative strength of interaction between the drugs and a water soluble β-cycodextrin polymer was determined by charge-transfer chromatography carried out on reversed-phase TLC layers. The relationship between capacity factors, physicochemical parameters and inclusion complex forming capacity of the monoamine oxidase inhibitory drugs were evaluated by stepwise regrerssion analysis and by principal component analysis (PCA) followed by two-dimensional nonlinear mapping and varimax rotation. Calculations indicated that the retention of monoamine oxidase inhibitory drugs on βCDP column is mainly governed by their steric and lipophylic parameters. Significant linear correlations were found between the corresponding coordinates of varimax rotation and two-dimensional nonlinear maps proving the suitability of both methods for the reduction of dimensionality of complicated data matrices.

Keywords: *β-cyclodextrin coated silica; principal component analysis; varimax rotation.*

Introduction

Cyclodextrins (CDs) and various CD derivatives have found growing acceptance and application in many fields of chromatography [1]. They have been used in reversed-phase thin-layer chromatography (RP-TLC) to study their interaction with various bioactive compounds such as barbiturates [2, 3], chlorophenol derivatives [4, 5] etc. CDs modify the effective mobilities of various inorganic ions in isotachophoresis [6], improve separation of peptides in capillary electrophoresis [7] and enhance the efficiency of enantiomeric separation in gas chromatography [8–11]. CDs are used in two different manners in high-performance liquid chromatography either by adding CDs to the eluent [12–15] or by covalently bonding CDs to the silica surface [16–19]. CDs are used either to improve separation of non chiral compounds [20] or to separate enantiomers both in direct and reversed-phase systems [21]. Silica columns with β-cyclodextrin polymer (βCDP) coatings have been prepared recently and their retention characteristics [22] and their capacity for enantiomer separation have been elucidated [23].

Propargylamine derivatives are selective inhibitors of B-type monoamine oxidase [24, 25], the determination of their lipophilicity [26, 27] and their behaviour in various adsorptive and reversed-phase TLC systems have been recently reported [28].

The objectives of our work were to study the retention characteristics of a β-cyclodextrin polymer coated silica column using propargylamine derivatives as solutes, to find relationship between retention behaviour and physicochemical parameters and to compare the applicability of various multivariate mathematical statistical methods for the evaluation of the retention behaviour of these solutes.

Experimental

The chemical structure of monoamine oxidase inhibitory drugs are compiled in Table 1.

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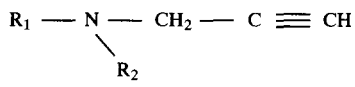
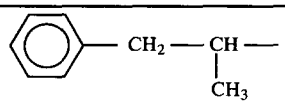
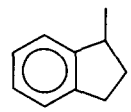
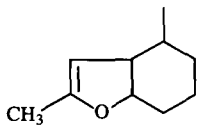
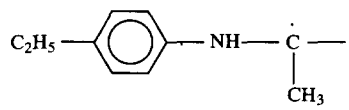
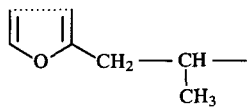
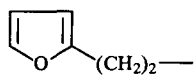
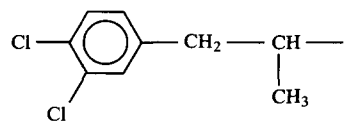
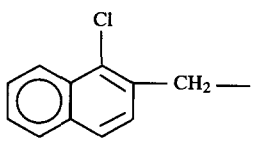
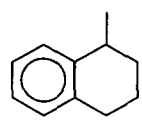
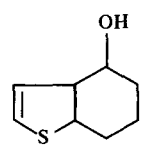
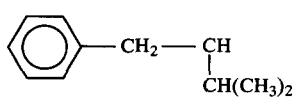
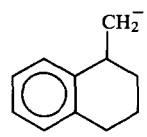
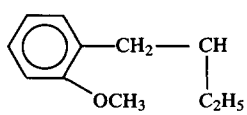
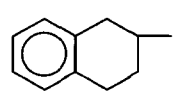
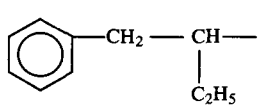
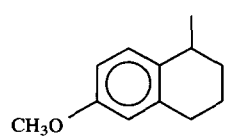
Table 1
Chemical structure of monomine oxidase inhibitory drugs



General structure

No. of compound	R_1	R_2	No. of compound	R_1	R_2
1	(+) 	$-CH_3$	10		$-CH_3$
2	(−)	$-CH_3$			
3		$-CH_3$	11		$-H$
4		$-H$			
5		$-CH_3$	12		$-CH_3$
6		$-CH_3$	13		$-CH_3$
			14		$-CH_3$
7		$-CH_3$	15		$-CH_3$
8		$-CH_3$	16		$-CH_3$
9		$-C_4H_7$	17		$-CH_3$

A. Determination of the retention behaviour of drugs by high performance liquid chromatography

The βCDP 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 analytical HPLC Packing Pump (Pittsburgh, USA) by the procedure normally used for the filling of reversed-phase columns. 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, California, USA), and a Waters 740 integrator (Milford, Massachusetts, USA). The flow-rate was 0.8 ml min^{-1} and the detection wavelength was 240 nm. The eluent was ethanol–0.05 M K_2HPO_4 (6:4 v/v). The drugs were dissolved in the eluent at a concentration of 0.05 mg ml^{-1}. The retention time of each compound was determined by three consecutive determinations. The capacity factor and the coefficient of variation capacity factor were calculated for each compound.

B. Determination of the interaction between drugs and a water-soluble β-cyclodextrin polymer by charge transfer thin-layer chromatography

Silica plates with fluorescence indicator (Silcoplat UV_{254}, Kavalier, Brno, Czech Republic) were impregnated with *n*-hexane–paraffin oil (95:5 v/v) by overnight predevelopment. Eluents were ethanol–water mixtures, the ethanol concentration varying between 35 and 60 vol% in steps of 5 vol%. To determine the strength of interaction between the drugs and βCD, a water-soluble β-CD polymer (further SCDP) was added to the eluent. Its concentration in the eluent varied between 0 and 20 mg ml^{-1}. The SCDP was prepared by cross-linking β-CD monomers with butylene glycol bis(epoxypropyl ether) in aqueous alkaline solution (βCD content 66.04%). The SCDP was purchased from CYCLOLAB Research and Development Laboratory (Budapest, Hungary). We have to emphasize that βCDP and SCDP were prepared with different process of polymerization, therefore, their inclusion forming capacity may also be different. After development the plates were dried at 105°C, and the spots were detected under UV light and with iodine vapour. Each determination was run in quadruplicate. The R_M values were calculated by $R_M = \log (1/R_f - 1)$. The dependence of R_M value on the eluent composition was calculated by

$$R_M = R_{M0} + b_1.C_1 + b_2.C_2, \qquad (1)$$

where R_M = actual R_M value of a compound determined at a given ethanol and SCDP concentrations; $R_{M0} = R_M$ value of a compound extrapolated to zero ethanol and SCDP concentrations (best estimation of molecular lipophilicity); b_1 = decrease in the R_M value caused by a 1% increase in the ethanol concentration in the eluent (related to the specific hydrophobic surface area of drugs); b_2 = decrease in the R_M value caused by 1 mg ml^{-1} change in the concentration of SCDP (indicator of the strength of drug-SCDP complex); C_1 and C_2 = ethanol and SCDP concentrations, respectively.

C. Calculation of relationships between retention behaviour and physicochemical parameters of propargylamine derivatives

To elucidate the influence of the physicochemical parameters of drugs on their retention behaviour on βCDP column stepwise regression analysis was applied [29]. The capacity factors of drugs determined on βCDP column were dependent variables and the independent variables were the following physicochemical parameters of drugs: π = Hansch–Fujita's substituent constant characterizing hydrophobicity; $H - Ac$ and $H - Do$ = indicator variables for proton acceptor and proton donor properties, respectively; $M - RE$ = molar refractivity; F and R = Swain–Lupton's electronic parameters characterizing the inductive and resonance effect, respectively; σ = Hammett's constant, characterizing the electron-withdrawing power of the substituent; Es = Taft's constant, characterizing steric effects of the substituent; B_1 and B_4 = Sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachment; as well as the parameters of equation 1 (R_{M0}, b_1 and b_2). The number of accepted independent variables was not limited and the acceptance limit was set to 95% significance level.

D. Comparison of various multivariate — mathematical statistical methods for the study of the retention behaviour of βCDP column Principal component analysis [30]

The capacity factors of drugs determined on βCDP column, their physicochemical and retention parameters in TLC system (see point C) were the variables and the monoamine oxidase drugs were the observations. The limit of the variance explained was set to 99%. As multidimensional systems cannot be easily evaluated nonlinear mapping technique [31] projects the variables or observations on a two-dimensional plane such a manner that the distances between the points on the plane be approximately the same as their distances in the multidimensional space. The two-dimen-

sional nonlinear map of PC loadings and variables as well as the varimax rotation around two axes were also calculated. The inclusion of both nonlinear mapping technique and varimax rotation in the evaluation was motivated by the consideration that varimax rotation and nonlinear mapping technique are theoretically similar, they calculate and visualize the relative distances between the members of data matrix. To compare their information content linear correlations were calculated between the corresponding coordinates

$$Y_{1-2} = a + b.X_{1-2}, \qquad (2)$$

where Y_{1-2} = coordinates of nonlinear map: X_{1-2} = coordinates of varimax rotation.

Results and Discussion

A. High-performance liquid chromatography

The retention order of propargylamine derivatives does not follow the lipophilicity order (Fig. 1). This finding suggests that the retention characteristics of βCDP column deviate from those of alkyl bonded silica columns where the retention order is determined by the lipophilicity order of the solutes. We assume that the following interactions may influence the retention behaviour of solutes on βCDP column.

1. Interactions of solutes with CD cavity. These interactions are determined by the size of 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 forces discussed above.

The logk' values and the coefficients of the variation are listed in Table 2. The logk' values show high diversity indicating that the drugs can be successfully separated on this column. The coefficients of variation is low indicating the good reproducibility of retention time on βCDP column.

B. Charge-transfer thin-layer chromatography

The parameters of equation 1 are compiled in Table 3. Compounds 1 and 2 were omitted from the calculations because they exhibited elongated spots in the eluents resulting in the inaccurate determination of their retention. Equation 1 fits well to the experimental data, the significance level being over 95% (see calculated F values) in each instance. The ratio of variance explained by the independent variables varied between 51 and 96% (see r^2 values). Both lipophilicity value (R_{M0}) and the

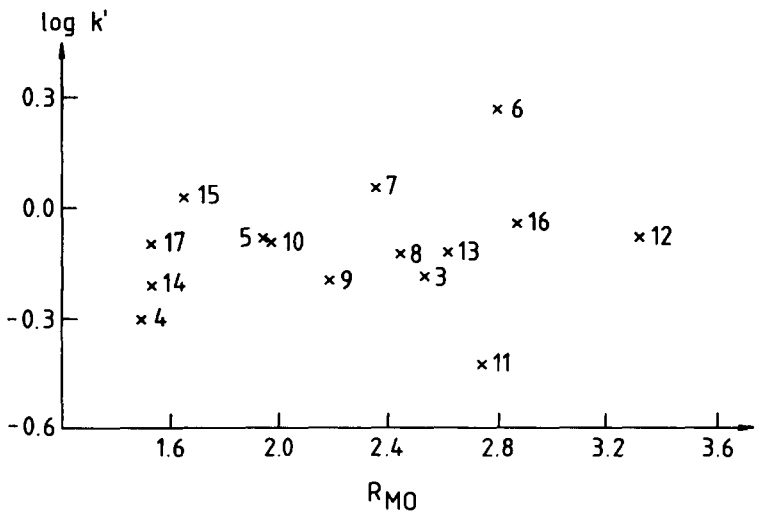


Figure 1
Relationship between the retention of monoamine oxidase inhibitory drugs on the β-cyclodextrin polymer-coated column and the measured hydrophobicity value (R_{M0}). Numbers refer to drugs in Table 1.

Table 2
Retention of monoamine oxidase inhibitory drugs on β-cyclodextrin polymer-coated silica column. Eluent: ethanol–50 mM K_2HPO_4 (6:4, v/v). Numbers refer to monoamine oxidase inhibitory drugs in Table 1

	log k'	
No. of compound	mean	coefficient of variation %
1	−0.127	0.24
2	−0.127	0.31
3	−0.182	0.52
4	−0.303	0.22
5	−0.082	0.36
6	0.270	0.29
7	0.053	0.23
8	−0.124	0.37
9	−0.191	0.21
10	−0.089	0.47
11	−0.423	0.55
12	−0.077	0.61
13	−0.122	0.43
14	−0.209	0.79
15	0.029	0.89
16	−0.038	0.95
17	−0.095	0.54

specific hydrophobic surface area (b_1) of the drugs differ considerably, indicating that these parameters can be separately included in the future Quantitative Structure Activity Relationship calculations.

C. Relationship between the retention behaviour and physicochemical parameters of drugs

Stepwise regression analysis indicated that the retention of monoamine oxidase inhibitory drugs significantly depend on their calculated lipophilicity

$$\log k' = -0.60 + (0.13 \pm 0.02).\pi \quad (3)$$
$$r_{calc.} = 0.8639 \qquad r_{99.9\%} = 0.7246$$
Standard error of the estimate = 0.09

Equation 3 clearly shows that the calculated hydrophobicity of solutes has the highest impact on their retention that is the retention mechanism of βCDP support may be similar to that of the traditional alkyl bonded silica phases. However, the relatively low ratio of variance explained (74%) indicates that factors other than molecular hydrophobicity may also have a significant influence on the retention. The results of stepwise regression analysis slightly contradict the results in Fig. 1. This discrepancy can be tentatively explained by the supposition that slight difference between the measured and calculated hydrophobicity values.

D. Comparison of various multivariate methods for the evaluation of the retention data

The results of PCA are summarized in Table 4. Five principal components explain the majority of variance indicating that the 15 original variables can be substituted by five background (composite) variables without considerable loss of information. Unfortunately, PCA does not prove the existence of such composite variables as concrete physicochemical entities only indicates their mathematical possibility. The log k' values — together with the measured hydrophobicity, complex forming capacity, steric and electronic parameters of drugs — have high loading in the second PC indicating the marked influence of these parameters on the mode of retention of the βCDP support. The distribution of variables on the two-dimensional nonlinear map of PC loadings supports our previous conclusions (Fig. 2), the log k' values form a loose cluster with the measured hydrophobicity, complex forming capacity, steric and electronic parameters of drugs. This finding indicates again the mixed retention mechanism of the βCDP support.

The propargylamine derivatives do not form distinct clusters on the two-dimensional non-linear map of principal component variables (Fig. 3). This result suggests that the character and position of each substituent may have similar impact on their retention behaviour.

Good linear correlation were found between the corresponding coordinates of nonlinear map and varimax rotation (r = 0.9963 and r = 0.9295 for the first and second coordinates, respectively) suggesting that the information content of the methods is similar. *We have to stress that the conclusion discussed above is not the result of theoretical considerations and hence is valid only for this data set.* We assume that the generalization of the conclusion may lead to severe misinterpretation.

It can be concluded from the data that βCDP support shows retention behaviour different from that of alkyl bonded silicas, the steric and electronic parameters of solutes markedly influencing the retention. Principal component analysis followed either with nonlinear mapping technique or with varimax rotation proved to be a useful tool for the elucidation of the effect of various molecular parameters on the retention.

Table 3
Relationship between the lipophilicity (R_{M0}) of monoamine oxidase inhibitory drugs and the concentration of ethanol (C_1) and water-soluble β-cyclodextrin polymer (C_2) in the eluent. Numbers refer to monoamine oxidase inhibitory drugs in Table 1. Compound 1 and 2 were omitted from calculations (n = 18)

$$R_M = R_{M0} + b_1.C_1 + b_2.C_2$$

	No. of compound						
Parameter	3	4	5	6	7	8	9
R_{M0}	2.53	1.50	1.94	2.80	2.35	2.44	2.17
$-b_1.10^{-2}$	4.82	3.66	4.26	5.24	3.00	4.43	1.62
$S_{b1}.10^{-3}$	2.7	3.0	2.8	3.5	7.3	3.4	4.4
b_1 (%)	80.13	85.51	80.00	81.25	69.77	—	—
$-b_2.10^{-2}$	1.22	6.15	1.00	1.42	1.39	—	—
$S_{b2}.10^{-3}$	2.8	2.7	2.9	3.6	3.7	—	—
b_2 (%)	19.87	14.49	20.00	18.75	30.23	—	—
r^2	0.9596	0.9179	0.9455	0.9415	0.8294	0.9099	0.9523
F_{calc}	178.43	78.01	130.22	120.83	14.59	161.68	14.70

Table 4
Similarities and dissimilarities between the physicochemical parameters of monoamine oxidase inhibitory drugs and their retention on β-cyclodextrin polymer coated silica column. Results of principal component analysis

No. of component		Eigenvalue			Total explained variance (%)	
1		5.45			36.38	
2		3.11			57.15	
3		2.64			74.53	
4		1.47			84.56	
5		0.93			90.79	
		Principal component loadings				
		1	2	3	4	5
1	π	0.12	0.14	−0.55	0.18	0.70
2	*H-Ac*	0.89	−0.38	0.17	0.05	0.01
3	*H-Do*	−0.82	0.29	0.29	−0.25	−0.02
4	*M-Re*	−0.61	0.34	0.17	0.50	0.20
5	*F*	0.81	−0.15	0.43	0.27	−0.01
6	*R*	0.40	0.68	−0.18	−0.52	0.02
7	σ	0.53	−0.46	−0.60	0.15	−0.21
8	*Es*	0.45	0.62	−0.30	−0.47	0.16
9	B_1	−0.82	−0.07	0.08	0.17	0.29
10	B_4	0.42	0.36	0.77	0.19	0.17
11	b_1	0.22	−0.06	0.90	−0.20	0.06
12	b_2	0.19	−0.59	−0.28	0.61	−0.24
13	R_M	0.79	−0.51	0.04	0.17	−0.04
14	logk'	0.11	0.90	0.03	0.17	−0.15

Table 3
Continued

No. of compound							
10	11	12	13	14	15	16	17
1.97	2.74	3.33	2.61	1.53	1.65	2.85	1.53
3.85	5.32	5.42	3.99	3.81	4.82	4.42	4.17
4.2	4.41	3.7	3.4	4.3	3.3	3.3	3.0
77.00	79.00	78.26	78.00	81.02	81.01	77.19	73.21
1.10	2.10	1.57	1.15	8.94	1.65	1.37	1.59
4.4	4.6	3.88	3.6	3.9	3.5	3.53	3.1
23.00	21.00	21.74	22.00	18.98	18.99	22.81	26.79
0.8934	0.9091	0.9403	0.9045	0.9525	0.9360	0.9253	0.9301
43.28	75.08	118.18	71.07	40.47	109.71	92.92	99.83

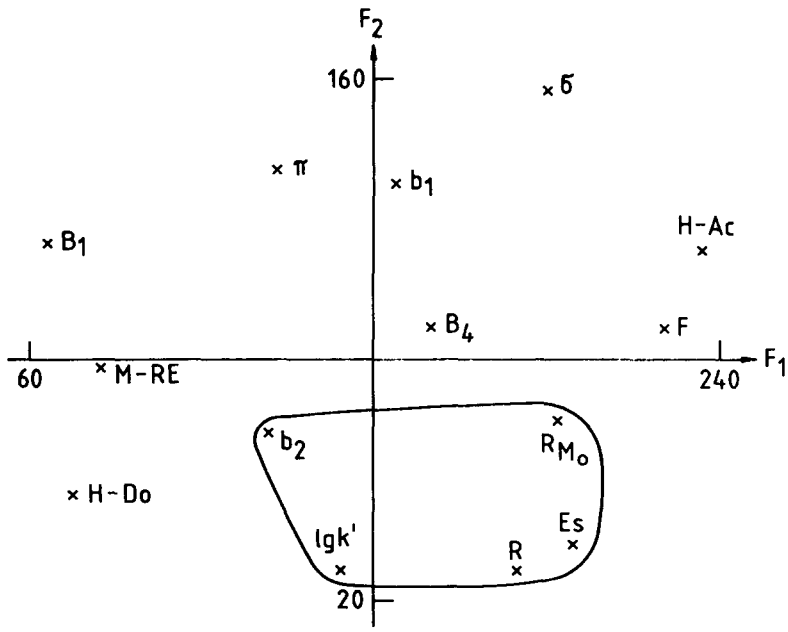


Figure 2
Two-dimensional nonlinear map of PC loadings. Number of iteration: 86. Maximum error: 6.29×10^{-3}. For symbols see Experimental.

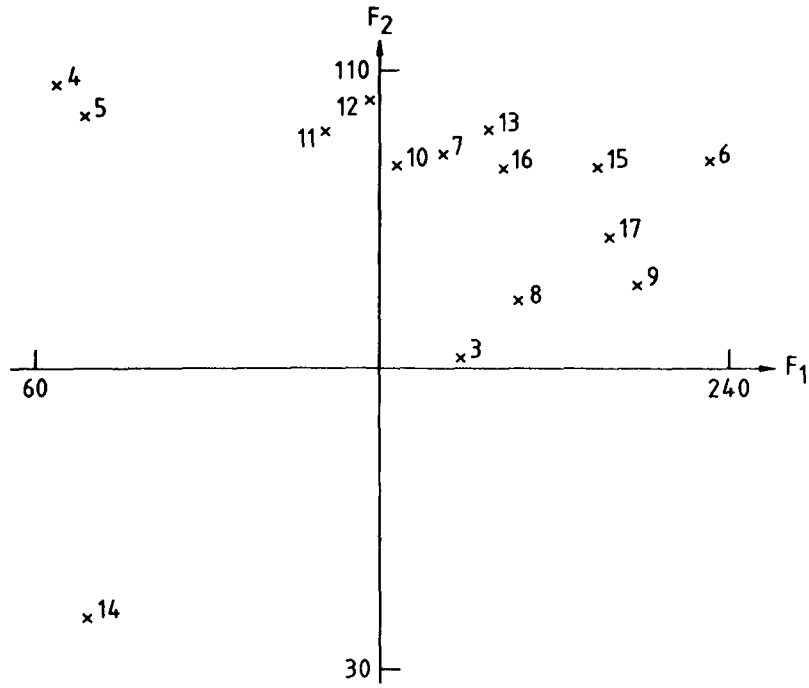


Figure 3
Two-dimensional nonlinear map of PC variables. Number of iteration: 250. Maximum error: 4.81×10^{-3}. Numbers refer to drugs in Table 1.

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