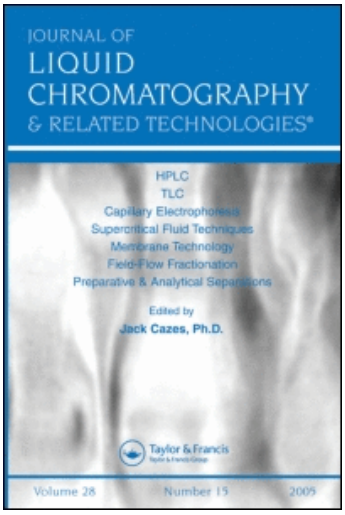


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# MIXED SUPPORTS IN THE REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY OF AMINO ACIDS

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ABSTRACT

Lipophilicity of amino acids was determined by reversed-phase thin-layer chromatography using silica, aluminium oxide, cellulose, diatomaceous earth and their mixtures as supports with water as eluent.

To compare the retention behaviour of supports principal component analysis /PCA/ was applied. The potency order and the selectivity of support mixtures was calculated by the spectral map technique. Linear and logarithmic correlations were calculated between the first PCA loadings and the potency values as de-



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pendent variables and the composition of supports as independent variables.

The first eigenvalue explained more than 90% of the total variance that is only one hidden factor influenced decisively the retention. On the basis of structural differences the retention strength of amino acids on support mixtures can not be explained adequately. The first principal component responsible for the 90% of change in the retention of amino acids is related to the logarithm of support composition that is the sorbents retain their original adsorptive character also after impregnation.



## INTRODUCTION

Quantitative Structure-Activity Relationship /QSAR/ studies have found growing acceptance in the up-to-date drug design. Numerous molecular parameters have been applied in QSAR calculations, but in the majority of cases the lipophilicity showed the strongest correlation with the biological activity /1-3/.

Different methods have been developed to determine the lipophilicity of bioactive compounds as the partition between water and n-octanol /4/, reversed-phase thin-layer chromatography /RPTLC/ /5-9/, high-performance liquid chromatography /10-12/, and gas-liquid chromatography /13-15/. The chromatographic methods have some advantages, they are rapid and accurate, the bioactive compounds need not to be very pure and the investigation requires only a fairly low quantity. However, in RPTLC the adsorptive characteristics of support can exert high influence on the retention also after impregnation /16,17/. In case of 5-nitro-imidazole derivatives it was proved that the quality of impregnation agent influenced considerably the $R_M$ value, the correlations between

the order of $R_M$ values determined on silica plates impregnated with various lipophilic agents were very good /18/. In the case of polar compounds not only the adsorptive strength but also the surface pH value of supports exerts a marked influence on the lipophilicity determination /19/. The lipophilicity determination of amino acids by RPTLC showed some unexpected difficulties, the retentions of amino acids were low on impregnated silica support most frequently used in RPTLC in common eluent systems /20,21/, therefore water insoluble alcohols saturated with water, with acetic acid or with ammonia solutions were applied as eluents /22,23/. Ion paring with trifluoro acetic acid has been applied to overcome problems with streaking and to obtain better separation /24/.

Retention strength and selectivity of supports determined experimentally depend considerably on the experimental conditions and on the solutes investigated /25/. With large number of supports and solutes computer assisted multivariate techniques offer the unique possibility to evaluate all retention data simultaneously /26/. Retention strength of supports can be expressed by the retention of a number of solutes. The usage of retention spectra is aimed at grouping of those supports which share the same /possibly unknown/ mechanism of retention. Usually the information on the spectral properties is disturbed by the relative retention strength of supports and for this reason any classification procedure has to be preceeded by the separation of the relative retention strength from the retention mechanism. The technique of spectral mapping complies with these requirements /27/. This technique has been succesfully applied to classify eluents in RPTLC /28/ and to compare the retention characteristics of some covalently

bonded silica /29/. The objectives of this work were to study the effect of mixed supports on the lipophilicity determination of the amino acids.

## MATERIALS AND METHODS

The following chromatographic sorbents were applied: Kieselgel 60 G /Merck/, MN-Aluminium oxide G /Macherey-Nagel/, Cellulosepulver MN 300 /Macherey-Nagel/ and Kieselgur G /Merck/. The composition of mixed supports is shown in Table 1.

A solution of 10% paraffin oil in n-hexane was added to each mixed support to obtain 9:1 support: paraffin oil weight ratio. These suspensions were shaken overnight at room temperature then the n-hexane was removed in a rotary vacuum evaporator. Layers of 0.25 mm thickness were prepared on 20 x 20 cm glass plates from each impregnated sorbent.

Amino acids were dissolved in water at a concentration of 1 mg/cm$^3$, 5 mm$^3$ of each solution was spot-



TABLE 1

Composition of Mixed Supports / in % /

| No. of support | Aluminium oxide | Kieselgel | Cellulose | Kieselgur |
|---|---|---|---|---|
| 1 | 50 | 25 | 25 | |
| 2 | 50 | | 25 | 25 |
| 3 | 50 | 25 | | 25 |
| 4 | 50 | 16.6 | 16.6 | 16.6 |
| 5 | 50 | | 50 | |
| 6 | 50 | | | 50 |
| 7 | 50 | 50 | | |
| 8 | 30 | 70 | | |
| 9 | 70 | 30 | | |

ted on the plates. Distilled water served as eluent. After development the plates were dried at 105°C and the amino acids were detected by ninhydrin reagent. For each experiment five independent parallel determinations were carried out.

To compare the retention behaviour of supports taking into consideration simultaneously the retention strength and selectivity of supports principal component analysis /PCA/ /30/ was applied to the data matrix /100•$R_f$ values of 21 amino acids on 9 different mixed supports/.

To treat separately the retention strength and selectivity the spectral map technique was applied. To elucidate the role of support composition in the retention behaviour linear and logarithmic correlations were calculated between the first PCA loadings and potency values of spectral map as dependent variables and the composition data of Table 1. as independent variables.



## RESULTS AND DISCUSSION

Some results of principal component analysis we compiled in Table 2. The first eigenvalue explains more than 90% of total variance that means that only one hidden factor influence at 90% level the retention of all amino acids on each support. The two dimensional linear map of supports shows clearly that supports 1, 3 and 4 exhibit similar retention behaviour and support 8 deviates the most strongly from the others /Fig. 1./. The two dimensional map of PCA variables shows the distribution of amino acids taking into consideration simultaneously their retentions on the 9 support mixtures. The amino acids without any additional functional group /Gly, Ala, Val, Leu, Ile/ form a loose cluster in the central part of the map, the

TABLE 2

Results of the Principal Component Analysis

| Eigenvalues | Sum of total variance explained % |
|---|---|
| 8.22 | 91.35 |
| 0.53 | 97.20 |

Principal component loadings

| No. of support | I | II |
|---|---|---|
| 1 | 0.987 | -0.089 |
| 2 | 0.934 | 0.326 |
| 3 | 0.986 | -0.089 |
| 4 | 0.987 | -0.032 |
| 5 | 0.955 | 0.263 |
| 6 | 0.912 | 0.356 |
| 7 | 0.971 | -0.205 |
| 8 | 0.904 | -0.387 |
| 9 | 0.962 | -0.129 |


FIGURE 1. Two dimensional map of PCA loadings
Numbers indicate mixed supports in Table 1.

other amino acids are distributed tangentially to it. This phenomenon suggests that all substitutions influence the retention by different ways. The dibasic amino acids Lys, Arg and Pro together with hydroxi-Pro form two distinct pairs that is the pairs behave similarly but differ considerably from the other amino acids. Dicarboxylic amino acids Glu, Asp and Tyr form a cluster, too, which proves the acidic character of phenolic OH group in Tyr. The amino acids with alcoholic OH groups /Ser, Thr/ are near to eachother and to the central group. This observation indicates that also this functional group influences the retention, but to a lesser extent than the other ones. Phe, Trp and Cys form a well defined cluster, however they are structurally different. The potency /retention strength/ order of support mixtures and amino acids are listed in Table 3.

As the spectral map technique calculates the potency on the basis of $R_f$ values, the higher potency means higher $R_f$ value. Since the eluent was always the same in our case, the higher potency means a lower retention strength of support mixtures and a lower retention of amino acids. The retention strength of mixed support decreases with growing concentration of silica /supports 7, 8 and 9/, the retention decreasing effect of cellulose and diatomaceous earth is lower than that of silica /supports 5, 6 and 7/ however in common chromatographic procedures silica is considered as a stronger sorbent than cellulose and diatomaceous earth. The retention of Trp, Cys and Phe were the strongest, this finding explains that they form a separate cluster in PCA. On the basis of structural differences the retention strength of amino acids on support mixtures can not be explained adequately. The spectral map is similar to the map of PCA loadings

TABLE 3

Potency Order of Support Mixtures and Amino Acids Calculated by the Spectral Map Technique

| Support | | Amino acid | |
|---|---|---|---|
| No. | Potency | Name | Potency |
| 1 | 2.46 | Trp | 3.4 |
| 2 | 2.18 | Cys | 5.1 |
| 3 | 2.44 | Phe | 6.5 |
| 4 | 2.36 | Asp | 7.2 |
| 5 | 2.29 | His | 12.2 |
| 6 | 1.71 | Arg | 12.4 |
| 7 | 2.27 | Tyr | 12.5 |
| 8 | 2.62 | Leu | 13.1 |
| 9 | 1.90 | Glu | 13.6 |
| | | Ile | 14.2 |
| | | Met | 14.3 |
| | | Asn | 14.8 |
| | | Lys | 15.1 |
| | | Thr | 16.7 |
| | | Ser | 18.7 |
| | | Gln | 19.4 |
| | | Val | 19.7 |
| | | Gly | 19.9 |
| | | Pro | 22.5 |
| | | Ala | 23.4 |
| | | OH-Pro | 24.4 |

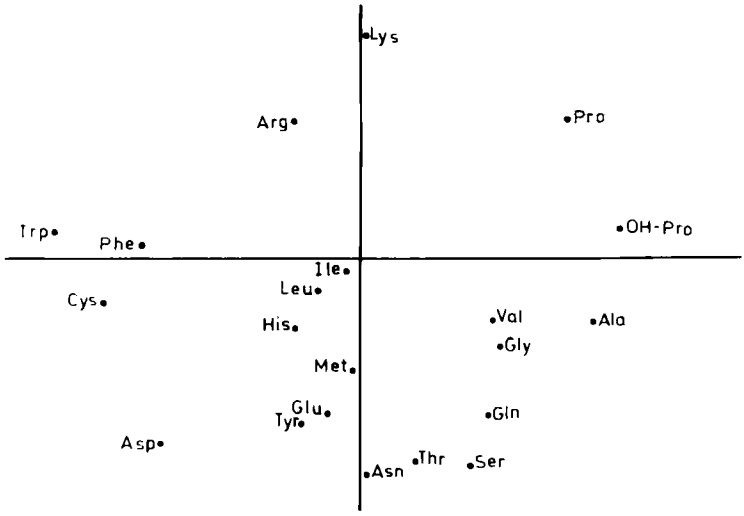


FIGURE 2. Distribution of amino acids according to their retention on mixed supports /Two dimensional map of PCA variables/

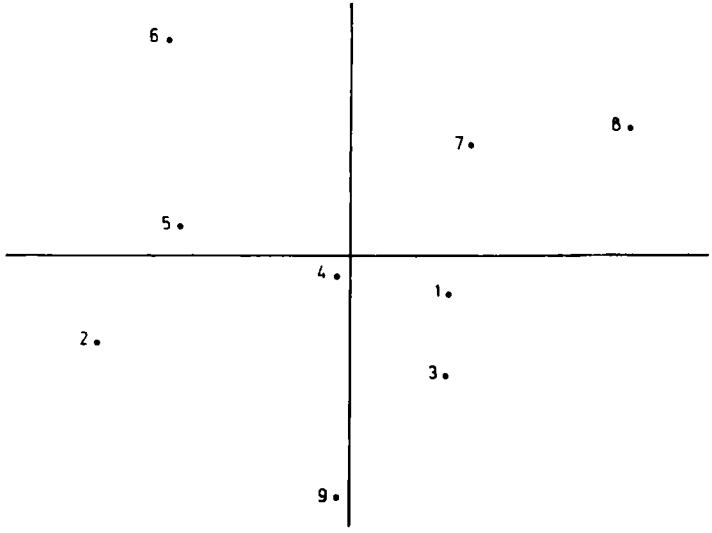


FIGURE 3. Two dimensional non-linear mapping of spectral data /Numbers indicate mixed supports in Table 1/
Number of iterations: 24
Error of mapping: $3.28 \cdot 10^{-2}$

/Fig. 3./ that is the spectral characteristics influence markedly the distribution of support mixtures. The F values of linear correlations between the loadings of first principal component /F = 1.53/, potency of spectral map /F = 4.31/ and the composition data of support mixtures were lower than those of logarithmic correlations /F = 3.87 and 5.34, respectively/. As no significant effect of Kieselgur content of support on the dependent variables was observed /partial t values 1.73 , 1.58 , 0.89 , 0.09 , respectively/ it was omitted from the data and the calculations were recalculated. The results are summarized in Table 4. The linear correlation between the loadings of first principal component and the composition of support did not reach the 90% significance level therefore its parameters were not included in Table 4.

Our calculation proved that the first principal component responsible for the 90% of change of amino acids on all supports can be identified as the logarithm of support composition /Eq. II in Table 4/. It proves that the sorbents – however they are heavily impregnated with paraffin oil – retain their adsorptive character and this is responsible for the retention behaviour and not the paraffin oil adsorbed on their surfaces. The path coefficients /$b'_i$ values/ show clearly that aluminium oxide, silica and cellulose have about the same impact on the retention of amino acids.

The potency values depend also on the support composition, the logarithmic function /Eq. III/ fits better to the experimental data than the linear one /Eq. II/. The growing aluminium oxide content of support increases the retention, the silica and cellulose content decreases it. This observation gives possibility to produce mixed supports with any retention

TABLE 4

Parameters of Correlations between the Loadings of First Principal Component /$Y_1$/, Potency Values /$Y_2$/ and Composition of Support Mixtures

$$Y_2 = a + b_1x_1 + b_2x_2 + b_3x_3 \qquad \text{I.}$$

$$Y_1 = a + b_1\log x_1 + b_2\log x_2 + b_3\log x_3 \qquad \text{II.}$$

$$Y_2 = a + b_1\log x_1 + b_2\log x_2 + b_3\log x_3 \qquad \text{III.}$$

where $x_1$ = aluminium oxide content of support in %
$x_2$ = silica content of support in %
$x_3$ = cellulose content of support in %

| Parameter | No. of equation I. | II. | III. |
|---|---|---|---|
| a | 239.3 | 0.61 | 506.4 |
| $b_1$ | -0.94 | 0.20 | -173.1 |
| $b_2$ | 0.84 | $1.85\cdot10^{-2}$ | 15.0 |
| $b_3$ | 0.95 | $1.38\cdot10^{-2}$ | 12.8 |
| r | 0.8334 | 0.8674 | 0.9175 |
| $r^2$ | 0.6945 | 0.7524 | 0.8417 |
| s | 28.3 | 0.03 | 28.5 |
| $s_{b1}$ | 0.81 | $7.76\cdot10^{-2}$ | 54.5 |
| $s_{b2}$ | 0.41 | $6.31\cdot10^{-3}$ | 4.43 |
| $s_{b3}$ | 0.50 | $6.17\cdot10^{-3}$ | 4.33 |
| $b_1'$ | -0.33 | 0.58 | -0.57 |
| $b_2'$ | 0.71 | 0.73 | 0.67 |
| $b_3'$ | 0.59 | 0.55 | 0.58 |
| F | 3.83 | 5.14 | 8.96 |
| $t_1$ | 1.16 | 2.24 | 2.96 |
| $t_2$ | 2.07 | 2.94 | 3.38 |
| $t_3$ | 1.89 | 2.56 | 3.18 |

$F_{90\%} = 3.62$ $F_{95\%} = 5.41$

$t_{90\%} = 2.02$ $t_{95\%} = 2.57$

strength. The path coefficients are similar supporting our previous finding that these three sorbents have about the same impact on the reversed-phase thin-layer chromatographic retention of amino acids.

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