ELSEVIER

Journal of Chromatography A, 668 (1994) 395–402

JOURNAL OF CHROMATOGRAPHY A

# Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silica column

Esther Forgács*, Tibor Cserháti

*Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary*

**Abstract**

The retentions of 45 barbituric acid derivatives were determined on a β-cyclodextrin (β-CD) polymer-coated silica column using unbuffered methanol–water, ethanol–water, acetonitrile–water, dioxane–water and tetrahydrofuran–water eluent mixtures. Stepwise regression analysis indicated that the retention of barbituric acid derivatives is mainly governed by the lipophilicity, molar refractivity (related to the solute volume) and to a lesser extent by the electronic parameters of substituents in each eluent system, suggesting the significant importance of these physico-chemical parameters in the determination of retention behaviour. The spectral map technique indicated that the solvent strength and selectivity of organic modifiers on a β-CD polymer-coated silica column depended on their bulkiness and electronic parameters, respectively.

## 1. Introduction

Cyclodextrins (CDs) can form inclusion complexes with a wide variety of compounds [1–3]. As the complex formation modifies the retention characteristics of the guest molecule, CDs have found growing acceptance and application in many fields of chromatography and electrophoresis (gas chromatography, thin-layer chromatography, high-performance liquid chromatography, isotachophoresis and electrophoresis). Both polar [4] and non-polar [5] cyclodextrin derivatives have been used in capillary gas chromatography for enantiomer separations. Reversed-phase thin-layer chromatography has been used to determine the inclusion complex stability of several chlorophenol derivatives [6]. The effective mobilities of various inorganic ions such as iodide, periodate and tetrathionate decreased with increasing concentration of α-, β- and γ-CDs in the isotachophoretic separation of these ions [7]. The application of α- and β-CDs in the capillary electrophoresis of peptides improved the separation [8]. CDs have been used extensively in HPLC either as eluent additives or covalently bonded to a silica surface. CDs added to the eluent modify the retention of aliphatic alcohols [9], drugs [10] and various steroids [11]. The application of a covalently bonded β-CD column for semi-preparative separations has also been reported [12]. Separations on silica columns with covalently bonded CDs are generally carried out in aqueous eluents similarly to the separations on traditional reversed-phase columns; CD derivatives for application to the adsorption separation of enantiomers have also been synthetized [13].

The objectives of these investigations were to determine the retentions of 45 barbituric acid derivatives on a β-CD polymer-coated silica column (β-CD column) using various eluents systems, to evaluate the retention data by multi-

* Corresponding author.

0021-9673/94/$07.00 
*SSDI* 0021-9673(93)E1038-2

variate statistical methods, to find the relationship between retention characteristics and physico-chemical parameters of the barbituric acid derivatives and to determine the influence of organic modifiers on the retention behaviour of barbiturates on a β-CD column.

## 2. Experimental

Monomeric β-CD polymerized on the surface of silica particles without binding the polymer covalently to the silanol groups, using a slight modification of the preparation method published recently [14]. A column of 250 × 4 mm I.D. was filled with the β-CD polymer-coated silica. The HPLC system consisted of a Liquopump Model 312 pump (LaborMIM, Budapest, Hungary), a Cecil Instruments (Cambridge, UK) CE-212 variable-wavelenth UV detector, a Valco (Houston, TX, USA) injector with a 20-μl sample loop and a Waters Model 740 integrator (Waters–Millipore, Milford, MA, USA). The flow-rate was 0.6 ml/min and the detection wavelength was set at 240 nm. The eluents were methanol–water, acetonitrile–water, ethanol–water, dioxane–water and tetrahydrofuran–water with 50% (v/v) organic modifier concentrations. Buffers were not used.

The structures of the barbituric acid derivatives are shown in Table 1. The barbituric acids were dissolved in each instance in the eluent at a concentration of 0.05 mg/ml. The dead volume of the column was determined by injecting sodium nitrate solution. Each determination was run in quadruplicate. The capacity factors (log $k'$) and asymmetry factors were calculated separately for each barbituric acid derivative in each eluent system [15].

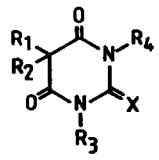
### 2.1. Determination of the relationship between the physico-chemical parameters of barbituric acid derivatives and their retention characteristics

To find the physico-chemical parameters of solutes that significantly influence their retention behaviour, stepwise regression analysis [16] was applied. In the common multivariate regression analysis the presence of independent variables that exert no significant influence on the dependent variable lessens the significance level of those independent variables which have a significant influence on the dependent variables. To overcome this difficulty, the stepwise regression analysis automatically eliminates from the selected equation the insignificant independent variables. In our calculations the dependent variables were always the logarithm of capacity factors and the independent variables were the different physico-chemical parameters of the barbituric acid derivatives. The acceptance level for the individual independent variables was set to the 95% significance level. Stepwise regression analysis was carried out five times, the log $k'$ values determined in the five eluent systems being separately the dependent variables. The physico-chemical parameters included in the calculation were the following: $\pi$, the Hansch–Fujita 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 $R$, the Swain–Lupton 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; and $B_1$ and $B_4$, Sterimol width parameters (determined by the distance of the substituents at their maximum point perpendicular to attachment bond axis).

### 2.2. Determination of the influence of organic modifiers on the retention behaviour of barbiturates on a β-CD column

To separate solvent strength and solvent selectivity, the "spectral map" technique [17,18] was applied. The data matrix consisted log $k'$ values of barbituric acid derivatives determined in five eluent systems. Calculations were carried out twice, the eluent systems and barbituric acid derivatives being the variables and observations, respectively. The potency values of the eluent systems and solutes were considered as the solvent strength and retention capacity, respectively. To visualize the spectral characteristics of

Table 1
Structure of barbituric acid derivatives




| No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | X |
|---|---|---|---|---|---|
| 1 | H | H | H | H | O |
| 2 | Methyl | Methyl | H | H | O |
| 3 | 3-Pentyl | Methyl | H | H | O |
| 4 | Methyl | 1-Methylpentyl | H | H | O |
| 5 | Ethyl | Ethyl | H | H | O |
| 6 | Ethyl | 1-Methylbutyl | H | H | O |
| 7 | Ethyl | 3-Methylbutyl | H | H | O |
| 8 | Ethyl | 1-Methylpropyl | H | H | O |
| 9 | Ethyl | *n*-Pentyl | H | H | O |
| 10 | Butyl | 1-Methylpropyl | H | H | O |
| 11 | Butyl | 1-Methylbutyl | H | H | O |
| 12 | Butyl | 3-Methylbutyl | H | H | O |
| 13 | Ethyl | *n*-Octyl | H | H | O |
| 14 | Ethyl | 3-Dimethyloctyl | H | H | O |
| 15 | Allyl | Isopropyl | H | H | O |
| 16 | Allyl | Isobutyl | H | H | O |
| 17 | Allyl | 1-Methylbutyl | H | H | O |
| 18 | Allyl | 1-Methylcyclohexenyl | H | H | O |
| 19 | Allyl | 2-Cyclopentyl | H | H | O |
| 20 | Ethyl | 1-Cyclohexenyl | H | H | O |
| 21 | Ethyl | Ethyl | H | H | S |
| 22 | Ethyl | 1-Methylbutyl | H | H | S |
| 23 | Allyl | 1-Methylbutyl | H | H | S |
| 24 | Ethyl | 1,3-Dimethylbutyl | H | H | S |
| 25 | Ethyl | Phenyl | H | H | O |
| 26 | Ethyl | Ethyl | Phenyl | H | O |
| 27 | Ethyl | Ethyl | Benzoyl | H | O |
| 28 | Ethyl | Ethyl | Benzoyl | Benzoyl | O |
| 29 | Ethyl | Ethyl | *p*-Cl-benzoyl | H | O |
| 30 | Ethyl | Ethyl | *p*-$NO_2$-benzoyl | H | O |
| 31 | Ethyl | Ethyl | *p*-$NO_2$-benzoyl | *p*-$NO_2$-benzoyl | O |
| 32 | Ethyl | Phenyl | Phenyl | H | O |
| 33 | Ethyl | Phenyl | Benzoyl | Methyl | O |
| 34 | Ethyl | Phenyl | *p*-$NH_2$-benzoyl | Methyl | O |
| 35 | Ethyl | Phenyl | *o*-$NO_2$-benzoyl | Methyl | O |
| 36 | Ethyl | Phenyl | *p*-$NO_2$-benzoyl | Methyl | O |
| 37 | Ethyl | Phenyl | *m*-$NO_2$-benzoyl | Methyl | O |
| 38 | Ethyl | Ethyl | *p*-$NO_2$-benzoyl | Methyl | O |
| 39 | Ethyl | Ethyl | Benzoyl | Methyl | O |
| 40 | Methyl | Phenyl | Benzoyl | H | O |
| 41 | Methyl | Phenyl | Benzoyl | Methyl | O |
| 42 | Ethyl | Phenyl | Benzoyl | H | O |
| 43 | Ethyl | Methyl | H | H | O |
| 44 | Ethyl | Propyl | H | H | O |
| 45 | Methyl | Methyl | Methyl | H | O |

Barbituric acid derivatives were synthesized by Professor J. Bojarski and co-workers (Department of Organic Chemistry, Academy of Medicine, Krakow, Poland).

the eluents and solutes, two-dimensional spectral maps [19] of barbituric acid derivatives and eluent systems were separately calculated.

The relationship between the physico-chemical parameters of the organic modifiers and their solvent strength was calculated by stepwise regression analysis. Stepwise regression analysis was carried out three times, as follows. (A) The independent variables were the various physico-chemical parameters of organic modifiers ($\pi$, H-Ac, H-Do, M-RE, $F$, $R$, $\sigma$, $Es$, $B_1$ and $B_4$ and the ratio $B_4$/M-RE). The inclusion of the combined variable $B_4$/M-RE was motivated by the assumption that the surface/volume ratio may influence the complex formation of solutes with the CD cavities on the support surface, resulting in a significant impact on the retention. The dependent variable was the solvent strength of the organic modifiers (potency values determined with the "spectral map" technique). (B, C) The independent variables were as in calculation (A) and the dependent variables were (B) the first and (C) the second coordinates of the two-dimensional spectral map of organic modifiers. The other parameters for the calculations were the same as in Section 2.1.

## 3. Results and discussion

The capacity factors and asymmetry factors of the barbituric acid derivatives are given in Table 2. Blank entries in Table 2 indicate that the corresponding barbiturate either eluted near the dead volume or its retention time was greater than 30 min. Both the quality of the organic modifier and the character of the substituents of the barbiturates considerably influenced the capacity factors, suggesting that the β-CD polymer column can be successfully used for the separation of these type of solutes. In each instance the asymmetry factors were near 1, that is, the tailing of peaks on this column was negligible. This suggests that the covering of the polar silica surface by the β-CD had been carried out satisfactorily.

### *3.1. Influence of physico-chemical parameters of barbituric acid derivatives on their retention on a β-CD column*

The parameters of the correlations describing the relationships between retention parameters and physico-chemical characteristics of barbituric acid derivatives are given in Table 3. The equations fit the experimental data well, the significance level being over 99% (see $F$ values). The physico-chemical parameters of barbituric acid derivatives explain 15–61% of the total variance, indicating that other parameters not included in the calculations may have a considerable influence on the retention.

The lipophilicity values and steric parameters of substituents have the greatest impact on the retention characteristics of barbiturates on the β-CD polymer-coated silica column (see $b_{\%}$ values in Table 3). The preponderant role of solute lipophilicity and steric parameters can be explained by the assumption that the retention of barbiturates on the β-CD support is influenced by the following interactions:

(1) Interactions of solutes with the cyclodextrin cavity: it is generally accepted that these interactions are determined by both the lipophilicity and the size of the guest molecules. The steric parameters define the capacity of the guest molecule to enter the cyclodextrin cavity and the lipophylicity of the guest molecule determines the strength of interaction with the hydrophobic inner surface of the CD cavity. The results in Table 3 demonstrate the importance of these interactions.

(2) Polar interactions between solutes and the polar groups on the β-CD polymer surface or between the polar substructures of the solutes and free silanol groups not covered by the β-CD polymer: these interactions are probably influenced by the electron-withdrawing and electron-donating properties of the polar substructures of the barbituric acid derivatives; however, as seen in Table 2, they are of secondary importance.

We emphasize that the interactions mentioned above can take place not only between the support and solutes but also between the support

Table 2
Log $k'$ values and asymmetry factors ($AS$) obtained with (I) methanol–water, (II) acetonitrile–water, (III) ethanol–water, (IV) dioxane–water and (V) tetrahydrofuran–water as mobile phases

| Compound[a] | I | | II | | III | | IV | | V | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Log $k'$ | $AS$ | Log $k'$ | $AS$ | Log $k'$ | $AS$ | Log $k'$ | $AS$ | Log $k'$ | $AS$ |
| 1 | −0.37 | 1.1 | – | – | −0.73 | 1.2 | −0.23 | 1.1 | −0.36 | 1.2 |
| 2 | −0.46 | 1.2 | – | – | – | – | −0.42 | 1.3 | −0.37 | 1.1 |
| 3 | 0.69 | 1.5 | 0.02 | 1.3 | 0.18 | 1.2 | 1.1 | 1.2 | −0.40 | 1.2 |
| 4 | 0.28 | 1.3 | −0.34 | 1.2 | −0.13 | 1.2 | −0.23 | 1.3 | −0.26 | 1.2 |
| 5 | −0.11 | 1.2 | −0.50 | 1.1 | −0.37 | 1.4 | −0.30 | 1.2 | −0.42 | 1.2 |
| 6 | 0.32 | 1.1 | 0.55 | 1.1 | 0.01 | 1.5 | −0.31 | 1.2 | −0.29 | 1.1 |
| 7 | 0.29 | 1.2 | −0.09 | 1.2 | −0.07 | 1.1 | −0.35 | 1.1 | −0.31 | 1.2 |
| 8 | 0.22 | 1.2 | −0.23 | 1.0 | −0.04 | 1.2 | −0.27 | 1.2 | 0.40 | 1.3 |
| 9 | 0.31 | 1.2 | −0.21 | 1.1 | −0.02 | 1.1 | −0.31 | 1.2 | −0.28 | 1.1 |
| 10 | 0.33 | 1.1 | −0.17 | 1.1 | 0.07 | 1.1 | −0.14 | 1.1 | −0.20 | 1.2 |
| 11 | 0.34 | 1.2 | −0.17 | 1.3 | 0.08 | 1.1 | −0.12 | 1.2 | −0.48 | 1.3 |
| 12 | 0.45 | 1.3 | −0.14 | 1.3 | 0.14 | 1.2 | −0.13 | 1.24 | −0.49 | 1.5 |
| 13 | 0.67 | 1.1 | 0.01 | 1.2 | 0.26 | 1.1 | 0.05 | 1.1 | −0.39 | 1.3 |
| 14 | – | – | 0.14 | 1.1 | 0.32 | 1.3 | −0.03 | 1.1 | −0.25 | 1.4 |
| 15 | −0.40 | 1.0 | −0.33 | 1.2 | −0.18 | 1.1 | −0.12 | 1.2 | −0.14 | 1.2 |
| 16 | 0.06 | 1.1 | −0.33 | 1.1 | −0.18 | 1.1 | −0.12 | 1.1 | −0.24 | 1.1 |
| 17 | 0.31 | 1.1 | −0.19 | 1.2 | −0.01 | 1.2 | −0.27 | 1.2 | −0.15 | 1.1 |
| 18 | 0.14 | 1.2 | −0.39 | 1.1 | −0.18 | 1.2 | −0.20 | 1.1 | −0.26 | 1.2 |
| 19 | 0.47 | 1.1 | −0.08 | 1.3 | 0.10 | 1.1 | −0.34 | 1.2 | −0.55 | 1.3 |
| 20 | 0.39 | 1.1 | −0.10 | 1.2 | 0.01 | 1.0 | −0.40 | 1.2 | −0.61 | 1.1 |
| 21 | 0.20 | 1.0 | −0.15 | 1.1 | 0.01 | 1.0 | −0.31 | 1.0 | −0.44 | 1.1 |
| 22 | 0.61 | 1.1 | 0.10 | 1.0 | 0.31 | 1.0 | 0.01 | 1.1 | −0.29 | 1.0 |
| 23 | 0.68 | 1.0 | 0.12 | 1.1 | 0.40 | 1.1 | 0.06 | 1.2 | −0.27 | 1.2 |
| 24 | 0.40 | 1.1 | −0.14 | 1.2 | 0.01 | 1.0 | −0.33 | 1.0 | −0.56 | 1.1 |
| 25 | 0.47 | 1.1 | −0.11 | 1.1 | 0.17 | 1.2 | −0.20 | 1.3 | −0.58 | 1.2 |
| 26 | 0.00 | 1.1 | −0.30 | 1.1 | −0.20 | 1.2 | −0.19 | 1.2 | −0.63 | 1.1 |
| 27 | 0.42 | 1.1 | −0.25 | 1.0 | 0.06 | 1.0 | −0.23 | 1.0 | −0.56 | 1.0 |
| 28 | – | – | 0.06 | 1.1 | 0.61 | 1.3 | 0.16 | 1.1 | −0.40 | 1.2 |
| 29 | 0.60 | 1.1 | 0.06 | 1.2 | 0.16 | 1.0 | 0.61 | 1.0 | −0.40 | 1.2 |
| 30 | 0.57 | 1.1 | −0.20 | 1.1 | 0.18 | 1.1 | 0.00 | 1.1 | 0.02 | 1.1 |
| 31 | 0.80 | 1.2 | 0.28 | 1.1 | 0.14 | 1.1 | 0.00 | 1.0 | 0.02 | 1.2 |
| 32 | −0.09 | 1.0 | −0.11 | 1.2 | 0.42 | 1.3 | 0.01 | 1.1 | 0.40 | 1.2 |
| 33 | 0.87 | 1.1 | 0.77 | 1.0 | 0.44 | 1.0 | 0.01 | 1.1 | −0.39 | 1.0 |
| 34 | 0.98 | 1.0 | −0.07 | 1.0 | 0.55 | 1.0 | −0.55 | 1.1 | −0.33 | 1.2 |
| 35 | 0.85 | 1.1 | −0.12 | 1.1 | −0.12 | 1.1 | 0.39 | 1.0 | 0.06 | 1.0 |
| 36 | 1.02 | 1.1 | −0.08 | 1.0 | 0.69 | 1.2 | −0.13 | 1.2 | −0.42 | 1.1 |
| 37 | 0.37 | 1.2 | −0.20 | 1.2 | 0.10 | 1.1 | −0.13 | 1.1 | −0.42 | 1.1 |
| 38 | 0.50 | 1.1 | −0.28 | 1.2 | 0.20 | 1.0 | −0.07 | 1.0 | −0.33 | 1.3 |
| 39 | 0.40 | 1.1 | −0.34 | 1.1 | 0.00 | 1.2 | −0.18 | 1.2 | −0.40 | 1.2 |
| 40 | 0.42 | 1.2 | −0.36 | 1.2 | 0.23 | 1.1 | −0.17 | 1.2 | −0.59 | 1.1 |
| 41 | −0.07 | 1.1 | −0.18 | 1.3 | 0.39 | 1.1 | −0.01 | 1.1 | −0.36 | 1.1 |
| 42 | 0.88 | 1.1 | −0.01 | 1.1 | 0.39 | 1.2 | −0.01 | 1.1 | −0.36 | 1.2 |
| 43 | −0.34 | 1.1 | −0.01 | 1.2 | −0.54 | 1.1 | −0.02 | 1.1 | −0.36 | 1.1 |
| 44 | −0.20 | 1.1 | −0.55 | 1.3 | −0.43 | 1.2 | −0.71 | 1.0 | −0.77 | 1.1 |
| 45 | −0.20 | 1.0 | −0.55 | 1.1 | −0.43 | 1.1 | 0.73 | 1.1 | 0.74 | 1.2 |

Concentrations of organic modifiers were 50% (v/v) in each instance.
[a] See Table 1.

Table 3
Effects of various physico-chemical parameters of barbituric acid derivatives on their retention behaviour on a β-CD column: results of stepwise regression analysis (number of samples = 45): $\log k' = a + b_1x_1 + b_2x_2$

| Parameter[a] | Eluent[b] | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| $a$ | −0.47 | 0.98 | −0.71 | −0.62 | −0.69 |
| $b_1$ | 0.02 | 0.19 | 0.17 | 0.05 | 0.06 |
| $x_1$ | M-RE | $\Sigma\pi$ | $\Sigma\pi$ | $\Sigma\pi$ | $\Sigma\pi$ |
| $S_{b2}$ | 0.01 | 0.04 | 0.903 | 0.03 | 0.01 |
| $b_{1\%}$ | 71.27 | – | 52.16 | 34.73 | – |
| $b_2$ | −0.29 | – | 0.31 | 0.01 | – |
| $x_2$ | $\sigma$ | – | H-AC | M-RE | – |
| $S_{b2}$ | 0.12 | – | 0.05 | 0.01 | – |
| $b_{2\%}$ | 28.72 | – | 47.84 | 65.24 | – |
| $r^2$ | 0.5321 | 0.3060 | 0.6127 | 0.4569 | 0.1421 |
| $F$ | 21.61 | 18.08 | 31.64 | 16.83 | 6.79 |

[a] $a$ = Intercept; $b_1$ and $b_2$ = regression coefficients; $s_{b1}$ and $s_{b2}$ = standard deviations of regression coefficients $b_1$ and $b_2$; $b_{1\%}$ and $b_{2\%}$ = path coefficients (dimensionless numbers indicating the relative impact of the individual independent variables on the dependent variable); $r^2$ = coefficient of determination (indicates the ratio of variance explained by the independent variables); $F$ = calculated value of the Fisher significance test.
[b] Eluent (1:1, v/v): I = methanol–water; II = acetonitrile–water; III = ethanol–water; IV = dioxane–water; V = tetrahydrofuran–water.

and the molecules of organic modifiers, which explains the slightly different character of the equations in Table 2. It can be assumed that the retention strength and selectivity on the β-CD polymer column are the result of the interplay of the various interactions discussed above.

### 3.2. *Influence of organic modifiers on the retention behaviour of a β-CD column*

The selectivity map of the barbituric acid derivatives is shown in Fig. 1. When we take into consideration the retention behaviour of barbiturates simultaneously in all five eluent systems, the solutes that have phenyl (32), benzoyl (33), *o*-nitrobenzoyl (35) and *p*-nitrobenzoyl (30) substituents at position $R_3$ differ in their retention characteristics from the other barbituric acid derivatives. This result indicates that these substituents have a considerable effect in each eluent system. The fact that the lipophilicity and molar refractivity (related to the bulkiness) of these substituents are high explains their marked role in the retention behaviour, and supports the conclusions drawn from data in Table 3.

The parameters of the equations describing the influence of organic modifiers on the retention behaviour of β-CD columns are given in Table 4. The equations fit the experimental data well, the significance level being over 99%. The physico-chemical parameters of the organic modifiers explain 83–90% of the total variance, indicating the good fit of the equations to the experimental values. The solvent strength of the organic modifiers is determined by the surface/volume ratio (see Eq. 1 in Table 4). As the inclusion complex formation depends markedly on the capacity of a guest molecule to enter the CD cavity (that is, on the steric parameters), this result suggests the involvement of inclusion complex formation in the retention mechanism of the β-DE columns. The selectivity of organic modifiers depends on their electronic parameters ($\sigma$) and molar refractivity (bulkines) (see Eqs. 2 and 3 in Table 4). These results emphasize again the impact of polar interactions and of inclusion complex formation between the molecules of organic modifiers and the surface of β-CD polymer-coated silica.

The selectivity map of the eluent systems is

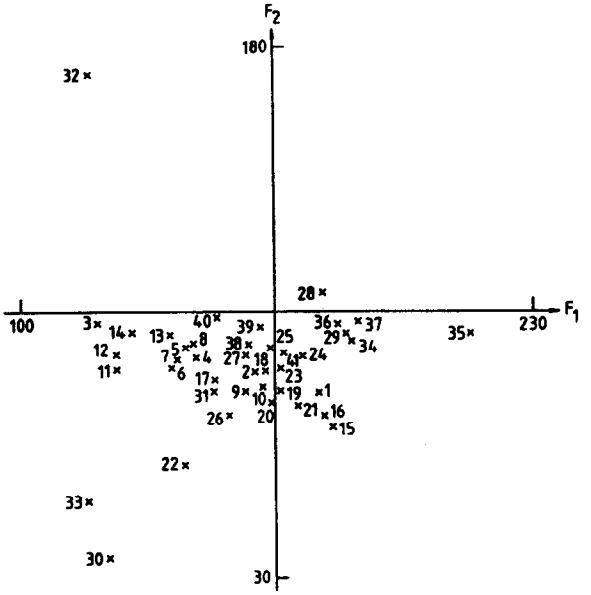



Fig. 1. Selectivity map of barbituric acid derivatives. Number of iterations, 78; maximum error, $2.13 \cdot 10^{-2}$. Numbers refer to barbituric acid derivatives in Table 1.

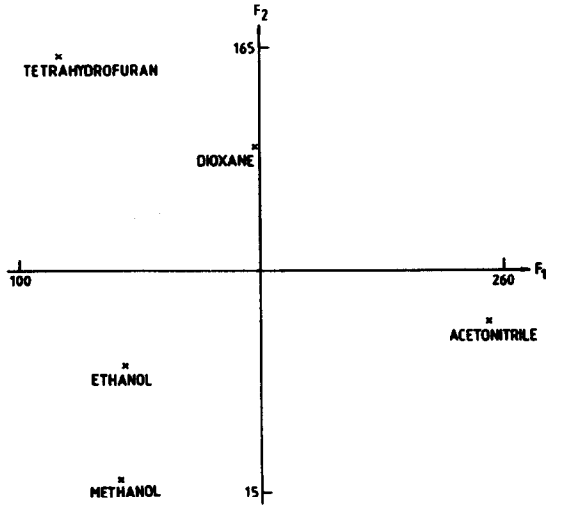



Fig. 2. Selectivity map of eluent systems. Number of iterations, 48; maximum error, $6.02 \cdot 10^{-3}$.

shown in Fig. 2. The organic modifiers form three loose clusters (methanol and ethanol; dioxane and tetrahydrofuran; acetonitrile). The distribution of organic modifiers entirely supports our previous conclusions that the steric characteristics are one of the main determinants of selectivity (bulky dioxane and tetrahydrofuran form separate clusters). As the polarity of –OH is different from that of ≡CN it is understandable that the selectivities of methanol and ethanol differ from that of acetonitrile, indicating again the importance of polar interactions between the molecules of organic modifiers and the surface of the β-CD support.

It can be concluded that babituric acid derivatives can be well separated on a β-CD polymer-coated silica column. Stepwise regression analysis confirmed that the retention of barbituric acid derivatives is mainly governed by the lipophilicity and steric parameters of the substituents. The spectral map technique indicated that the solvent strength and selectivity of organic modifiers on a β-CD polymer-coated silica column depended on their bulkiness and electronic parameters, respectively.

Table 4
Relationships between solvent strength and selectivity of organic modifiers and their physico-chemical parameters: results of stepwise regression analysis: $y = a + bx$

| Parameter[a] | Equation No.[b] | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| $a$ | 7.05 | 102.10 | −39.91 |
| $b$ | 19.63 | 273.07 | 8.01 |
| $x$ | $B_4$/M-RE | $\sigma$ | M-RE |
| $S_b$ | 5.00 | 67.93 | 1.49 |
| $r$ | 0.9147 | 0.9183 | 0.9516 |

[a] For symbols, see Table 3.

[b] (1) $y$ = solvent strength of eluent systems (roman numbers refer to eluent systems in Table 3): I, 2.39; II, 1.57; III, 0.19; IV, 1.17; V, 2.83. (2) $y$ = first coordinate of selectivity map of eluent systems. (3) $y$ = second coordinate of selectivity map of eluent systems.

## 4. Acknowledgement

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

## 5. References


[1] J. Szejtli, in J.L. Atwood, J.E. Davis and D.D. McNicol (Editors), *Inclusion Compounds*, Vol. III, Academic Press, London, 1984.

[2] J. Szejtli, *Cyclodextrins and Their Inclusion Complexes*, Akadémiai Kiadó, Budapest, 1982.

[3] J. Szejtli, B. Zsadon and T. Cserháti, in W.L. Hinze and D.W. Armstrong (Editors), *Ordered Media in Chemical Separation (ACS Symposium Series*, No. 342), American Chemical Society, Washington, DC, 1987, pp. 200–217.

[4] D.W. Armstrong, W. Li, C.-D. Chang and J. Pitha, *Anal. Chem.*, 62 (1990) 914–923.

[5] D.W. Armstrong, W. Li and J. Pitha, *Anal. Chem.*, 62 (1990) 215–217.

[6] T. Cserháti, J. Szejtli and M. Szögyi, *J. Chromatogr.*, 509 (1990) 255–262.

[7] K. Fukushi and K. Hiiro, *J. Chromatogr.*, 518 (1990) 189–198.

[8] J. Liu, K.A. Cobb and M. Novotny, *J. Chromatogr.*, 519 (1990) 189–197.

[9] M. Gosselet and B. Sebille, *J. Chromatogr.*, 552 (1991) 563–573.

[10] B. Agnus, B. Sebille and M. Gosselet, *J. Chromatogr.*, 552 (1991) 583–592.

[11] K. Shimada, T. Oe and M. Suzuki, *J. Chromatogr.*, 558 (1991) 1306–1310.

[12] R.R. West and J.H. Cardellina, II, *J. Chromatogr.*, 539 (1991) 15–23.

[13] D.W. Armstrong, A.M. Stalcup, M.L. Hilton, J.D. Duncan, J.R. Faulkner, Jr., and S.C. Chang, *Anal. Chem.*, 62 (1990) 1610–1615.

[14] N. Thuaud, B. Sebille, A. Deratini, B. Popping and C. Pellet, *Chromatographia*, 36 (1993) 373.

[15] L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1973, pp. 222–224.

[16] H. Mager, *Moderne Regressionsanalyse*, Salle, Sauerlander, Frankfurt/Main, 1982, pp. 135–157.

[17] P.J. Lewi, *Arzneim.-Forsch.*, 26 (1976) 1290.

[18] T. Cserháti and E. Forgács, *Anal. Chim. Acta*, 279 (1993) 107.

[19] J.W. Sammon, Jr., *IEEE Trans. Comput.*, C18 (1969) 401.