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# RETENTION BEHAVIOUR OF β-CARBOLINES IN NORMAL-PHASE CHROMATOGRAPHY

## SILICA AND AMINO PHASES IN HIGH-PERFORMANCE AND THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The retention behaviour of a series of β-carbolines in normal-phase chromatography was studied. Silica and amino phases in high-performance liquid (HPLC) and thin-layer chromatographic (TLC) systems were analyzed. The Snyder–Soczewinski approach was used to interpret the retention mechanism in relation to different solute, solvent and stationary phase characteristics. Amino and silica stationary phases, TLC and HPLC techniques and various solvent selectivities were compared. The molecular structure–retention relationship was also studied by means of the group contributions to retention and the electronic constant.

INTRODUCTION

The chromatographic retention behaviour of polar solutes in normal-phase (NP) systems is determined by specific solute–stationary and mobile phase interactions[1–4]. The semiempirical adsorption model of Snyder[5,6] has proved to be a valuable tool in investigating how the solute molecular structure, the mobile phase composition and the stationary phase structure affect retention in both thin-layer (TLC) and high-performance liquid chromatographic (HPLC) systems. Studies on the retention behaviour of various solute classes in different chromatographic systems may be helpful to clarify the rôle each interaction plays in the retention mechanism. For this purpose, TLC seems to be a very promising technique because of its speed and applicability to a wide range of solute and solvent types[5,7,8]. Therefore it is very important to estimate TLC as a pilot technique replacing HPLC.

In this study the retention of β-carbolines (βCCs) on NP systems was analyzed and TLC and HPLC systems were compared. βCCs are a class of drugs, chemically

unrelated to benzodiazepine, able to interact to various degrees with the benzodiazepine receptors in the mammal central nervous system[9,10]. In a previous work the retention of $\beta$CCs, in reversed-phase (RP) systems, particularly in an ion-pairing system, was studied in order to determine their lipophilic character[11]. In the present work the Snyder approach was applied. The slopes and intercepts of the linear relationships between retention and mobile phase composition are discussed in relation to the solute, solvent and stationary phase characteristics in order to obtain information about the retention mechanism and the structure–retention relationships.

EXPERIMENTAL

The HPLC measurements were performed with a Waters 600 multisolvent delivery system equipped with a Waters 990 photodiode array detector. A Wisp Model 712 was used to inject 10 $\mu$l of the standard solutions. The columns used were 30 cm $\times$ 3.9 mm I.D. $\mu$Porasil and $\mu$Bondapak NH$_2$ from Waters Assoc.

The TLC measurements were carried out using Merck HPTLC NH$_2$ and silica plates. A Camag Nanomat was used to spot about 100 nl of the $\beta$CC solutions on the plates. HPLC grade 2-propanol, ethyl acetate, dioxane, tetrahydrofuran (THF), acetonitrile, $n$-hexane and dichloromethane were obtained from Rudi Pont. For each chromatographic system, a minimum of seven different compositions of mixtures with $n$-hexane as the diluent (or dichloromethane for acetonitrile) were studied. The only exception to this was ethyl acetate on HPLC NH$_2$ where only the measurements with 100% solvent were possible.

The $\beta$CCs, obtained from Sigma Chemicals (St. Louis, MO, U.S.A.), were dissolved in 2-propanol or in 2-propanol–water mixtures in a concentration range between 100 and 500 $\mu$g/ml. The molecular structures of the $\beta$CCs studied are shown in Fig. 1.

RESULTS AND DISCUSSION

Both the Snyder and the Soczewinski approaches[5,12] draw a linear relationship between retention (as log $k'$ or $R_M$ values) and the mobile phase composition (as $X_S$ = mole fraction of the strong solvent)

$$\log k' = R_M = c - n \log X_S \tag{1}$$

from the intercept, $c$, of which it is possible to estimate the strength of the solute interaction with the stationary phase, while the slope, $n$ (as the ratio between the molecular area of the solute and solvent at the adsorbent surface), indicates the number of active solute molecule adsorption sites interacting with the stationary phase.

The validity of eqn. 1 was experimentally confirmed for all stationary and mobile phases examined: the correlation coefficients were excellent for HPLC (mean 0.996 $\pm$ 0.0016) and satisfactory for TLC (mean 0.984 $\pm$ 0.100). The values of the intercept and slope are presented in Tables I and II for amino and silica HPLC and TLC stationary phases when 2-propanol and ethyl acetate were the strong solvents. A comparison of the intercept values calculated for the different chromatographic
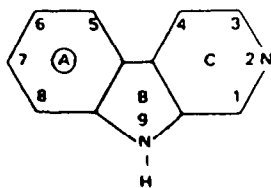
| Cpd | Name | $R_1$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | Ring C |
|---|---|---|---|---|---|---|---|---|
| 1 | Nor-harman | H | H | H | H | H | H | Arom. |
| 2 | Harman | $CH_3$ | H | H | H | H | H | Arom. |
| 3 | Harmine | $CH_3$ | H | H | H | H | $OCH_3$ | Arom. |
| 4 | Harmol | $CH_3$ | H | H | H | H | OH | Arom. |
| 5 | | $CH_2OH$ | H | H | H | H | H | Arom. |
| 6 | -CCM | H | $COOCH_3$ | H | H | H | H | Arom. |
| 7 | -CCE | H | $COOC_2H_5$ | H | H | H | H | Arom. |
| 8 | PR-CC | H | $COOC_3H_7$ | H | H | H | H | Arom. |
| 9 | DMCM | H | $COOCH_3$ | $C_2H_5$ | H | $OCH_3$ | $OCH_3$ | Arom. |
| 10 | ZK 91296 | H | $COOC_2H_5$ | $CH_2OCH_3$ | $OCH_2C_6H_5$ | H | H | Arom. |
| 11 | ZK 93426 | H | $COOC_2H_5$ | $CH_3$ | $OiC_3H_7$ | H | H | Arom. |
| 12 | | H | $COONHCH_3$ | H | H | H | H | Arom. |
| 13 | Harmaline | $CH_3$ | H | H | H | H | $OCH_3$ | Non ar. |
| 14 | Harmalol | $CH_3$ | H | H | H | H | OH | Non ar. |
| 15 | ZK 93423 | H | $COOC_2H_5$ | $CH_2OCH_3$ | H | $OCH_2C_6H_5$ | H | Arom. |

Fig. 1. Molecular structures of the βCCs studied.

systems shows that for this series of solutes the amino phase is more retentive than silica. This pattern is more evident from the data calculated from measurements with ethyl acetate as the strong solvent. As predicted theoretically, when ethyl acetate was used as the strong solvent, larger slopes and intercepts were obtained. Table III reports the linear relationships calculated when the intercept values with ethyl acetate and 2-propanol are correlated with one another for the different systems (eqns. 2–4). The poor correlations may be interpreted in terms of solvent selectivity according to the relative importance of hydrogen bonding vs. dipole interaction. 2-Propanol, as a strong proton acceptor and donor, exhibits different specific interactions toward

TABLE I

INTERCEPT, *c*, AND SLOPE, *n*, OF EQN. 1 ON HPLC AND TLC AMINO PHASES

| | 2-Propanol | | | | Ethyl acetate | | |
| | HPLC | | TLC | | HPLC | TLC | |
| | *c* | *n* | *c* | *n* | *c*[a] | *c* | *n* |
|---|---|---|---|---|---|---|---|
| 1 | −0.381 | 0.811 | −0.544 | 1.497 | 0.92 | 0.351 | 2.219 |
| 2 | −0.446 | 0.982 | −0.526 | 1.468 | 0.90 | 0.368 | 2.138 |
| 3 | −0.284 | 1.043 | −0.471 | 1.612 | 1.09 | 0.531 | 2.984 |
| 4 | −0.079 | 2.690 | 0.117 | 2.601 | − | − | − |
| 5 | −0.308 | 2.296 | −0.245 | 2.335 | − | 1.107 | 3.332 |
| 6 | −0.342 | 0.994 | −0.239 | 1.268 | 1.00 | 1.042 | 2.832 |
| 7 | −0.368 | 0.834 | −0.277 | 1.222 | 0.98 | 0.946 | 3.306 |
| 8 | −0.402 | 0.781 | −0.328 | 1.281 | 0.90 | 0.914 | 2.629 |
| 9 | −0.225 | 1.330 | −0.197 | 1.867 | 1.26 | 1.306 | 3.992 |
| 10 | −0.503 | 1.073 | −0.374 | 1.689 | 0.85 | 0.851 | 3.673 |
| 11 | −0.583 | 1.120 | −0.435 | 1.281 | 0.71 | 0.684 | 3.303 |
| 12 | −0.335 | 1.595 | −0.328 | 2.422 | 0.90 | 0.741 | 3.165 |
| 13 | −0.372 | 1.069 | −0.335 | 1.636 | 1.15 | 0.990 | 2.703 |
| 14 | −0.145 | 2.839 | 0.050 | 2.791 | − | − | − |
| 15 | −0.475 | 1.121 | −0.379 | 1.480 | 0.72 | 0.755 | 3.635 |

[a] Only experimental measurements with 100% ethyl acetate were possible.

TABLE II

INTERCEPT, *c*, AND SLOPE, *n*, VALUES OF EQN. 1 ON HPLC AND TLC SILICA PHASES

| | 2-Propanol | | Ethyl acetate | | | |
| | HPLC | | HPLC | | TLC | |
| | *c* | *n* | *c* | *n* | *c* | *n* |
|---|---|---|---|---|---|---|
| 1 | −0.413 | 0.981 | 0.549 | 2.145 | 0.871 | 1.386 |
| 2 | −0.463 | 1.041 | 0.511 | 2.346 | 0.854 | 1.526 |
| 3 | −0.317 | 1.092 | 0.906 | 2.501 | 1.252 | 1.767 |
| 4 | −0.109 | 2.687 | 1.044 | 3.162 | 1.46[a] | − |
| 5 | −0.331 | 2.375 | 0.729 | 3.194 | 1.278 | 3.705 |
| 6 | −0.376 | 1.073 | 0.520 | 2.507 | 0.714 | 2.084 |
| 7 | −0.409 | 0.943 | 0.458 | 2.286 | 0.569 | 2.399 |
| 8 | −0.468 | 0.887 | 0.297 | 2.179 | 0.418 | 2.190 |
| 9 | −0.254 | 1.407 | 0.685 | 3.591 | 1.103 | 2.276 |
| 10 | −0.683 | 1.391 | 0.147 | 3.644 | 0.365 | 3.552 |
| 11 | −0.695 | 1.460 | 0.143 | 2.958 | 0.353 | 2.359 |
| 12 | −0.425 | 1.871 | 0.343 | 3.832 | 0.615 | 2.839 |
| 13 | −0.375 | 1.084 | 0.868 | 1.981 | 1.248 | 1.705 |
| 14 | −0.009 | 2.365 | 1.021 | 2.711 | 1.46[a] | − |
| 15 | −0.642 | 1.391 | 0.160 | 3.481 | 0.416 | 3.337 |

[a] Only experimental measurements with 100% ethyl acetate were possible.

TABLE III

CORRELATIONS BETWEEN INTERCEPT VALUES, $c$, OBTAINED FOR 2-PROPANOL AND ETHYL ACETATE (EQNS. 2–4) AND IN CORRESPONDING TLC AND HPLC SYSTEMS (EQNS. 5–7)

| | | | |
|---|---|---|---|
| $c(\text{2-propanol}) = -0.887\ (\pm 0.093) + 0.521\ (\pm 0.096)\ c(\text{ethyl acetate})$ | | HPLC NH$_2$ | (2) |
| $n = 12$ | $r = 0.863$ $\quad s = 0.052$ $\quad F = 29.19$ | | |
| $c(\text{2-propanol}) = -0.664\ (\pm 0.026) + 0.374\ (\pm 0.030)\ c(\text{ethyl acetate})$ | | TLC NH$_2$ | (3) |
| $n = 13$ | $r = 0.966$ $\quad s = 0.030$ $\quad F = 151.93$ | | |
| $c(\text{2-propanol}) = -0.711\ (\pm 0.045) + 0.560\ (\pm 0.070)\ c(\text{ethyl acetate})$ | | HPLC Si | (4) |
| $n = 15$ | $r = 0.911$ $\quad s = 0.082$ $\quad F = 63.13$ | | |
| $c(\text{TLC}) = 0.130\ (\pm 0.049) + 1.093\ (\pm 0.131)\ c(\text{HPLC})$ | | NH$_2$ 2-propanol | (5) |
| $n = 11$ (cpds. 4–15) | $r = 0.935$ $\quad s = 0.063$ $\quad F = 68.28$ | | |
| $c(\text{TLC}) = -0.021\ (\pm 0.141) + 0.949\ (\pm 0.153)\ c(\text{HPLC})$ | | NH$_2$ ethyl acetate | (6) |
| $n = 8$ (cpds. 6–13 and 15) | $r = 0.908$ $\quad s = 0.029$ $\quad F = 43.61$ | | |
| $c(\text{TLC}) = 0.148\ (\pm 0.050) + 1.284\ (\pm 0.078)\ c(\text{HPLC})$ | | Si ethyl acetate | (7) |
| $n = 15$ | $r = 0.977$ $\quad s = 0.091$ $\quad F = 268.15$ | | |

$\beta$CC solutes when compared to ethyl acetate, a solvent characterized by strong dipole interactions and lower proton donor and acceptor strength[13,14]. The worst correlation obtained with data on the HPLC amino phase (eqn. 2) may be due to the fact that experimental ethyl acetate measurements were related to calculated 2-propanol intercept values.

*TLC–HPLC correlation*

By comparing the intercept values reported in Tables I and II the correlation between retention values in the corresponding TLC and HPLC systems was calculated (eqn. 5–7 in Table III). Eqns. 5 and 6 show that for the amino phase the slope of the TLC–HPLC correlation does not differ from unity, thus suggesting that the same retention mechanism occurs in both TLC and HPLC. Moreover, the equality of the parameters for both 2-propanol and ethyl acetate solvents may suggest this similarity is independent of eluent type. The linear relationship between $R_M$ and log $k'$ values is statistically significant only when compounds 1–3 (which do not contain an ester or hydroxyl group) are excluded from the correlation. In Fig. 2 one may note that these compounds deviate most from the straight line, thus showing that the HPLC system exerts a specific, stronger retention towards them. The different solute selectivities of the systems may be explained by the different distribution of the mobile phase over the stationary phase and by some specific effects of the stationary phase[7–15]. When the silica phase is considered (eqn. 7 in Table III), the slope value 1.3 may suggest some differences in the retention properties of the TLC and HPLC silica systems. However, the statistical parameters of eqn. 7 are very good, thus excluding any evident specific selectivity of the two systems towards particular solutes. Since the TLC data on silica are very highly correlated with those of HPLC for all the $\beta$CCs studied, silica plates were chosen to study solvent selectivity effects.
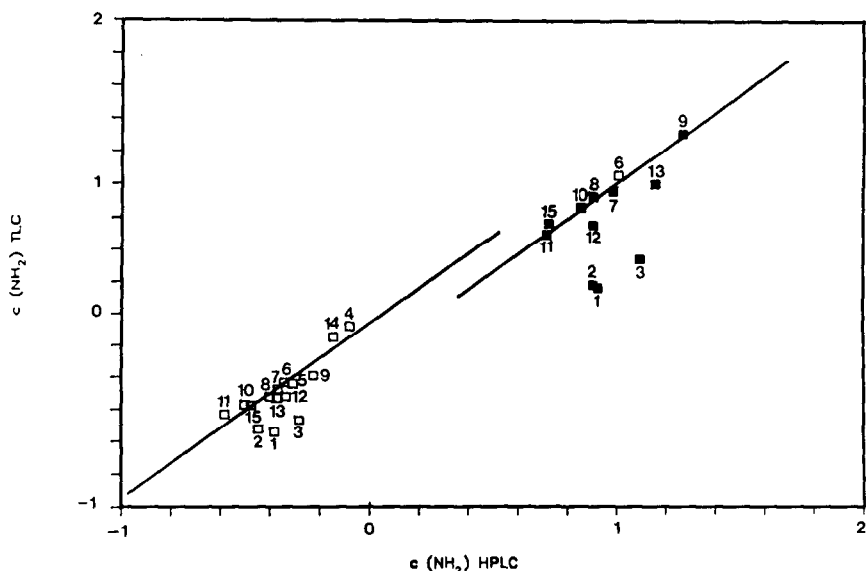
Fig. 2. Relationships between $R_M$ and log $k'$ (both as intercept values of eqn. 1) on the amino stationary phase with 2-propanol ($\square$, eqn. 5, Table III) or ethyl acetate ($\%$, eqn. 6, Table III) as the strong solvent.

TABLE IV

CORRELATIONS BETWEEN THE INTERCEPT, $c$, AND SLOPE, $n$, OBTAINED IN CORRESPONDING SILICA AND AMINO SYSTEMS

| | | |
|---|---|---|
| $c(Si) = -0.013\ (\pm0.042) + 1.490\ (\pm0.114)\ c(NH_2)$ | HPLC 2-propanol | (8) |
| $n = 15 \quad r = 0.958 \quad s = 0.056 \quad F = 146.12$ | | |
| $n(Si) = 0.333\ (\pm0.095) + 0.829\ (\pm0.065)\ n(NH_2)$ | HPLC 2-propanol | (9) |
| $n = 15 \quad r = 0.964 \quad s = 0.160 \quad F = 172.95$ | | |
| $c(Si) = -0.858\ (\pm0.252) + 1.396\ (\pm0.262)\ c(NH_2)$ | HPLC ethyl acetate | (10) |
| $n = 12 \quad r = 0.860 \quad s = 0.140 \quad F = 28.45$ <br> (cpds. 1–3, 6–13 and 15) | | |
| $c(Si) = -0.636\ (\pm0.138) + 1.072\ (\pm0.148)\ c(NH_2)$ | HPLC ethyl acetate | (10') |
| $n = 8 \quad r = 0.947 \quad s = 0.069 \quad F = 52.27$ <br> (cpds. 6–12 and 15) | | |
| $c(Si) = -0.925\ (\pm0.326) + 1.610\ (\pm0.319)\ c(NH_2)$ | HPLC ethyl acetate | (10'') |
| $n = 4 \quad r = 0.963 \quad s = 0.069 \quad F = 25.33$ <br> (cpds. 1–3 and 13) | | |
| $c(Si) = -0.414\ (\pm0.235) + 1.086\ (\pm0.254)\ c(NH_2)$ | TLC ethyl acetate | (11') |
| $n = 8 \quad r = 0.867 \quad s = 0.135 \quad F = 18.25$ <br> (cpds. 6–12 and 15) | | |
| $c(Si) = 0.732\ (\pm0.208) + 0.580\ (\pm0.338)\ c(NH_2)$ | TLC ethyl acetate | (11'') |
| $n = 4 \quad r = 0.771 \quad s = 0.174 \quad F = 2.94$ <br> (cpds. 1–3 and 13) | | |

*Amino–silica comparison*

The silica and amino phases show very similar selectivities when 2-propanol is the strong solvent, according to the statistical parameters of the correlations reported in Table IV (eqns. 8 and 9). The quality of the relationship is lower with ethyl acetate (eqn. 10). Nonetheless, a statistically significant improvement is achieved when two groups of compounds are considered: one containing ester, amide or hydroxyl groups and the other without them (eqns. 10' and 10" in Table IV). The TLC stationary phases exhibit the same behaviour, however the quality of the correlations is worse (eqns. 11' and 11" in Table IV). This behaviour may be interpreted assuming that the amino phase exerts a strong specific retention towards the carbonyl and the hydroxyl functions present in the solute molecules.

*Structure–retention relationship*

Similarly to the intercept values, the slopes increase in the order of solute polarity. This order, apparently similar to the solute hydrogen-bonding ability, is therefore, about the same on both the phases. When ethyl acetate is the strong solvent, the slope assumes values of about 2 for compounds 1 and 2; *i.e.*, either these βCC molecules contain two groups which are strongly adsorbed on the stationary phase, or a single βCC molecule is able to interact simultaneously with two silanol or amino groups. This value may be due to the binding of the βCC molecule to the column by the two hydrogen-bonding acceptor aliphatic N atoms in positions 2 and 9. When one or more ester or ether substituents are present in the molecule (compounds 3, 6–11, 13 and 15) the slope increases by about 0.3 (on silica) or 0.5 (on amino) for each group, indicating the partial adsorption of these groups. Moreover, when a larger number of functional groups are present in the solute molecule, the values of $c$ and $n$ are smaller than the simple sums of the corresponding values for monofunctional compounds. This scattering effect may be due to very specific solute–solvent interactions. When an hydroxyl or an amide group is present in the molecule (compounds 4, 5, 12 and 14) the slope increases by about 1, thus indicating that these hydrogen-bonding donor and acceptor groups are one more active site for βCC adsorption. When 2-propanol is the strong solvent, the solutes containing an OH group (4, 5 and 14) show exceptionally high slopes (2.3–2.8). This behaviour reflects the solvent solvation properties: 2-propanol strongly manifests its electron donor properties relative to proton donor groups, thus determining specific solvation interactions with solutes containing such groups[13,16,17].

*Group contributions*

In order to correlate the retention behaviour with molecular structure, the contribution of different substituents to retention was calculated as $\Delta c$ and $\Delta n$. The intercept and slope contributions increased approximately in the same order, while a clearer trend was evident for the $\Delta n$ values. The slope increased in the order

$$OH > CONHCH_3 > COOCH_3 \approx OCH_3 > COOR > OR > CH_3$$

for the amino phase, and

$$CONHCH_3 > OH > COOCH_3 \approx OCH_3 \approx OR > COOR \approx CH_3$$

for the silica phase. These values as in accord with the literature data, with the exception of the unusually low increment for the ester group, particularly on silica[18-20]. This behaviour may be determined by a shielding effect, probably due to the resonance of the conjugated double bond system going from $C=O$ to $N_9$ through $N_2$, as confirmed by the crystal structure of $\beta$-CCM[21]. Support for this hypothesis is also provided by the pattern of the linear free energy correlation between the electronic constant and chromatographic retention. As widely reported for NP chromatographic systems[22,23], this relationship is expressed by:

$$\log k' = a + \rho\sigma \tag{12}$$

The linearity is confirmed for this class of $\beta$CCs in HPLC systems when the electronic constant is expressed as the Taft inductive constant, $\sigma_i$[24] (Table V). However, Fig. 3 evidences the presence of two approximately parallel straight lines where the one corresponding to an ester function (or substituents in $\beta$CCs containing an ester group) is shifted to lower retention values. This behaviour is consistent with the previous hypothesis that intramolecular interactions systematically decrease the adsorptive strength of $\beta$CC solutes containing an ester group. The substituent interaction effect on retention reflects mainly the influence of substituents on the hydrogen-bonding ability of the solute molecule with the stationary phase. Therefore, the positive $\rho$ value in eqn. 12 indicates that the $\beta$CC molecule, as a whole, acts as a proton acceptor in hydrogen-bonding interactions with the silica and amino stationary phases.

TABLE V

SUBSTITUENT CONTRIBUTIONS, CALCULATED AS $\log k'$ WITH 2-PROPANOL, AND INDUCTIVE ELECTRONIC CONSTANT, $\sigma_i$

| Substituents | log k' on silica | log k' on amino | $\sigma_i$ |
|---|---|---|---|
| (A) $CH_3$ | −0.03 | −0.06 | −0.04 |
| (B) $CH_2OH$ | 0.08 | 0.07 | 0.05 |
| (C) $OCH_3$ | 0.15 | 0.16 | 0.23 |
| (D) OH | 0.35 | 0.37 | 0.31 |
| (E) COOR | −0.01 | 0.01 | 0.31 |
| (F) $CONHCH_3$ | −0.01 | 0.05 | 0.33 |
| (G) $OC_3H_7$-*iso* + $CH_3$ | −0.32 | −0.21 | 0.23 |
| (H) $CH_2OCH_3$ + $OCH_2C_6H_5$ | −0.25 | −0.12 | 0.26 |
| (I) $C_2H_5$ + 2 $OCH_3$ | 0.12 | 0.11 | 0.43 |

*Modifier selectivity*

In order to study the solvent effects on $\beta$CC retention on silica, the TLC measurements were extended to acetonitrile, dioxane and tetrahydrofuran as strong solvents. The Snyder–Soczewinski relationship (eqn. 1) was obeyed by these solvents as well. Table VI reports the intercept, *c*, and slope, *n*, values. The elution strength (evaluated in terms of the intercept values) shows the decreasing order:
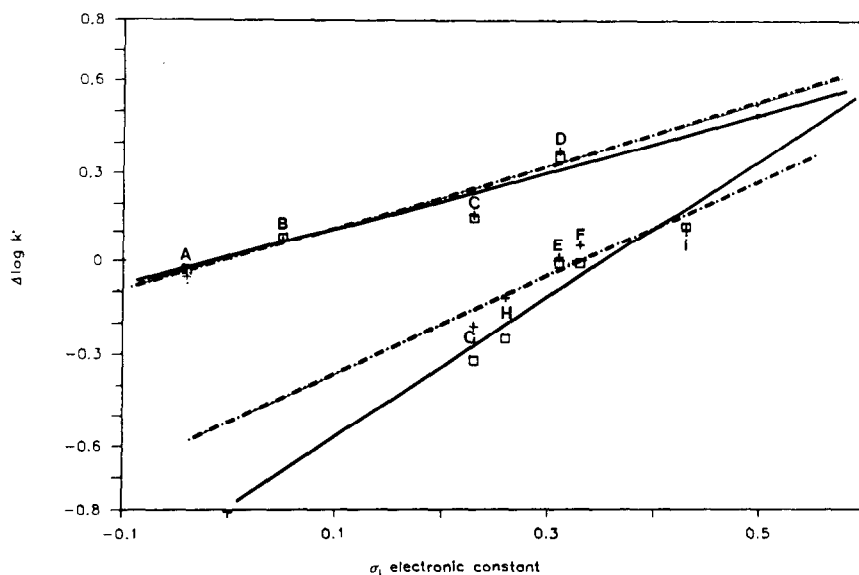
2-propanol > acetonitrile > THF $\approx$ dioxane > ethyl acetate

Fig. 3. Relationships between substituent contributions (Δlog $k'$ with 2-propanol) and the electronic constant $\sigma_i$. □, Silica; +, amino.

This sequence closely parallels the eluotropic series, except for the unusually low strength of ethyl acetate. Table VII reports the correlations between the intercept values with the different solvents and ethyl acetate as a reference. The slope being quite close to unity in eqns. 13 and 14 may be interpreted by these solvents (THF, dioxane

TABLE VI

INTERCEPT, $c$, AND SLOPE, $n$, OF EQN. 1 ON THE TLC SILICA PHASE WITH ACETONITRILE, DIOXANE AND THF AS STRONG SOLVENTS

|    | Acetonitrile | | Dioxane | | THF | |
|----|------|------|------|------|------|------|
|    | c | n | c | n | c | n |
| 1  | 0.540 | 1.400 | 0.176 | 1.603 | 0.216 | 3.153 |
| 2  | 0.558 | 1.343 | 0.151 | 1.798 | 0.171 | 3.050 |
| 3  | 1.14[a] | — | 0.381 | 2.019 | 0.412 | 3.608 |
| 4  | 1.34[a] | — | 0.663 | 3.418 | 0.664 | 4.754 |
| 5  | 1.20[a] | — | 0.479 | 3.364 | 0.475 | 4.733 |
| 6  | −0.259 | 0.743 | −0.091 | 1.919 | 0.170 | 3.466 |
| 7  | −0.498 | 0.752 | −0.200 | 1.780 | 0.003 | 4.007 |
| 8  | −0.572 | 0.676 | −0.297 | 1.706 | −0.130 | 4.185 |
| 9  | −0.045 | 0.752 | 0.090 | 2.103 | 0.201 | 4.096 |
| 10 | −0.773 | 1.058 | −0.339 | 2.100 | −0.285 | 4.708 |
| 11 | −0.797 | 1.233 | −0.282 | 2.026 | −0.271 | 3.975 |
| 12 | −0.409 | 2.238 | −0.263 | 2.567 | −0.028 | 5.049 |
| 13 | 1.03[a] | — | 0.476 | 2.189 | 0.476 | 3.107 |
| 14 | 1.34[a] | — | 0.701 | 3.530 | 0.674 | 4.591 |
| 15 | −0.570 | 1.667 | −0.248 | 2.277 | −0.244 | 4.363 |

[a] Only measurements with 100% acetonitrile were possible.

TABLE VII

CORRELATIONS BETWEEN INTERCEPT VALUES, $c$, OBTAINED IN TLC ON SILICA FOR DIFFERENT SOLVENTS

| | | | |
|---|---|---|---|
| $c$(THF) = $-0.451$ ($\pm 0.097$) $+$ 0.734 ($\pm 0.083$) $c$(ethyl acetate) | | TLC Si | (13) |
| $n = 15$  $r = 0.981$  $s = 0.045$  $F = 78.21$ | | | |
| $c$(dioxane) = $-0.674$ ($\pm 0.055$) $+$ 0.887 ($\pm 0.058$) $c$(ethyl acetate) | | TLC Si | (14) |
| $n = 15$  $r = 0.973$  $s = 0.088$  $F = 234.41$ | | | |
| $c$(acetonitrile) = $-1.467$ ($\pm 0.165$) $+$ 1.944 ($\pm 0.174$) $c$(ethyl acetate) | | TLC Si | (15) |
| $n = 15$  $r = 0.952$  $s = 0.264$  $F = 125.35$ | | | |

and ethyl acetate) exerting very similar effects on $\beta$CC retention. On the other hand, the slope value for acetonitrile and for 2-propanol (1.9 and 0.6, respectively) may suggest that these solvents exert some specific solvent–solute and/or solvent–stationary phase interactions compared to ethyl acetate.

When the correlations between retention values for different solvent pairs are calculated, the smallest values of the correlation coefficient, $r$, must be associated with the maximum differences in solvent selectivity. Snyder *et al.*[25] considered this effect to be a result of interactions produced by solvent and solute localization, *e.g.*, direct interaction of a polar solute molecule with a corresponding adsorption site on the adsorbent surface. The results of these correlations are summarized in Table VIII. THF, dioxane and ethyl acetate show good correlation with each other, implying an absence of solvent-specific localization for these mobile phases. According to the Snyder classification of solvent properties[13], polarity index and selectivity parameters —indicating the relative importance of hydrogen bonding *vs.* dipole interaction— are all quite similar for these three solvents. The pairs acetonitrile–ethyl acetate and acetonitrile–THF exhibit lower $r$ values suggesting specific solvent–solute–stationary phase interactions most likely due to the high polarity of acetonitrile (dipole moment 4.0[26]). Snyder *et al.*[25] calculated a much higher solvent selectivity parameter, related to the polarity or adsorption energy of the solvent molecule, for acetonitrile than for ethyl acetate and THF. However, this hypothesis contrasts with the high $r$ value calculated for the correlation between acetonitrile and dioxane. Strong solute–solvent localization may also explain the low slope, $n$, values (Table VI) calculated for acetonitrile. Fig. 4 shows that the selectivity of acetonitrile results in a particularly strong silica retention of those $\beta$CC molecules which do not contain an ester group (compounds 1–3, 5 and

TABLE VIII

CORRELATION COEFFICIENTS, $r$, BETWEEN INTERCEPT VALUES OBTAINED FOR DIFFERENT SOLVENT PAIRS

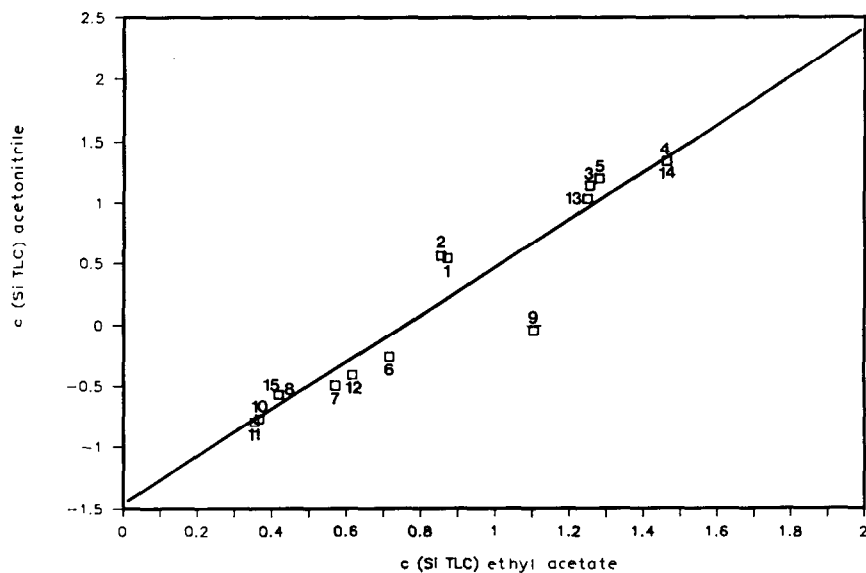| | THF | Dioxane | Ethyl acetate | Acetonitrile |
|---|---|---|---|---|
| THF | 1 | 0.970 | 0.979 | 0.954 |
| Dioxane | 0.970 | 1 | 0.978 | 0.980 |
| Ethyl acetate | 0.979 | 0.978 | 1 | 0.952 |
| Acetonitrile | 0.954 | 0.980 | 0.952 | 1 |

Fig. 4. Relationship between retention (as intercept $c$ values) for acetonitrile and ethyl acetate (eqn. 15, Table VII).

13). The quite good correlation between dioxane and THF confirms that for both these solvents the competition for silanol between the solute and modifier molecule is the predominant adsorption mechanism[14]. However, some specific selectivity of dioxane *vs.* THF is seen in the plot in Fig. 5. The points corresponding to solutes with only one
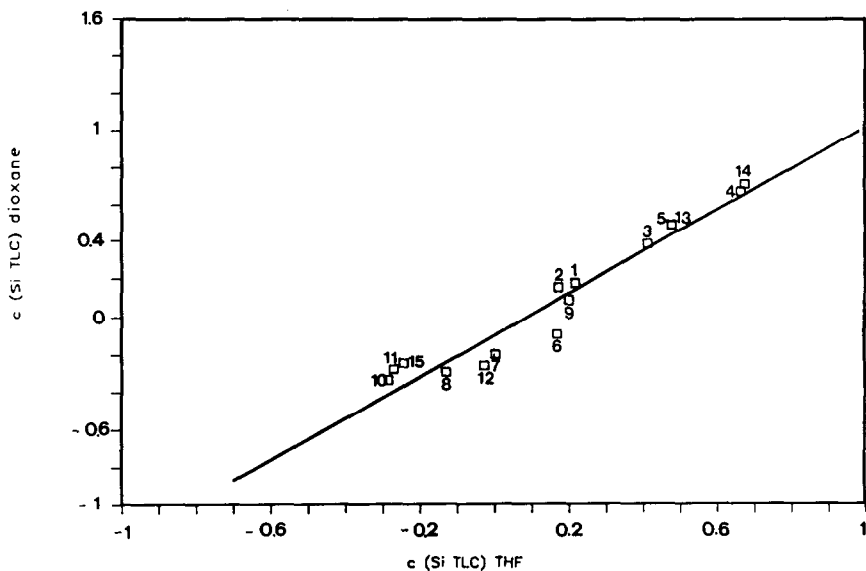


Fig. 5. Relationship between retention (as intercept values) for dioxane and tetrahydrofuran (see Table VIII).

carbonyl group (compounds 6–8 and 12) are shifted down in relation to the line, thus indicating a relatively weaker adsorption in the dioxane system. This effect may be produced by the more complex adsorption mechanism, e.g., coadsorption which takes place when the solute and exposed ether oxygens of the dioxane molecules interact, the latter being adsorbed as a monolayer on the silica surface[27,28].

The substituent contributions to retention were calculated for TLC systems with all the solvents studied and related to $\sigma_i$ values. In general, the TLC data are more scattered than HPLC ones and therefore the quality of the $\Delta c$ vs. $\sigma_i$ relationship is lower. Two approximately parallel straight lines were found for all solvents. Moreover, mean slope value (1.53 $\pm$ 0.49) is in accord with the HPLC value (1.49 $\pm$ 0.59). This result further confirms that the interactions (particularly specific electronic effects) controlling the retention mechanism are all quite similar in both TLC and HPLC systems.

CONCLUSIONS

The Snyder–Soczewinski approach seems a suitable tool for interpreting the retention mechanism of $\beta$CCs in NP chromatographic systems. Since retention is related to hydrogen-bonding ability, it is possible to identify the active sites in the $\beta$CC molecule which determine their adsorption on the silica and amino surfaces. The comparison between TLC and HPLC data suggests the possible use of TLC as a pilot technique replacing the more precise but time-consuming HPLC. The selectivity effects shown by different modifier and stationary phases evidence that specific intramolecular interactions play a significant rôle in determining retention. Complex equilibria, such as competitive solvation, displacement of the solvent molecule and coadsorption of the solute molecule, simultaneously affect solute retention.

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