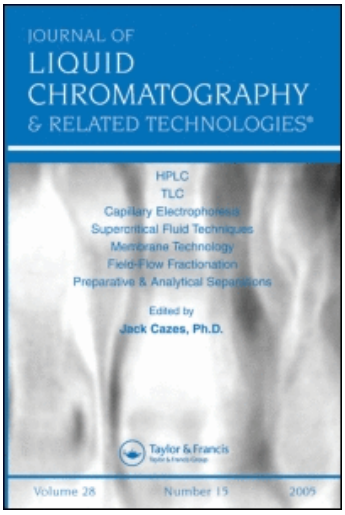


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# Influence of the Physicochemical Parameters of Propargylamine Derivatives on Their Retention on α-Cyclodextrin Polymer-Coated Support

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# INFLUENCE OF THE PHYSICOCHEMICAL PARAMETERS OF PROPARGYLAMINE DERIVATIVES ON THEIR RETENTION ON β-CYCLODEXTRIN POLYMER-COATED SUPPORT

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ABSTRACT

The retention of 17 propargylamine derivatives was determined on a ß-cyclodextrin polymer (ß-CDP)-coated silica column using dioxane-0.05 M $K_2HPO_4$ (6:4, v/v) as eluent. The inclusion complex formation between the propargylamine derivatives and a water-soluble ß-CD polymer was studied by charge-transfer chromatography carried out on reversed-phase TLC layers. The capacity factors were correlated with the various physicochemical parameters of the solutes. Principal component analysis proved that the hydrophobicity and steric parameters have the highest influence on the retention of propargylamine derivatives in HPLC. The result suggest that the selectivity of the ß-CDP-coated silica support may be different from that of the traditional alkyl bonded reversed-phase supports.

## INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides which have the ability to form inclusion complexes with many organic and inorganic compounds of various chemical structures (1,2). Cyclodextrins (CDs) and modified CDs have been used in many fields of chromatography (3). They were applied in reversed-phase thin-layer chromatography (RP-TLC) to study the formation of inclusion complexes with barbiturates (4,5) and with chlorophenol derivatives (6,7). CDs influenced the mobility of inorganic ions in isotachophoresis (8), improved the separation of peptides in capillary electrophoresis (9) and enhance the efficiency of enantomeric separation in gas chromatography (10). CDs are used in high performance liquid chromatography either by adding CDs to the eluent (11) or by covalently bonding CDs to the silica surface (12). CDs have been used to improve separation of non chiral compounds (13) and to separate enantiomers both in direct and reversed-phase seoparation mode (14). Silica supports with ß-cyclodextrin polymer (ßCDP) on their surface have been recently prepared, and their retention behavior (15) and their capacity to separate enantiomers have been studied in detail (16).

Propargylamine derivatives are selective inhibitors of B-type monoamine oxidase (17,18), the determination of their lipophilicity (19,20) and their behavior in adsorptive and reversed-phase TLC have been recently reported (21).

The objectives of our work were to study the retention behavior of propargylamine derivatives on

the ß-cyclodextrin polymer coated silica support, to find the physicochemical parameters of solutes accounting for the retention and to compare the various mulivariate mathematical statistical methods for the evaluation of the retention data.

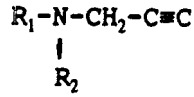
## MATERIALS AND METHODS

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

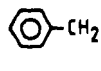
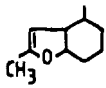
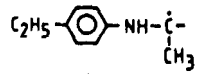
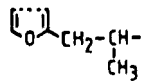
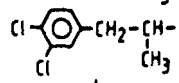
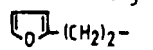
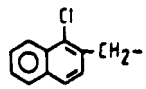
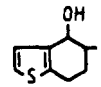
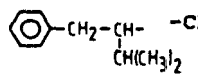
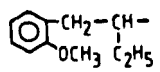
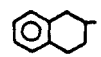
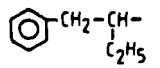
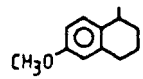
### A. Determination of the retention behaviour of drugs by high performance liquid chromatography

The ßCDP coated silica support was prepared at the CYCLOLAB Research and Development Laboratory (Budapest, Hungary). A 25 cm x 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 2 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 and the detection wavelength was 240 nm. The eluent was dioxane-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. The retention time of each compound was determined by three consecutive determinations. The capacity factor and the coefficient of

TABLE 1
Chemical Structures of Monoamine Oxidase Inhibitory Drugs



General structures

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

variation capacity factor were calculated for each compound.

B. Determination of the interaction between drugs and a water-soluble ß-cyclodextrin polymer by reversed-phase 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 dioxane:water mixtures, the dioxane concentration varying between 35-60 vol% in steps of 5 vol%. To determine the strength of interaction between the drugs and ßCD, a water-soluble ß-CD polymer (further SCDP) was added to the eluent. Its concentration in the eluent varied between 0-20 mg/ml. The SCDP was prepared by crosslinking ß-CD monomers with butylene glycol bis(epoxypropyl ether) in aqueous alkaline solution (ßCD content 66.04%). The SCDP was purchased from CYCLOLAB Research and Development Laboratory (Budapest, Hungary). It has to be emphasized that ßCDP and SCDP were prepared with different process of polymerization, therefore their capacity to form inclusion complexes may 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 dioxane and SCDP concentrations; $R_{M0}$ = $R_M$ value

of a compound exrapolated to zero dioxane and SCDP concentrations (best estimation of molecular lipophilicity); $b_1$ = decrease in the $R_M$ value caused by a 1% increase in the dioxane concentration in the eluent (related to the specific hydrophobic surface area of drugs); $b_2$ = decrease in the $R_M$ value caused by 1mg/ml change in the concentration of SCDP (indicator of the strength of drug-SCDP complex); $C_1$ and $C_2$ = dioxane and SCDP concentrations, respectively.

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

Principal component analysis (PCA) (22): The capacity factors of drugs determined on ßCDP column, their physicochemical and retention parameters in TLC system were the variables and the monoamine oxidase drugs were the observations. The physicochemical parameters included in the calculation were: $\pi$ = 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; $\sigma$ = 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 attachement. The limit of the variance explained was set to 99%. The two-dimensional non-linear map of PC loadings and variables (23) and

the cluster analysis of the original data matrix and the PC loadings and variables was also calculated (24). The inclusion of both nonlinear mapping technique and cluster analysis in the evaluation was motivated by the consideration that each of them are theoretically similar, they calculate and visualize the relative distances between the members of data matrix.

## RESULTS AND DISCUSSION

The log k' values and the coefficients of the variation (standard deviations expressed in percent of the mean value) are listed in Table 2. The log k' values are different suggesting that the drugs can be sucessfully separated on this column by an appropriate mixture of dioxane-water. The coefficients of variation are low indicating the good reproducibility of the retention time on ßCDP column.

B. Reversed-phase thin-layer chromatography

The parameters of eq.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.

Propargylamine derivatives did not form inclusion complexes with SCDP in the presence of dioxane ($b_2$ value in eq.1. was never significant), however, the ring structures in the drug molecules can easily fit into the CD cavity. This finding can be explained by the supposition that the bulky dioxane molecule also enter the CD cavity and inhibits competitively the interac-

TABLE 2.
Retention of Propargylamine Derivatives on ß-Cyclodexdextrin Polymer-Coated Silica Column. Eluent: Dioxane--50 mM $K_2HPO_4$ (6:4, v/v). Numbers Refer to Propargylamine Derivatives in TABLE 1.

| | log k' | |
|---|---|---|
| No. | Mean | Coefficent of variation % |
| 1. | -0.462 | 0.53 |
| 2. | -0.456 | 0.22 |
| 3. | -0.430 | 0.88 |
| 4. | -1.617 | 0.82 |
| 5. | -0.153 | 0.47 |
| 6. | -0.168 | 0.25 |
| 7. | -0.276 | 0.64 |
| 8. | -0.428 | 0.21 |
| 9. | 0.017 | 0.85 |
| 10. | -0.386 | 0.56 |
| 11. | -0.625 | 0.69 |
| 12. | -0.246 | 0.75 |
| 13. | -0.211 | 0.42 |
| 14. | -0.680 | 0.45 |
| 15. | -0.300 | 0.42 |
| 16. | -0.415 | 0.59 |
| 17. | -0.287 | 0.38 |



tion between the drugs and the complex forming centers of SCDP. Eq.1. fits well to the experimental data, the significance level being over 99.9% (see calculated F values) in each instance. The ratio of variance explained by the independent variables varied between 63 and 96 % (see $r^2$ values).

Both lipophilicity values ($R_{M0}$) and the specific hydrophobic surface areas ($b_1$) of the drugs differ considerably, indicating that these parameters can be separately included in future quantitative structure activity relationship calculations.

The parameters of PCA are compiled in Table 4.

TABLE 3.
Relationship Between the $R_M$ Value of Propargylamine Derivatives and the Concentration of Dioxane ($C_1$) and Water-Soluble ß-Cyclodextrin Polymer ($C_2$) in the Eluent. Number Refer to Propargylamine Derivatives in TABLE 1. ($b_2$ never was significant).

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

| Parameter | Compound no. | | | | |
|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 |
| n | 17 | 17 | 17 | 17 | 17 |
| $R_{M0}$ | 1.61 | 1.29 | 1.61 | 2.90 | 2.83 |
| $-b_1.10^2$ | 3.05 | 3.40 | 3.48 | 4.76 | 4.35 |
| $s_{b1}.10^3$ | 2.72 | 3.06 | 3.05 | 2.96 | 2.29 |
| $F_{calc.}$ | 125.99 | 125.63 | 130.67 | 258.31 | 362.41 |
| $r^2$ | 0.8936 | 0.8933 | 0.8970 | 0.9451 | 0.9603 |
| | 8 | 9 | 10 | 11 | 12 |
| n | 17 | 16 | 18 | 18 | 9 |
| $R_{M0}$ | 2.36 | 3.72 | 1.50 | 1.84 | 3.55 |
| $-b_1.10^2$ | 4.03 | 4.73 | 2.85 | 3.90 | 6.33 |
| $s_{b1}.10^3$ | 2.51 | 4.00 | 4.02 | 5.19 | 6.80 |
| $F_{calc.}$ | 257.63 | 140.28 | 50.13 | 56.42 | 86.72 |
| $r^2$ | 0.9450 | 0.9093 | 0.7580 | 0.7790 | 0.9253 |
| | 13 | 14 | 15 | 16 | 17 |
| n | 18 | 18 | 18 | 18 | 18 |
| $R_{M0}$ | 2.28 | 0.91 | 2.40 | 1.76 | 2.09 |
| $-b_1.10^2$ | 3.43 | 2.50 | 3.73 | 3.18 | 3.30 |
| $s_{b1}.10^3$ | 4.81 | 4.81 | 4.35 | 4.07 | 4.65 |
| $F_{calc.}$ | 50.75 | 26.98 | 73.41 | 61.37 | 50.39 |
| $r^2$ | 0.7603 | 0.6277 | 0.8210 | 0.7932 | 0.7590 |

Five principal components explain the majority of variance indicating that the 13 original variables can be substituted by 5 background (abstract) variables with only 7% loss of information. Unfortunately, PCA does not prove the existence of such background variables as concrete physicochemical entities, but only indicates their mathematical possibility. The log k

TABLE 4.
Similarities and Dissimilarities Between the Physicochemical Parameters of Propargylamine Derivatives and their Retention on ß-Cyclodextrin Polymer Coated Silica Column. Results of Principal Component Analysis.

| No. of component | Eigenvalue | Variance explained % | Sum of variance explained % |
|---|---|---|---|
| 1 | 5.74 | 44.13 | 44.13 |
| 2 | 2.52 | 19.40 | 63.53 |
| 3 | 2.39 | 18.38 | 81.91 |
| 4 | 0.87 | 6.69 | 88.60 |
| 5 | 0.56 | 4.29 | 92.89 |

| Parameters | Principal component loadings No of principal component 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| log k' | 0.68 | 0.38 | -0.01 | 0.28 | 0.22 |
| $R_{M0}$ | 0.89 | -0.10 | 0.13 | 0.18 | 0.28 |
| $b_1$ | 0.72 | -0.42 | 0.40 | 0.20 | 0.23 |
| $\pi$ | 0.88 | 0.30 | -0.27 | -0.06 | 0.01 |
| H-Ac | -0.77 | 0.03 | 0.49 | -0.25 | 0.16 |
| H-Do | -0.60 | 0.18 | 0.34 | 0.63 | -0.19 |
| M-RE | 0.78 | 0.53 | -0.04 | 0.00 | -0.28 |
| F | 0.44 | -0.64 | 0.57 | -0.11 | -0.14 |
| R | 0.51 | -0.28 | -0.76 | 0.15 | 0.03 |
| $\sigma$ | 0.49 | -0.69 | 0.49 | 0.04 | -0.11 |
| Es | -0.81 | 0.14 | 0.08 | 0.33 | 0.27 |
| $B_1$ | 0.43 | 0.59 | 0.61 | 0.11 | -0.24 |
| $B_4$ | 0.30 | 0.67 | 0.51 | -0.30 | 0.25 |

values - together with the measured and calculated hydrophobicity values, the Taft constant characterizing the steric effect of substituents and electronic parameters of drugs - have high loading in the first PC indicating the marked influence of these parameters on the mode of retention of the ß-CDP support. It can be assumed the interactions of solutes with the CD cavity can exert a considerable influence on the retention behaviour of solutes on ßCDP column. These interac-

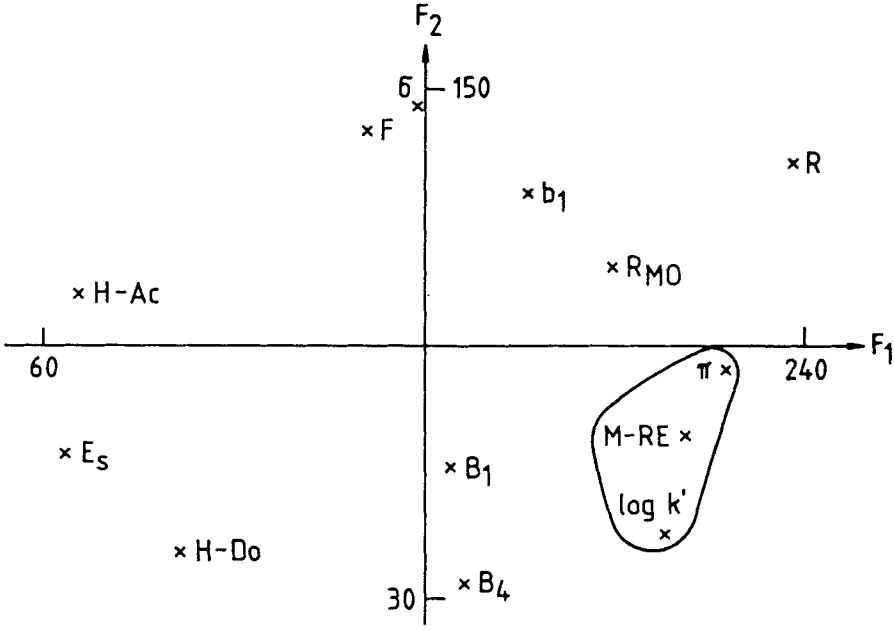



Figure 1. Similarities and dissimilarities between the physicochemical parameters and log k' of propargylamine derivatives on ß-cyclodextrin polymer coated column. Two dimensional nonlinear map of principal component loadings. Number of iterations: 162; max.error: $3.83.10^{-2}$. For symbols see MATERIALS AND METHODS.



tions 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. It is probable that, as a consequence of polymerization, access to the sites of inclusion complex formation is hindered to various degrees. Furthermore, secondary

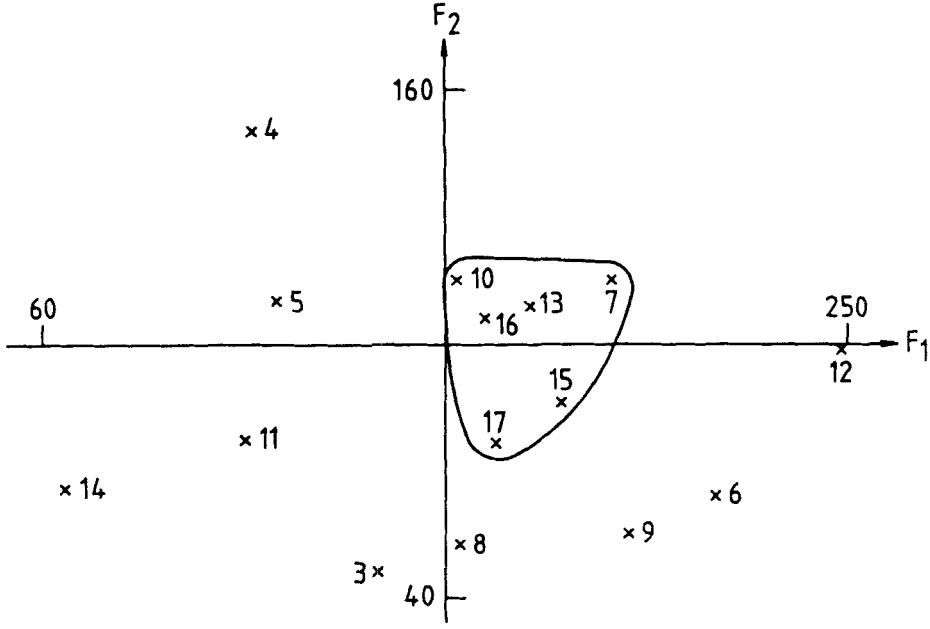



Figure 2. Similarities and dissimilarities between the propargylamine derivatives on ß-cyclodextrin polymer coated column. Two dimensional nonlinear map of principal component variables. Number of iterations: 124; max.error: $3.07.10^{-2}$. Numbers refer to propargylamine derivatives in Table 1.



cavities of different dimensions are formed between the polymer network. These dimensions are at least commensurable with the dimensions of ß-CD cavities, resulting in different inclusion copmlex formation and hence in different retention characteristics. The retention of solutes is probably determined by the interplay of the various binding forces discussed above.

The distribution of variables on the two-dimensional nonlinear map of PC loadings supports our previous assumptions (Fig.1) that both steric and hydro-

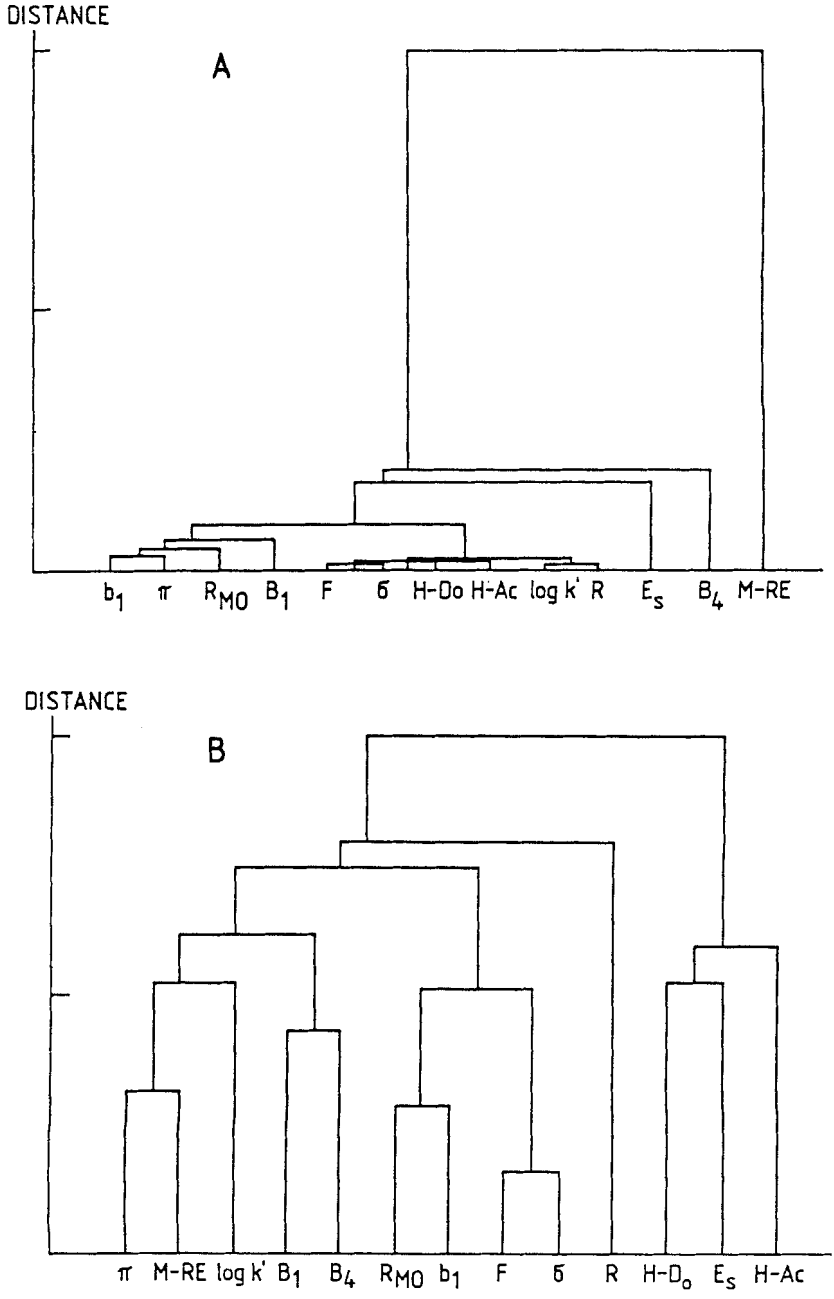



Figure 3. Similarities and dissimilarities between the physicochemical parameters and log k' of propargylamine derivatives on ß-cyclodextrin polymer coated column. Cluster dendograms calculated from the original data matrix (A) and from the principal component loadings (B). For symbols see MATERIALS AND METHODS.

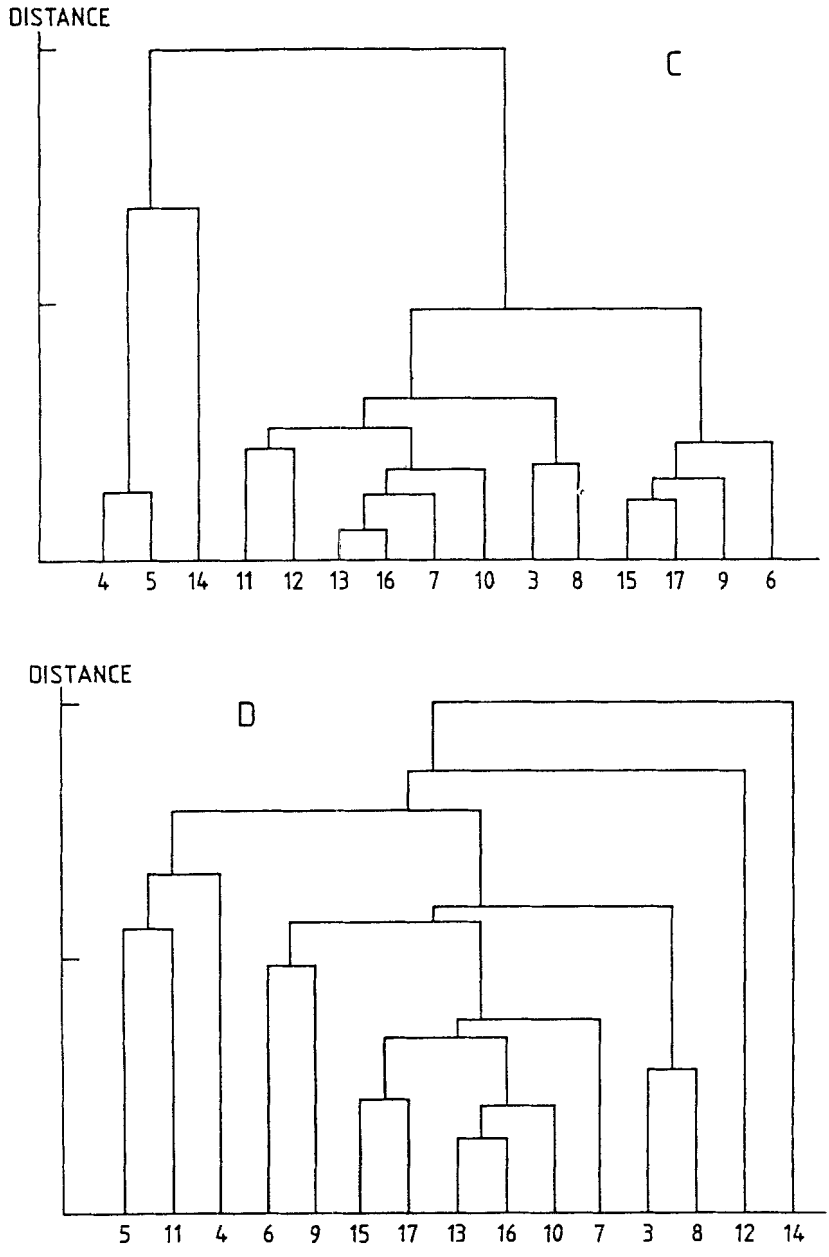



Figure 4. Similarities and dissimilarities between the propargylamine derivatives on ß-cyclodextrin polymer coated column. Cluster dendograms calculated from the original data matrix (C) and from the principal component variables (D). Numbers refer to propargylamine derivatives in Table 1.

phobic parameters influence the retention of drugs on the ßCDP column. The log k' values form a distinct cluster with the molar refraction (related to the bulkiness of the drug) and the calculated lipophilicity. This finding indicates again the mixed retention mechanism of the ßCDP support. The solutes with condensed ring structures are near to each other on the two-dimensional nonlinear map of principal component variables (Fig.2) suggesting again the importance of steric conditions in the retention.

The dendograms of cluster analysis calculated from the original data matrix and from the principal component loadings are shown in Fig 3. The information content 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. Due to its higher dimensionality we strongly advocate the application of the two-dimensional nonlinear mapping technique instead of cluster analysis. We assume that the two dimensional nonlinear map may contain more information than the one dimensional structure of clusters.

We have to 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.

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