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# Evaluation and modulation of selectivity in reversed-phase high-performance liquid chromatography

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## Abstract

Retention factors for 31 solutes of widely different types were determined on five columns of different characteristics using acetonitrile–water and methanol–water as the mobile phases, in a composition range of 20–70% (v/v) of organic modifiers. Calculating selectivity factors,  $\alpha$ , for various types of solute pairs, different types of selectivities were determined and evaluated as hydrophobic or methylene selectivity, chemical or polar selectivity and relative retention of different types of polar solutes. It was demonstrated that chromatographic selectivity is a very complex phenomenon depending on the characteristics of the stationary phase, the type of the organic modifier, the composition of the mobile phase and also on the type and structural characteristics of the compounds to be separated. © 1999 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

There is still some debate about the exact mechanism of RP-HPLC in the literature, but it is generally recognized that the acting stationary phase is a ternary combination of the bonded organic moiety, the sorbed solvent molecules and the residual silanols on the surface of the support [1–3]. The volume and composition of the stationary phase depend on the type and chain length of the bonded moiety, the type of the organic modifier, the composition of the mobile phase and the residual silanol activity of the support [4–6]. All of these parameters will influence differently both the retention and the relative retention of various types of solutes. It is also well recognized that selectivity is the most

important parameter to influence chromatographic separation [1,7,8]. It reflects the difference between two solutes in terms of Gibbs free energy of transfer from the mobile phase to the stationary phase.

$$\alpha = \frac{k_j}{k_i}, \quad \ln \alpha = - \frac{\Delta(\Delta G)}{RT} \quad (1)$$

where  $k_i$  and  $k_j$  are retention factors for solutes  $i$  and  $j$ ,  $\Delta G$  is the Gibbs energy,  $R$  is the gas constant and  $T$  is the absolute temperature.

Generally it is not recognized that selectivity is a very complex phenomenon comprising of different mechanisms. The importance and contribution of the different mechanisms in the retention process depends on the type of the stationary phase (column selectivity), on the type and composition of the mobile phase (mobile phase selectivity) and, to a lesser extent, on the temperature of separation (temperature selectivity).

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Table 1  
Characteristics of the columns

| Column                               | Manufacturer       | Dimensions<br>(mm×mm<br>I.D.) | Ligand<br>type  | Particle<br>size<br>( $\mu\text{m}$ ) | Pore<br>size<br>(nm) | Surface<br>area<br>( $\text{m}^2/\text{g}$ ) | % C  | End<br>capping | Symbol              | Chromato-<br>graphic<br>character |
|--------------------------------------|--------------------|-------------------------------|-----------------|---------------------------------------|----------------------|--|------|----------------|---------------------|-----------------------------------|
| LiChrospher<br>100 RP-18e            | Merck<br>(Germany) | 125×4.0                       | C <sub>18</sub> | 5.0                                   | 10                   | 350  | 21.6 | +              | M-C <sub>18</sub> e | Well<br>balanced –<br>neutral     |
| Purospher<br>RP-18e                  | Merck              | 125×4.0                       | C <sub>18</sub> | 5.0                                   | 12                   | 350  | 18.0 | +              | M-PURe              | Well<br>balanced –<br>neutral     |
| LiChrospher<br>100 RP-8              | Merck              | 125×4.0                       | C <sub>8</sub>  | 5.0                                   | 10                   | 350  | 12.5 | –              | M-C <sub>8</sub>    | Acidic/<br>basic                  |
| SymmetryShield<br>RP-C <sub>18</sub> | Waters<br>(USA)    | 150×3.9                       | C <sub>18</sub> | 5.0                                   | 10                   | 340  | 21.2 | +              | Sym-C <sub>18</sub> | Basic<br>(carbamate)              |
| Symmetry-Shield<br>RP-C <sub>8</sub> | Waters             | 150×3.9                       | C <sub>8</sub>  | 5.0                                   | 10                   | 340  | 15.0 | +              | Sym-C <sub>8</sub>  | Basic<br>(carbamate)              |

Table 2  
Log *k* values in acetonitrile–water on M-C<sub>18</sub>e column

| Compound<br>Name            | Symbol | Log <i>k</i>           |        |        |        |        |        |
|-----------------------------|--------|------------------------|--------|--------|--------|--------|--------|
|                             |        | Acetonitrile (% , v/v) |        |        |        |        |        |
|                             |        | 20                     | 30     | 40     | 50     | 60     | 70     |
| Aniline                     | A      | 0.701                  | 0.542  | 0.355  | 0.210  | 0.101  | –0.019 |
| Methylbenzoate              | MBO    | 1.520                  | 1.209  | 0.878  | 0.638  | 0.424  | 0.241  |
| Toluene                     | T      | 2.030                  | 1.635  | 1.261  | 0.954  | 0.701  | 0.473  |
| Ethylbenzene                | EB     | 2.440                  | 1.954  | 1.510  | 1.147  | 0.859  | 0.602  |
| <i>p</i> -Cresol            | PCR    | 1.121                  | 0.830  | 0.550  | 0.340  | 0.174  | 0.024  |
| 2,6-Dimethylphenol          | DP26   | 1.483                  | 1.160  | 0.825  | 0.568  | 0.357  | 0.172  |
| Ethylbenzoate               | EBO    | 1.920                  | 1.533  | 1.138  | 0.830  | 0.588  | 0.375  |
| Chlorobenzene               | CB     | 2.200                  | 1.696  | 1.271  | 0.954  | 0.696  | 0.469  |
| Bromobenzene                | BRB    | 2.350                  | 1.792  | 1.346  | 1.015  | 0.749  | 0.513  |
| Caffeine                    | CAF    | 0.097                  | –0.019 | –0.140 | –0.271 | –0.283 | –0.301 |
| <i>o</i> -Toluidine         | OT     | 1.000                  | 0.773  | 0.546  | 0.357  | 0.210  | 0.069  |
| Benzyl cyanide              | BC     | 1.303                  | 0.999  | 0.708  | 0.464  | 0.268  | 0.119  |
| $\alpha$ -Naphthylamine     | NA     | 1.543                  | 1.212  | 0.829  | 0.563  | 0.354  | 0.164  |
| <i>o</i> -Nitrotoluene      | ONT    | 1.790                  | 1.463  | 1.054  | 0.750  | 0.506  | 0.285  |
| Hydroquinone                | HQ     | 0.046                  | 0.031  | –0.100 | –0.167 | –0.226 | –0.289 |
| Phenol                      | P      | 0.796                  | 0.591  | 0.377  | 0.206  | 0.070  | –0.053 |
| <i>o</i> -Cresol            | OCR    | 1.168                  | 0.917  | 0.602  | 0.384  | 0.210  | 0.053  |
| 3,5-Dimethylphenol          | DP35   | 1.350                  | 1.050  | 0.744  | 0.481  | 0.263  | 0.084  |
| $\beta$ -Naphthol           | BNA    | 1.643                  | 1.264  | 0.807  | 0.513  | 0.295  | 0.109  |
| Benzyl alcohol              | BA     | 0.628                  | 0.443  | 0.239  | 0.106  | 0.000  | –0.097 |
| Acetophenone                | AP     | 1.161                  | 0.896  | 0.628  | 0.438  | 0.288  | 0.094  |
| Dimethyl phtalate           | PDM    | 1.347                  | 1.010  | 0.678  | 0.438  | 0.288  | 0.094  |
| <i>p</i> -Ethylphenol       | PEP    | 1.505                  | 1.161  | 0.770  | 0.513  | 0.316  | 0.119  |
| $\alpha$ -Naphthol          | ANA    | 1.746                  | 1.302  | 0.893  | 0.589  | 0.357  | 0.146  |
| Pyridine                    | PYR    | 0.388                  | 0.264  | 0.131  | 0.042  | –0.013 | –0.074 |
| Anisole                     | AN     | 1.558                  | 1.201  | 0.949  | 0.687  | 0.473  | 0.282  |
| <i>N,N</i> -Dimethylaniline | DMA    | 1.760                  | 1.400  | 1.131  | 0.850  | 0.621  | 0.410  |
| Methylparaben               | MP     | 0.956                  | 0.671  | 0.363  | 0.178  | 0.036  | –0.097 |
| Ethylparaben                | EP     | 1.325                  | 0.951  | 0.576  | 0.331  | 0.148  | –0.013 |
| Propylparaben               | PP     | 1.741                  | 1.279  | 0.819  | 0.513  | 0.291  | 0.099  |
| Butylparaben                | BP     | 2.200                  | 1.626  | 1.078  | 0.713  | 0.447  | 0.219  |

Column selectivity can be classified according to the type of molecular interactions between the stationary phase and the solutes [1,8]. Hydrophobic selectivity depends on the hydrophobic interaction between the stationary phase and the compounds investigated. Hydrophobic selectivity is generally defined as the relative retention of adjacent members of homologous series differing only in one CH<sub>2</sub> group. For this reason it is often referred as methylene selectivity. It has been reported that hydrophobic or methylene selectivity increases with the chain length of ligand [9–11], bonded phase carbon loading [12,13] and surface coverage [14,15]. It has been established, however, that in addition to the charac-

teristics of the stationary phase, the type of the organic modifier and the composition of the mobile phase will also influence methylene selectivity [16,17].

Polar or chemical selectivity arises from strong polar interactions, such as hydrogen bonding, dipole and ionic interactions, complexation between the solute molecules and specific active sites, such as silanol groups or trace metal contaminants on the silica surface [1,8,18]. These effects are relatively unimportant for nonpolar solutes, but can furnish unique opportunities to separate polar solutes with similar structure and physical characteristics. In addition to the polarity of the stationary phase polar

Table 3  
Log *k* values in methanol–water on M-C<sub>18</sub>e column

| Compound symbol | Log <i>k</i>     |       |        |        |        |        |
|-----------------|------------------|-------|--------|--------|--------|--------|
|                 | Methanol (% v/v) |       |        |        |        |        |
|                 | 20               | 30    | 40     | 50     | 60     | 70     |
| A               | 0.809            | 0.612 | 0.467  | 0.288  | 0.147  | -0.031 |
| MBO             | 1.998            | 1.641 | 1.338  | 0.993  | 0.686  | 0.383  |
| T               | 2.610            | 2.120 | 1.709  | 1.388  | 1.067  | 0.724  |
| EB              | 2.980            | 2.460 | 2.060  | 1.684  | 1.303  | 0.908  |
| PCR             | 1.520            | 1.315 | 0.895  | 0.667  | 0.414  | 0.156  |
| DP26            | 1.847            | 1.523 | 1.200  | 0.934  | 0.639  | 0.341  |
| EBO             | 2.450            | 2.030 | 1.650  | 1.281  | 0.914  | 0.558  |
| CB              | 2.660            | 2.120 | 1.742  | 1.405  | 1.057  | 0.701  |
| BRB             | 2.830            | 2.300 | 1.852  | 1.499  | 1.135  | 0.766  |
| CAF             | 0.933            | 0.530 | 0.251  | 0.081  | -0.136 | -0.255 |
| OT              | 1.208            | 0.941 | 0.742  | 0.518  | 0.320  | 0.097  |
| BC              | 1.472            | 1.136 | 0.862  | 0.667  | 0.443  | 0.222  |
| NA              | 1.879            | 1.505 | 1.183  | 0.848  | 0.543  | 0.250  |
| ONT             | 2.017            | 1.698 | 1.409  | 1.085  | 0.768  | 0.450  |
| HQ              | 0.212            | 0.022 | -0.019 | -0.179 | -0.224 | -0.352 |
| P               | 1.034            | 0.797 | 0.621  | 0.415  | 0.227  | 0.018  |
| OCR             | 1.553            | 1.220 | 0.968  | 0.703  | 0.450  | 0.192  |
| DP35            | 1.937            | 1.581 | 1.271  | 0.937  | 0.621  | 0.298  |
| BNA             | 2.100            | 1.748 | 1.385  | 1.008  | 0.657  | 0.327  |
| BA              | 1.046            | 0.789 | 0.607  | 0.400  | 0.219  | 0.012  |
| AP              | 1.546            | 1.203 | 0.934  | 0.666  | 0.412  | 0.168  |
| PDM             | 1.890            | 1.402 | 1.022  | 0.666  | 0.375  | 0.106  |
| PEP             | 1.650            | 1.493 | 1.292  | 0.956  | 0.635  | 0.298  |
| ANA             | 1.890            | 1.746 | 1.460  | 1.084  | 0.727  | 0.386  |
| PYR             | 0.813            | 0.564 | 0.407  | 0.239  | 0.108  | -0.051 |
| AN              | 1.794            | 1.544 | 1.296  | 1.019  | 0.743  | 0.448  |
| DMA             | 1.960            | 1.736 | 1.475  | 1.186  | 0.897  | 0.594  |
| MP              | 1.536            | 1.167 | 0.871  | 0.546  | 0.294  | 0.046  |
| EP              | 2.006            | 1.576 | 1.213  | 0.816  | 0.490  | 0.188  |
| PP              | 2.580            | 2.027 | 1.600  | 1.133  | 0.736  | 0.371  |
| BP              | 3.080            | 2.540 | 1.980  | 1.469  | 1.004  | 0.572  |

selectivity depends also on the type of the organic modifier and the mobile phase composition.

There is a third type of column selectivity, steric or shape selectivity, which may be important in the separation of polycondensed multiring systems according to their molecular shape [19,20]. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), steroids and carotenoids can often be separated on the basis of molecular shape [21,22].

Mobile phase can influence selectivity in two ways. Solvent strength selectivity (SSS) arises by variation of the organic modifier concentration using the same modifier. Modifier selectivity comes about from using different organic modifiers [23–25].

Recently it has been shown, that in addition to the stationary phase and the mobile phase, temperature can also have considerable influence on selectivity [26,27]. In general, selectivity increases with decreasing temperature, but depends heavily on the type of the molecules investigated.

In the literature only three types of selectivities, hydrophobic or methylene selectivity, polar or chemical selectivity and steric or shape selectivity, are discussed in some detail. There are very few studies and no general conclusions on the separation of different types of polar solutes. Secondary interactions (hydrogen bonding, dipole or ionic forces) with specific binding sites of the packing material contrib-

Table 4  
Log  $k$  values in acetonitrile–water on M-PURc column

| Compound symbol | Log $k$              |        |        |        |        |        |
|-----------------|----------------------|--------|--------|--------|--------|--------|
|                 | Acetonitrile (% v/v) |        |        |        |        |        |
|                 | 20                   | 30     | 40     | 50     | 60     | 70     |
| A               | 0.740                | 0.557  | 0.367  | 0.269  | 0.150  | 0.020  |
| MBO             | 1.610                | 1.254  | 0.915  | 0.685  | 0.482  | 0.294  |
| T               | 2.095                | 1.673  | 1.291  | 1.003  | 0.753  | 0.522  |
| EB              | 2.525                | 1.994  | 1.540  | 1.200  | 0.912  | 0.652  |
| PCR             | 1.062                | 0.803  | 0.554  | 0.377  | 0.209  | 0.050  |
| DP26            | 1.522                | 1.133  | 0.836  | 0.604  | 0.399  | 0.204  |
| EBO             | 2.050                | 1.578  | 1.172  | 0.886  | 0.646  | 0.425  |
| CB              | 2.181                | 1.710  | 1.301  | 1.005  | 0.750  | 0.515  |
| BRB             | 2.302                | 1.806  | 1.376  | 1.067  | 0.804  | 0.562  |
| CAF             | 0.379                | -0.070 | -0.102 | -0.208 | -0.231 | -0.269 |
| OT              | 1.036                | 0.796  | 0.563  | 0.407  | 0.258  | 0.106  |
| BC              | 1.338                | 1.018  | 0.722  | 0.506  | 0.315  | 0.156  |
| NA              | 1.587                | 1.215  | 0.849  | 0.605  | 0.399  | 0.204  |
| ONT             | 1.846                | 1.476  | 1.079  | 0.797  | 0.557  | 0.333  |
| HQ              | 0.064                | 0.012  | -0.110 | -0.127 | -0.187 | -0.269 |
| P               | 0.820                | 0.597  | 0.375  | 0.246  | 0.114  | -0.028 |
| OCR             | 1.193                | 0.907  | 0.607  | 0.421  | 0.246  | 0.085  |
| DP35            | 1.340                | 1.023  | 0.747  | 0.506  | 0.291  | 0.111  |
| BNA             | 1.668                | 1.304  | 0.810  | 0.549  | 0.331  | 0.132  |
| BA              | 0.648                | 0.450  | 0.249  | 0.155  | 0.046  | -0.065 |
| AP              | 1.215                | 0.933  | 0.655  | 0.482  | 0.287  | 0.127  |
| PDM             | 1.382                | 1.028  | 0.694  | 0.482  | 0.304  | 0.127  |
| PEP             | 1.528                | 1.147  | 0.778  | 0.624  | 0.321  | 0.146  |
| ANA             | 1.774                | 1.304  | 0.905  | 0.624  | 0.397  | 0.169  |
| PYR             | 0.444                | 0.301  | 0.156  | 0.114  | 0.052  | -0.007 |
| AN              | 1.655                | 1.356  | 0.980  | 0.745  | 0.529  | 0.330  |
| DMA             | 1.905                | 1.570  | 1.164  | 0.908  | 0.679  | 0.464  |
| MP              | 0.996                | 0.664  | 0.390  | 0.226  | 0.076  | -0.065 |
| EP              | 1.361                | 0.940  | 0.599  | 0.374  | 0.192  | 0.020  |
| PP              | 1.771                | 1.267  | 0.823  | 0.553  | 0.331  | 0.127  |
| BP              | 2.274                | 1.614  | 1.081  | 0.746  | 0.482  | 0.248  |

ute more severely to the retention of polar solutes, resulting in unexpectedly large gaps between peaks or even reversal of the elution order. In a recent publication [28], we defined polar or chemical selectivity as the relative retention of polar test solutes compared to a nonpolar solute toluene. We have used this approach to characterize and compare secondary polar interactions in the evaluation of different commercially available RP columns [28].

However, the relative retention of different types of polar solutes, as acidic, basic, acidic to basic and basic to acidic will vary differently depending on the polar character of the stationary phase, the type of the organic modifier and the composition of the

mobile phase. These questions have not been discussed yet in the literature. The polar or chemical selectivity defined above characterizes the sum of the secondary interactions between the stationary phase and the solutes. But depending on the structure and functionality of the solutes as well as on the characteristics of the phase system, different types of polar interactions may dominate the retention process.

In this study we wish to illustrate the complexity of the simple word ‘selectivity’ and how the type and characteristics of the column, the type of the organic modifier and the composition of the mobile phase can influence the different types of selectivities. Furthermore, we want to demonstrate that in addition

Table 5  
Log *k* values in methanol–water on M-PURE column

| Compound symbol | Log <i>k</i>     |       |        |        |        |        |
|-----------------|------------------|-------|--------|--------|--------|--------|
|                 | Methanol (% v/v) |       |        |        |        |        |
|                 | 20               | 30    | 40     | 50     | 60     | 70     |
| A               | 0.964            | 0.661 | 0.446  | 0.222  | 0.068  | 0.069  |
| MBO             | 2.240            | 1.750 | 1.390  | 1.081  | 0.755  | 0.537  |
| T               | 2.850            | 2.270 | 1.815  | 1.438  | 1.111  | 0.838  |
| EB              | 3.350            | 2.710 | 2.197  | 1.747  | 1.363  | 1.024  |
| PCR             | 1.601            | 1.270 | 0.930  | 0.634  | 0.378  | 0.227  |
| DP26            | 1.989            | 1.578 | 1.251  | 0.926  | 0.629  | 0.420  |
| EBO             | 2.740            | 2.191 | 1.757  | 1.383  | 1.002  | 0.699  |
| CB              | 2.822            | 2.312 | 1.873  | 1.460  | 1.110  | 0.810  |
| BRB             | 2.986            | 2.483 | 1.989  | 1.556  | 1.192  | 0.877  |
| CAF             | 1.017            | 0.569 | 0.218  | 0.047  | -0.116 | -0.212 |
| OT              | 1.372            | 1.034 | 0.738  | 0.520  | 0.298  | 0.180  |
| BC              | 1.665            | 1.220 | 0.888  | 0.608  | 0.320  | 0.219  |
| NA              | 2.060            | 1.620 | 1.207  | 0.893  | 0.562  | 0.340  |
| ONT             | 2.248            | 1.856 | 1.465  | 1.158  | 0.824  | 0.573  |
| HQ              | 0.323            | 0.045 | -0.193 | -0.336 | -0.368 | -0.301 |
| P               | 1.149            | 0.848 | 0.575  | 0.353  | 0.167  | 0.069  |
| OCR             | 1.619            | 1.258 | 0.940  | 0.674  | 0.421  | 0.245  |
| DP35            | 1.985            | 1.570 | 1.205  | 0.890  | 0.609  | 0.359  |
| BNA             | 2.330            | 1.822 | 1.372  | 1.008  | 0.655  | 0.390  |
| BA              | 1.111            | 0.833 | 0.554  | 0.343  | 0.155  | 0.058  |
| AP              | 1.710            | 1.353 | 0.992  | 0.707  | 0.420  | 0.270  |
| PDM             | 2.012            | 1.517 | 1.056  | 0.707  | 0.420  | 0.270  |
| PEP             | 2.158            | 1.652 | 1.268  | 0.945  | 0.621  | 0.362  |
| ANA             | 2.465            | 1.933 | 1.446  | 1.087  | 0.726  | 0.449  |
| PYR             | 1.014            | 0.723 | 0.448  | 0.251  | 0.105  | 0.063  |
| AN              | 1.984            | 1.676 | 1.364  | 1.075  | 0.790  | 0.561  |
| DMA             | 2.295            | 1.877 | 1.554  | 1.256  | 0.959  | 0.706  |
| MP              | 1.618            | 1.174 | 0.831  | 0.521  | 0.244  | 0.104  |
| EP              | 2.087            | 1.590 | 1.191  | 0.821  | 0.473  | 0.248  |
| PP              | 2.610            | 2.119 | 1.582  | 1.150  | 0.740  | 0.436  |
| BP              | 3.100            | 2.560 | 1.999  | 1.499  | 1.025  | 0.641  |

to the phase system and operating conditions the selectivity of separation depends also on the type and structural characteristics of the compounds to be separated.

The results of this study has furnished an insight and some general guidelines for the selection of column type and mobile phase components in order to modulate selectivity for the separation of different types of compounds.

In a subsequent publication we shall illustrate how the selectivity can be explained and estimated by using a linear free energy relationship (LFER) or solvation equation derived by Abraham and co-workers [29–31].

## 2. Experimental

Retention data were measured on a Merck–Hitachi Lichrograph consisting of a L-6200 programmable pump, a Rheodyne 7215 injector with a 10  $\mu$ l loop and a L-4250 UV–Vis detector operating at 220 nm. Data acquisition was performed by the D-7000 HPLC System Manager Software.

The RP-HPLC columns investigated in this study are listed in Table 1, together with their main characteristics as provided by the manufacturers. The first two columns are well balanced quasi neutral  $C_{18}$  columns [32]. The third one is a nonendcapped  $C_8$  column showing both acidic and basic character. The

Table 6  
Log  $k$  values in acetonitrile–water on M- $C_8$  column

| Compound symbol | Log $k$                |       |       |        |        |        |
|-----------------|------------------------|-------|-------|--------|--------|--------|
|                 | Acetonitrile (% , v/v) |       |       |        |        |        |
|                 | 20                     | 30    | 40    | 50     | 60     | 70     |
| A               | 0.679                  | 0.586 | 0.429 | 0.234  | 0.129  | 0.034  |
| MBO             | 1.408                  | 1.130 | 0.800 | 0.536  | 0.358  | 0.201  |
| T               | 1.800                  | 1.419 | 1.062 | 0.751  | 0.525  | 0.330  |
| EB              | 2.180                  | 1.694 | 1.268 | 0.902  | 0.638  | 0.418  |
| PCR             | 1.042                  | 0.759 | 0.539 | 0.321  | 0.180  | 0.045  |
| DP26            | 1.347                  | 1.036 | 0.753 | 0.493  | 0.313  | 0.154  |
| EBO             | 1.770                  | 1.402 | 1.006 | 0.694  | 0.479  | 0.292  |
| CB              | 1.780                  | 1.469 | 1.081 | 0.760  | 0.532  | 0.333  |
| BRB             | 1.880                  | 1.545 | 1.137 | 0.802  | 0.566  | 0.354  |
| CAF             | 0.174                  | 0.159 | 0.006 | -0.123 | -0.158 | -0.156 |
| OT              | 0.923                  | 0.770 | 0.546 | 0.341  | 0.211  | 0.086  |
| BC              | 1.218                  | 0.983 | 0.690 | 0.440  | 0.263  | 0.119  |
| NA              | 1.412                  | 1.118 | 0.775 | 0.500  | 0.313  | 0.154  |
| ONT             | 1.631                  | 1.337 | 0.959 | 0.651  | 0.430  | 0.244  |
| HQ              | 0.131                  | 0.120 | 0.012 | -0.085 | -0.125 | -0.182 |
| P               | 0.754                  | 0.622 | 0.398 | 0.227  | 0.111  | 0.000  |
| OCR             | 1.074                  | 0.870 | 0.581 | 0.359  | 0.211  | 0.066  |
| DP35            | 1.150                  | 0.963 | 0.694 | 0.431  | 0.246  | 0.076  |
| BNA             | 1.522                  | 1.145 | 0.759 | 0.465  | 0.276  | 0.086  |
| BA              | 0.612                  | 0.503 | 0.295 | 0.137  | 0.051  | -0.031 |
| AP              | 1.095                  | 0.881 | 0.605 | 0.397  | 0.250  | 0.114  |
| PDM             | 1.255                  | 0.978 | 0.661 | 0.411  | 0.250  | 0.114  |
| PEP             | 1.382                  | 1.084 | 0.721 | 0.436  | 0.316  | 0.114  |
| ANA             | 1.612                  | 1.228 | 0.831 | 0.524  | 0.316  | 0.114  |
| PYR             | 0.585                  | 0.463 | 0.372 | 0.212  | 0.100  | 0.020  |
| AN              | 1.417                  | 1.156 | 0.838 | 0.576  | 0.383  | 0.216  |
| DMA             | 1.627                  | 1.332 | 0.992 | 0.693  | 0.487  | 0.304  |
| MP              | 0.974                  | 0.699 | 0.408 | 0.216  | 0.092  | -0.012 |
| EP              | 1.306                  | 0.936 | 0.578 | 0.338  | 0.176  | 0.050  |
| PP              | 1.674                  | 1.212 | 0.774 | 0.477  | 0.279  | 0.114  |
| BP              | 2.080                  | 1.495 | 0.982 | 0.624  | 0.383  | 0.190  |

last two columns have basic character because of the carbamate groups embedded in the alkyl chain [32,33].

The 31 test solutes investigated were of analytical grade and were purchased from different manufacturers. They were selected to cover a wide range of chemical properties.

On all five columns retention time of test solutes were measured in duplicate or triplicate using acetonitrile–water and methanol–water mobile phases, respectively, in a composition range of 20–70% (v/v) of organic modifiers. Log *k* values obtained with the two organic modifiers investigated are given

in Tables 2–11. Acetonitrile, methanol and water were of chromatographic grade obtained from Merck (Darmstadt, Germany). For calculation of retention factors, column dead time was determined by injecting 0.05 *M* sodium nitrate solution. Reproducibility of sequential measurements were excellent, with an average deviation of 1% in *k* (retention factor). In order to evaluate and compare the different packing materials without modification of surface properties, water was used without any additive for pH and ionic strength adjustment [34]. Sample mixtures were prepared using the actual mobile phase composition to approximately 2 mg/ml concentration,

Table 7  
Log *k* values in methanol–water on M-C<sub>8</sub> column

| Compound symbol | Log <i>k</i>     |       |        |        |        |        |
|-----------------|------------------|-------|--------|--------|--------|--------|
|                 | Methanol (% v/v) |       |        |        |        |        |
|                 | 20               | 30    | 40     | 50     | 60     | 70     |
| A               | 0.778            | 0.570 | 0.337  | 0.267  | 0.182  | 0.045  |
| MBO             | 1.710            | 1.450 | 1.136  | 0.878  | 0.590  | 0.333  |
| T               | 2.070            | 1.780 | 1.390  | 1.080  | 0.802  | 0.497  |
| EB              | 2.510            | 2.150 | 1.720  | 1.350  | 0.995  | 0.636  |
| PCR             | 1.346            | 1.050 | 0.765  | 0.573  | 0.350  | 0.158  |
| DP26            | 1.737            | 1.414 | 1.103  | 0.778  | 0.509  | 0.277  |
| EBO             | 2.140            | 1.810 | 1.457  | 1.133  | 0.767  | 0.461  |
| CB              | 2.210            | 1.820 | 1.363  | 1.111  | 0.782  | 0.493  |
| BRB             | 2.360            | 1.930 | 1.457  | 1.186  | 0.842  | 0.536  |
| CAF             | 0.700            | 0.390 | 0.237  | 0.126  | -0.025 | -0.123 |
| OT              | 1.116            | 0.939 | 0.581  | 0.455  | 0.289  | 0.128  |
| BC              | 1.399            | 1.145 | 0.846  | 0.603  | 0.322  | 0.158  |
| NA              | 1.727            | 1.429 | 0.966  | 0.724  | 0.450  | 0.219  |
| ONT             | 1.875            | 1.612 | 1.170  | 0.931  | 0.630  | 0.370  |
| HQ              | 0.238            | 0.203 | -0.125 | -0.077 | -0.111 | -0.164 |
| P               | 0.929            | 0.798 | 0.459  | 0.364  | 0.209  | 0.066  |
| OCR             | 1.450            | 1.143 | 0.830  | 0.593  | 0.371  | 0.178  |
| DP35            | 1.510            | 1.325 | 0.992  | 0.724  | 0.509  | 0.254  |
| BNA             | 1.962            | 1.643 | 1.142  | 0.857  | 0.531  | 0.270  |
| BA              | 0.943            | 0.795 | 0.448  | 0.356  | 0.206  | 0.066  |
| AP              | 1.460            | 1.220 | 0.792  | 0.605  | 0.374  | 0.182  |
| PDM             | 1.800            | 1.438 | 0.908  | 0.643  | 0.374  | 0.158  |
| PEP             | 1.779            | 1.509 | 1.059  | 0.816  | 0.518  | 0.254  |
| ANA             | 2.140            | 1.698 | 1.201  | 0.915  | 0.580  | 0.307  |
| PYR             | 1.011            | 0.800 | 0.495  | 0.391  | 0.256  | 0.128  |
| AN              | 1.598            | 1.392 | 1.000  | 0.808  | 0.555  | 0.330  |
| DMA             | 1.848            | 1.543 | 1.247  | 0.961  | 0.685  | 0.433  |
| MP              | 1.450            | 1.260 | 0.890  | 0.541  | 0.304  | 0.114  |
| EP              | 1.940            | 1.550 | 1.180  | 0.765  | 0.454  | 0.209  |
| PP              | 2.380            | 1.960 | 1.387  | 1.025  | 0.639  | 0.333  |
| BP              | 2.800            | 2.310 | 1.737  | 1.294  | 0.846  | 0.475  |

Table 8  
Log *k* values in acetonitrile–water on Sym-C<sub>18</sub> column

| Compound symbol | Log <i>k</i>         |        |        |        |        |        |
|-----------------|----------------------|--------|--------|--------|--------|--------|
|                 | Acetonitrile (% v/v) |        |        |        |        |        |
|                 | 20                   | 30     | 40     | 50     | 60     | 70     |
| A               | 0.573                | 0.459  | 0.290  | 0.159  | 0.033  | -0.075 |
| MBO             | 1.520                | 1.107  | 0.825  | 0.535  | 0.337  | 0.168  |
| T               | 2.001                | 1.501  | 1.140  | 0.833  | 0.585  | 0.384  |
| EB              | 2.402                | 1.805  | 1.344  | 1.012  | 0.727  | 0.501  |
| PCR             | 1.036                | 0.820  | 0.580  | 0.352  | 0.184  | 0.043  |
| DP26            | 1.401                | 1.148  | 0.862  | 0.565  | 0.357  | 0.184  |
| EBO             | 1.798                | 1.407  | 1.036  | 0.720  | 0.485  | 0.289  |
| CB              | 1.950                | 1.546  | 1.143  | 0.850  | 0.598  | 0.396  |
| BRB             | 2.130                | 1.643  | 1.206  | 0.912  | 0.652  | 0.446  |
| CAF             | -0.089               | -0.187 | -0.250 | -0.368 | -0.376 | -0.396 |
| OT              | 0.837                | 0.692  | 0.554  | 0.295  | 0.144  | 0.011  |
| BC              | 1.084                | 0.903  | 0.649  | 0.385  | 0.187  | 0.043  |
| NA              | 1.394                | 1.152  | 0.859  | 0.548  | 0.339  | 0.157  |
| ONT             | 1.561                | 1.330  | 1.006  | 0.676  | 0.429  | 0.220  |
| HQ              | 0.062                | -0.044 | 0.060  | -0.116 | -0.268 | -0.313 |
| P               | 0.747                | 0.606  | 0.502  | 0.250  | 0.073  | -0.042 |
| OCR             | 1.110                | 0.908  | 0.630  | 0.429  | 0.226  | 0.083  |
| DP35            | 1.325                | 1.011  | 0.740  | 0.493  | 0.292  | 0.124  |
| BNA             | 1.574                | 1.304  | 0.941  | 0.617  | 0.374  | 0.187  |
| BA              | 0.501                | 0.369  | 0.304  | 0.053  | -0.048 | -0.125 |
| AP              | 0.965                | 0.799  | 0.621  | 0.341  | 0.168  | 0.033  |
| PDM             | 1.086                | 0.872  | 0.676  | 0.394  | 0.168  | 0.011  |
| PEP             | 1.366                | 1.132  | 0.842  | 0.510  | 0.304  | 0.128  |
| ANA             | 1.980                | 1.437  | 1.045  | 0.699  | 0.458  | 0.256  |
| PYR             | 0.169                | 0.054  | 0.010  | -0.060 | -0.157 | -0.200 |
| AN              | 1.341                | 1.157  | 0.881  | 0.665  | 0.319  | 0.206  |
| DMA             | 1.618                | 1.330  | 1.005  | 0.701  | 0.465  | 0.329  |
| MP              | 0.910                | 0.687  | 0.460  | 0.224  | 0.069  | -0.061 |
| EP              | 1.250                | 0.961  | 0.650  | 0.381  | 0.187  | 0.028  |
| PP              | 1.589                | 1.287  | 0.918  | 0.571  | 0.337  | 0.144  |
| BP              | 1.967                | 1.616  | 1.149  | 0.765  | 0.492  | 0.271  |

which corresponded to the 0.008–4015 mg/g stationary phase. It was described in the literature that retention of even basic solutes is independent of the sample size if the linear capacity range of around 0.1 mg sample per g stationary phase is not exceeded [35].

Selectivity factors,  $\alpha$ , for selected pairs of solutes were calculated for all columns in the whole range of mobile phase compositions. In a subsequent publication the database given in Tables 2–11 will be used also to determine the regression coefficients of the LFER equations for each column by multivariate linear regression technique [29–31].

### 3. Results and discussion

#### 3.1. Hydrophobic or methylene selectivity

Hydrophobic or methylene selectivity depends first of all on the hydrophobic strength of the stationary phase, but also on the type of the organic modifier and varies considerably with mobile phase composition. Among the various selectivity terms, hydrophobic or methylene selectivity has been the most extensively studied property of RP phase systems. Notwithstanding, there are several contradictory conclusions in the literature regarding hydrophobic



Table 9  
Log *k* values in methanol–water on Sym-C<sub>18</sub> column

| Compound symbol | Log <i>k</i>     |       |       |        |        |        |
|-----------------|------------------|-------|-------|--------|--------|--------|
|                 | Methanol (% v/v) |       |       |        |        |        |
|                 | 20               | 30    | 40    | 50     | 60     | 70     |
| A               | 0.920            | 0.620 | 0.433 | 0.230  | 0.047  | -0.078 |
| MBO             | 1.940            | 1.502 | 1.180 | 0.860  | 0.540  | 0.287  |
| T               | 2.580            | 2.070 | 1.610 | 1.222  | 0.883  | 0.587  |
| EB              | 3.090            | 2.500 | 1.950 | 1.493  | 1.098  | 0.750  |
| PCR             | 1.486            | 1.220 | 0.960 | 0.612  | 0.395  | 0.171  |
| DP26            | 1.906            | 1.590 | 1.283 | 0.942  | 0.627  | 0.365  |
| EBO             | 2.400            | 1.890 | 1.490 | 1.113  | 0.737  | 0.433  |
| CB              | 2.680            | 2.180 | 1.680 | 1.269  | 0.907  | 0.594  |
| BRB             | 2.810            | 2.290 | 1.780 | 1.365  | 0.988  | 0.662  |
| CAF             | 0.815            | 0.450 | 0.170 | -0.128 | -0.254 | -0.318 |
| OT              | 1.113            | 0.867 | 0.695 | 0.446  | 0.223  | 0.052  |
| BC              | 1.349            | 1.046 | 0.803 | 0.540  | 0.261  | 0.089  |
| NA              | 1.871            | 1.534 | 1.237 | 0.877  | 0.557  | 0.301  |
| ONT             | 1.936            | 1.635 | 1.360 | 1.003  | 0.672  | 0.397  |
| HQ              | 0.373            | 0.244 | 0.077 | -0.103 | -0.254 | -0.366 |
| P               | 1.081            | 0.862 | 0.693 | 0.441  | 0.213  | 0.042  |
| OCR             | 1.536            | 1.264 | 1.038 | 0.656  | 0.453  | 0.226  |
| DP35            | 1.898            | 1.480 | 1.170 | 0.852  | 0.565  | 0.315  |
| BNA             | 2.240            | 1.923 | 1.566 | 1.144  | 0.768  | 0.458  |
| BA              | 0.940            | 0.698 | 0.542 | 0.307  | 0.098  | -0.053 |
| AP              | 1.392            | 1.080 | 0.843 | 0.544  | 0.284  | 0.094  |
| PDM             | 1.661            | 1.221 | 0.880 | 0.514  | 0.216  | 0.005  |
| PEP             | 1.780            | 1.584 | 1.296 | 0.929  | 0.591  | 0.315  |
| ANA             | 2.360            | 2.045 | 1.690 | 1.263  | 0.876  | 0.551  |
| PYR             | 0.588            | 0.352 | 0.200 | 0.054  | -0.098 | -0.190 |
| AN              | 1.682            | 1.421 | 1.210 | 0.893  | 0.602  | 0.360  |
| DMA             | 1.930            | 1.575 | 1.356 | 1.037  | 0.737  | 0.483  |
| MP              | 1.606            | 1.255 | 0.870 | 0.565  | 0.309  | 0.094  |
| EP              | 2.013            | 1.604 | 1.150 | 0.802  | 0.490  | 0.216  |
| PP              | 2.410            | 2.000 | 1.623 | 1.136  | 0.725  | 0.392  |
| BP              | 2.810            | 2.380 | 1.950 | 1.443  | 0.971  | 0.575  |

or methylene selectivity [1,6,25,26]. In a recent publication we have compared and evaluated 15 RP columns of different types and obtained from different manufacturers [28]. It has been established that methylene selectivity strongly depends on the type of stationary phase, i.e. alkyl-chain length, bonding density and pore size. Highly covered, endcapped stationary phases furnished the highest methylene selectivity while C<sub>8</sub> columns had lower selectivities than C<sub>18</sub> columns. Our results have also shown that methylene selectivity is not identical with the hydrophobicity or hydrophobic strength of the stationary phase as it depends on the retention mechanism

involved [28]. In this study we have investigated the effect of mobile phase composition and the type of the organic modifier on methylene selectivity.

In Fig. 1a, the variation of the  $\alpha$  selectivity factor of ethylbenzene (EB) to toluene (T) is shown as a function of the mobile phase composition with acetonitrile modifier for the five columns investigated. In Fig. 1b, the same correlations are being shown obtained with methanol as organic modifier.

It can be seen in both figures that methylene selectivity considerably increases with increasing water content of the mobile phase. The explanation of this phenomenon is that there is a stronger

Table 10  
Log  $k$  values in acetonitrile–water on Sym-C<sub>8</sub> column

| Compound symbol | Log $k$              |        |        |        |        |        |
|-----------------|----------------------|--------|--------|--------|--------|--------|
|                 | Acetonitrile (% v/v) |        |        |        |        |        |
|                 | 20                   | 30     | 40     | 50     | 60     | 70     |
| A               | 0.922                | 0.637  | 0.410  | 0.250  | 0.121  | 0.017  |
| MBO             | 1.460                | 1.140  | 0.825  | 0.567  | 0.363  | 0.196  |
| T               | 1.840                | 1.457  | 1.105  | 0.803  | 0.554  | 0.349  |
| EB              | 2.190                | 1.741  | 1.309  | 0.956  | 0.671  | 0.438  |
| PCR             | 1.273                | 0.917  | 0.599  | 0.421  | 0.248  | 0.100  |
| DP26            | 1.562                | 1.181  | 0.810  | 0.596  | 0.387  | 0.207  |
| EBO             | 1.801                | 1.414  | 1.036  | 0.728  | 0.492  | 0.292  |
| CB              | 2.050                | 1.523  | 1.048  | 0.827  | 0.576  | 0.366  |
| BRB             | 2.200                | 1.609  | 1.108  | 0.878  | 0.617  | 0.399  |
| CAF             | 0.125                | -0.093 | -0.170 | -0.243 | -0.269 | -0.274 |
| OT              | 1.049                | 0.750  | 0.488  | 0.359  | 0.208  | 0.082  |
| BC              | 1.284                | 1.002  | 0.695  | 0.466  | 0.266  | 0.122  |
| NA              | 1.605                | 1.202  | 0.773  | 0.583  | 0.370  | 0.196  |
| ONT             | 1.771                | 1.306  | 0.915  | 0.699  | 0.456  | 0.257  |
| HQ              | 0.406                | 0.130  | 0.000  | -0.060 | -0.144 | -0.179 |
| P               | 0.997                | 0.697  | 0.434  | 0.307  | 0.157  | 0.043  |
| OCR             | 1.326                | 0.974  | 0.629  | 0.466  | 0.284  | 0.135  |
| DP35            | 1.500                | 1.148  | 0.820  | 0.542  | 0.333  | 0.178  |
| BNA             | 1.920                | 1.350  | 0.842  | 0.629  | 0.400  | 0.217  |
| BA              | 0.746                | 0.446  | 0.247  | 0.143  | 0.027  | -0.048 |
| AP              | 1.201                | 0.852  | 0.548  | 0.410  | 0.235  | 0.091  |
| PDM             | 1.360                | 0.967  | 0.592  | 0.423  | 0.235  | 0.091  |
| PEP             | 1.604                | 1.187  | 0.752  | 0.556  | 0.343  | 0.167  |
| ANA             | 2.020                | 1.475  | 0.946  | 0.719  | 0.477  | 0.275  |
| PYR             | 0.540                | 0.300  | 0.090  | -0.023 | -0.093 | -0.116 |
| AN              | 1.513                | 1.240  | 0.799  | 0.613  | 0.398  | 0.224  |
| DMA             | 1.728                | 1.370  | 1.016  | 0.721  | 0.494  | 0.307  |
| MP              | 1.100                | 0.805  | 0.504  | 0.331  | 0.157  | 0.043  |
| EP              | 1.420                | 1.055  | 0.699  | 0.470  | 0.266  | 0.118  |
| PP              | 1.903                | 1.384  | 0.848  | 0.664  | 0.391  | 0.217  |
| BP              | 2.310                | 1.696  | 1.057  | 0.835  | 0.523  | 0.315  |

dispersion interaction between the bonded alkyl phase and the solutes than between the aqueous mobile phase and the solutes [6]. Comparing the two figures it can be seen that methanol as the organic modifier furnishes higher methylene selectivity than acetonitrile. As it was explained, methylene selectivity depends also on the hydrophobic strength of the column. It can be seen that C<sub>8</sub> columns give lower methylene selectivity than C<sub>18</sub> columns.

### 3.2. Polar or chemical selectivity

Polar selectivity arises from secondary polar interactions as hydrogen-bonding (HB), dipole or ionic

interactions. Generally speaking polar selectivity can be defined by the relative retention of polar solutes to that of a nonpolar solute. In a recent publication we defined polar or chemical selectivity as the relative retention of polar test solutes compared to toluene as a nonpolar solute [28]. This polar selectivity computed in this way accounts for specific polar properties of the stationary phase. This type of selectivity characterizes generally the extent of secondary interactions between the stationary phase and the solutes. The higher the value of  $\alpha$  is, the more strongly the polar solute is retained compared to toluene.

In Fig. 2a and b, the  $\alpha$  relative retentions of two basic solutes, caffeine and aniline, compared to

Table 11  
Log *k* values in methanol–water on Sym-C<sub>8</sub> column

| Compound symbol | Log <i>k</i>     |       |       |        |        |        |
|-----------------|------------------|-------|-------|--------|--------|--------|
|                 | Methanol (% v/v) |       |       |        |        |        |
|                 | 20               | 30    | 40    | 50     | 60     | 70     |
| A               | 0.711            | 0.603 | 0.446 | 0.230  | 0.118  | 0.027  |
| MBO             | 2.001            | 1.700 | 1.200 | 0.840  | 0.520  | 0.307  |
| T               | 2.490            | 2.140 | 1.580 | 1.107  | 0.768  | 0.511  |
| EB              | 2.930            | 2.500 | 1.901 | 1.354  | 0.955  | 0.644  |
| PCR             | 1.404            | 1.215 | 0.971 | 0.650  | 0.421  | 0.235  |
| DP26            | 1.722            | 1.497 | 1.225 | 0.880  | 0.605  | 0.377  |
| EBO             | 2.401            | 2.040 | 1.501 | 1.074  | 0.696  | 0.428  |
| CB              | 2.290            | 1.980 | 1.600 | 1.174  | 0.812  | 0.536  |
| BRB             | 2.360            | 2.040 | 1.670 | 1.257  | 0.879  | 0.589  |
| CAF             | 0.633            | 0.344 | 0.150 | -0.030 | -0.179 | -0.211 |
| OT              | 1.038            | 0.878 | 0.678 | 0.475  | 0.255  | 0.125  |
| BC              | 1.380            | 1.200 | 0.825 | 0.554  | 0.293  | 0.165  |
| NA              | 1.723            | 1.465 | 1.167 | 0.846  | 0.525  | 0.307  |
| ONT             | 1.796            | 1.576 | 1.298 | 0.979  | 0.645  | 0.405  |
| HQ              | 0.463            | 0.344 | 0.191 | 0.073  | -0.077 | -0.129 |
| P               | 1.039            | 0.898 | 0.701 | 0.503  | 0.273  | 0.133  |
| OCR             | 1.443            | 1.253 | 1.012 | 0.680  | 0.470  | 0.278  |
| DP35            | 1.789            | 1.580 | 1.210 | 0.840  | 0.583  | 0.346  |
| BNA             | 2.110            | 1.834 | 1.482 | 1.093  | 0.716  | 0.444  |
| BA              | 0.881            | 0.737 | 0.549 | 0.364  | 0.161  | 0.047  |
| AP              | 1.285            | 1.067 | 0.822 | 0.574  | 0.306  | 0.153  |
| PDM             | 1.571            | 1.237 | 0.911 | 0.574  | 0.284  | 0.108  |
| PEP             | 1.790            | 1.568 | 1.268 | 0.934  | 0.597  | 0.353  |
| ANA             | 2.240            | 1.939 | 1.587 | 1.199  | 0.810  | 0.521  |
| PYR             | 0.630            | 0.415 | 0.260 | 0.101  | -0.017 | -0.073 |
| AN              | 1.521            | 1.341 | 1.102 | 0.834  | 0.549  | 0.343  |
| DMA             | 1.870            | 1.596 | 1.215 | 0.934  | 0.640  | 0.420  |
| MP              | 1.601            | 1.324 | 1.007 | 0.660  | 0.391  | 0.201  |
| EP              | 1.950            | 1.644 | 1.297 | 0.880  | 0.553  | 0.309  |
| PP              | 2.360            | 2.040 | 1.627 | 1.177  | 0.752  | 0.448  |
| BP              | 2.850            | 2.420 | 1.977 | 1.462  | 0.970  | 0.605  |

toluene are shown as a function of mobile phase composition with methanol and acetonitrile as organic modifiers on three columns of different types.

In Fig. 2c and d, the  $\alpha$  relative retentions of two acidic solutes, *p*-cresol and 3,5-dimethylphenol, compared to toluene are shown. Polar selectivity decreases considerably with increasing water content of the mobile phase for all solutes, because of the masking effect of the increasingly polar aqueous mobile phase. Acetonitrile as the less polar organic modifier furnishes higher polar selectivity than methanol. As regards column type, the M-C<sub>8</sub> column possessing both acidic and basic properties, furnishes higher polar selectivities for both basic and acidic

solutes indicating enhanced secondary interactions. Basic type (Sym C<sub>18</sub>) columns furnish higher polar selectivity for acidic solutes, while for basic solutes, the polar selectivity is practically the same as for a neutral type of column (M-C<sub>18</sub>e).

### 3.3. Relative retention of various polar solutes

The selectivity of separation of various types of polar solutes depends considerably on the acidic or basic character of the solutes to be separated. In addition, it depends also on the acidic or basic properties of the columns investigated.

In Fig. 3a and b,  $\alpha$  relative retentions of acidic

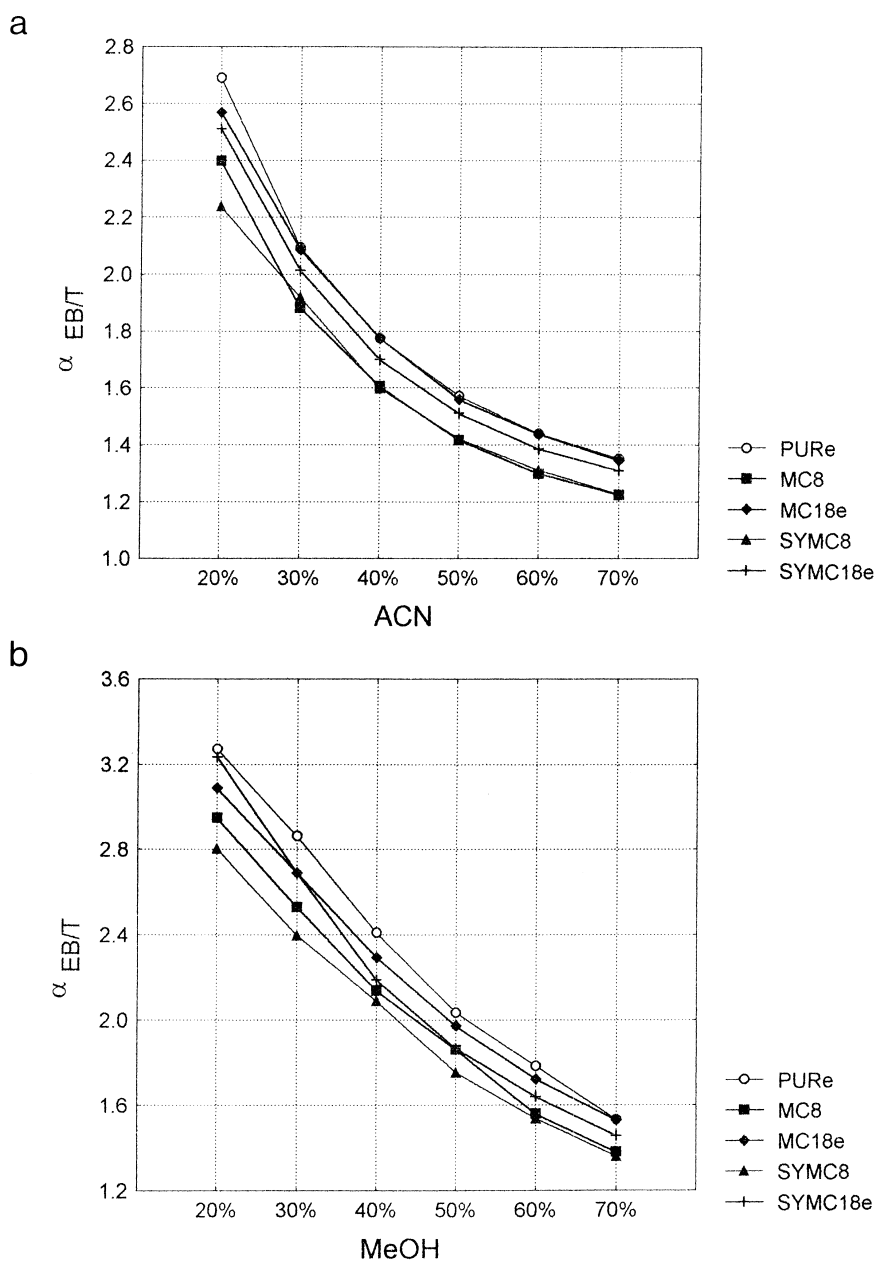


Fig. 1. Methylene selectivity: relative retention of ethylbenzene (EB) to toluene (T) on the columns investigated as a function of the mobile phase composition. (a) Acetonitrile as organic modifier; (b) methanol as organic modifier.

solutes (ethylparaben/*p*-cresol and 2,6-dimethylphenol/*o*-cresol) are shown as a function of mobile phase composition with methanol and acetonitrile as organic modifiers. Relative retention of acidic solutes – in general relative retention of polar to polar

solutes – increases with increasing water content of the mobile phase. Methanol furnishes higher selectivities than acetonitrile. Column type has little influence on selectivities.

In Fig. 4a and b,  $\alpha$  relative retentions of basic

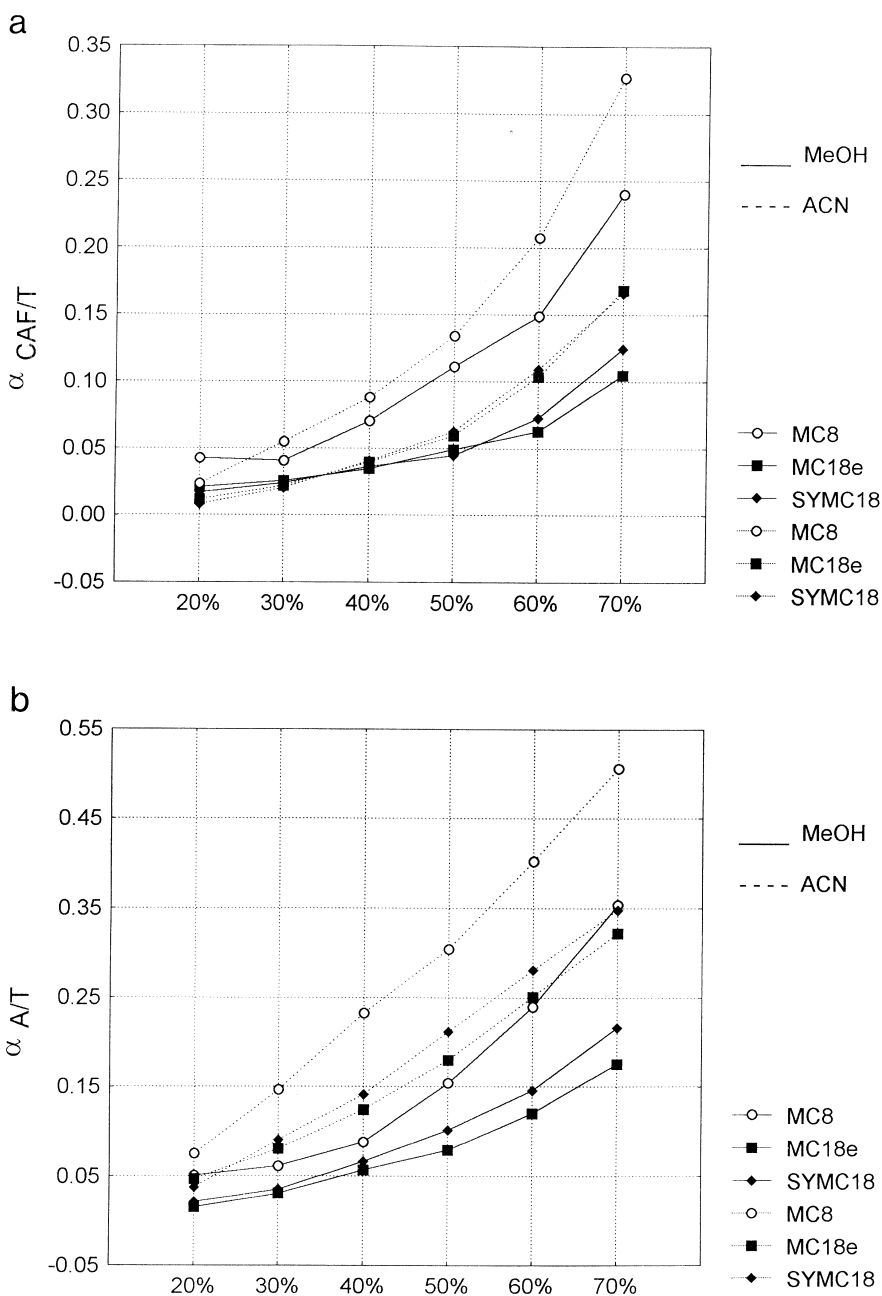


Fig. 2. Polar selectivity: relative retention of polar solutes to toluene on three columns of different types as a function of the mobile phase composition. (a) Caffeine/toluene; (b) aniline/toluene; (c) *p*-cresol/toluene; (d) 3,5-dimethylphenol/toluene.

solutes, methylbenzoate/benzyl cyanide and ethylbenzoate/dimethylaniline, are shown as a function of mobile phase composition for the two organic modifiers. Here again the relative retentions increase

with increasing water content of the mobile phase. Methanol furnishes considerably higher selectivities than acetonitrile. Column type has little influence on selectivities.

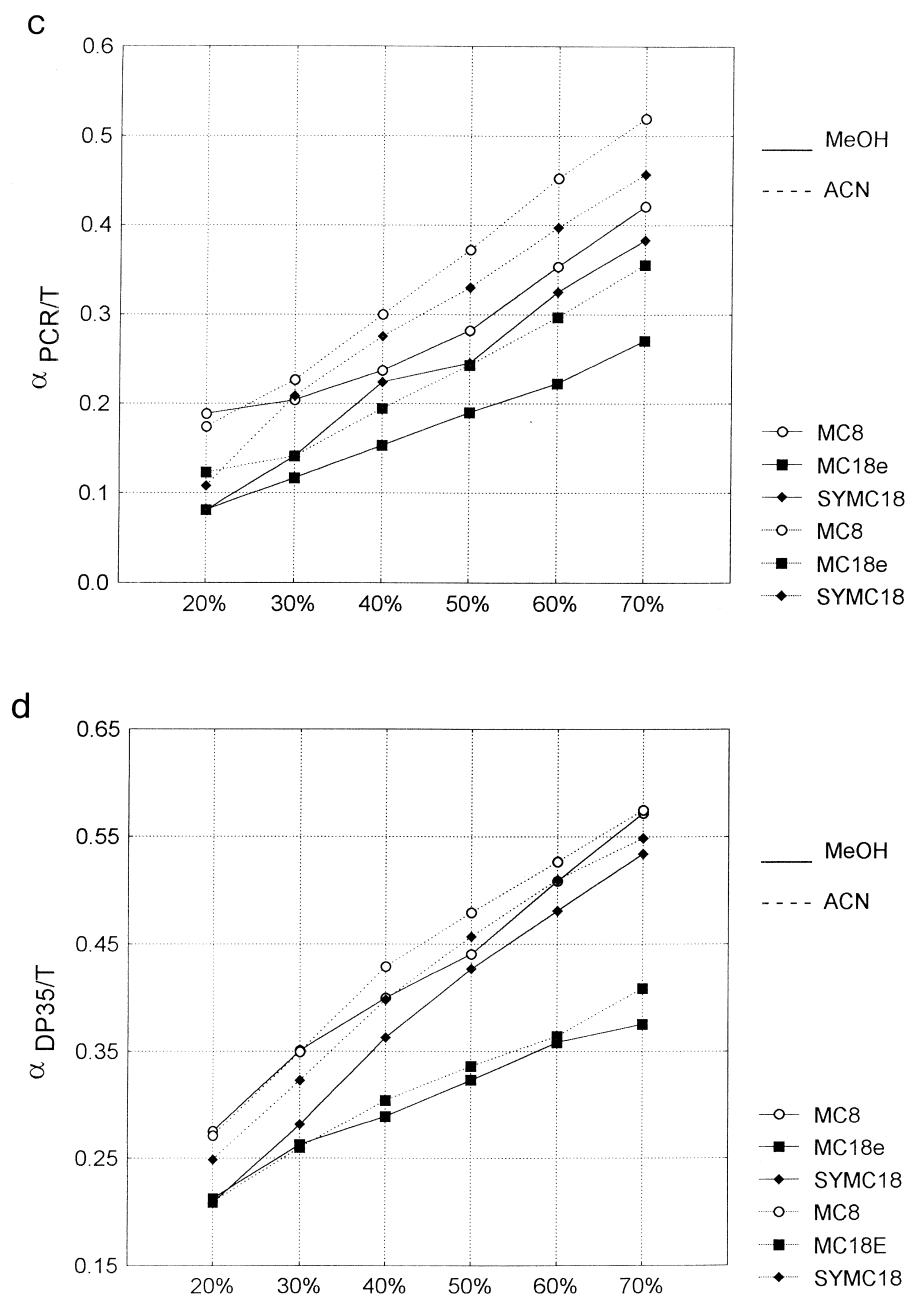


Fig. 2. (continued)

In Fig. 5a and b,  $\alpha$  relative retentions of acidic to basic solutes, *p*-ethylphenol/acetophenone and 2,6-dimethylphenol/*o*-toluidine, are shown as a function of mobile phase composition for the two organic modifiers. It is seen that relative retention of acidic

to basic solutes increases also with increasing water content. Methanol furnishes higher selectivities than acetonitrile. Basic type (Sym) columns furnish higher selectivities because of the preferential retention of acidic solutes.

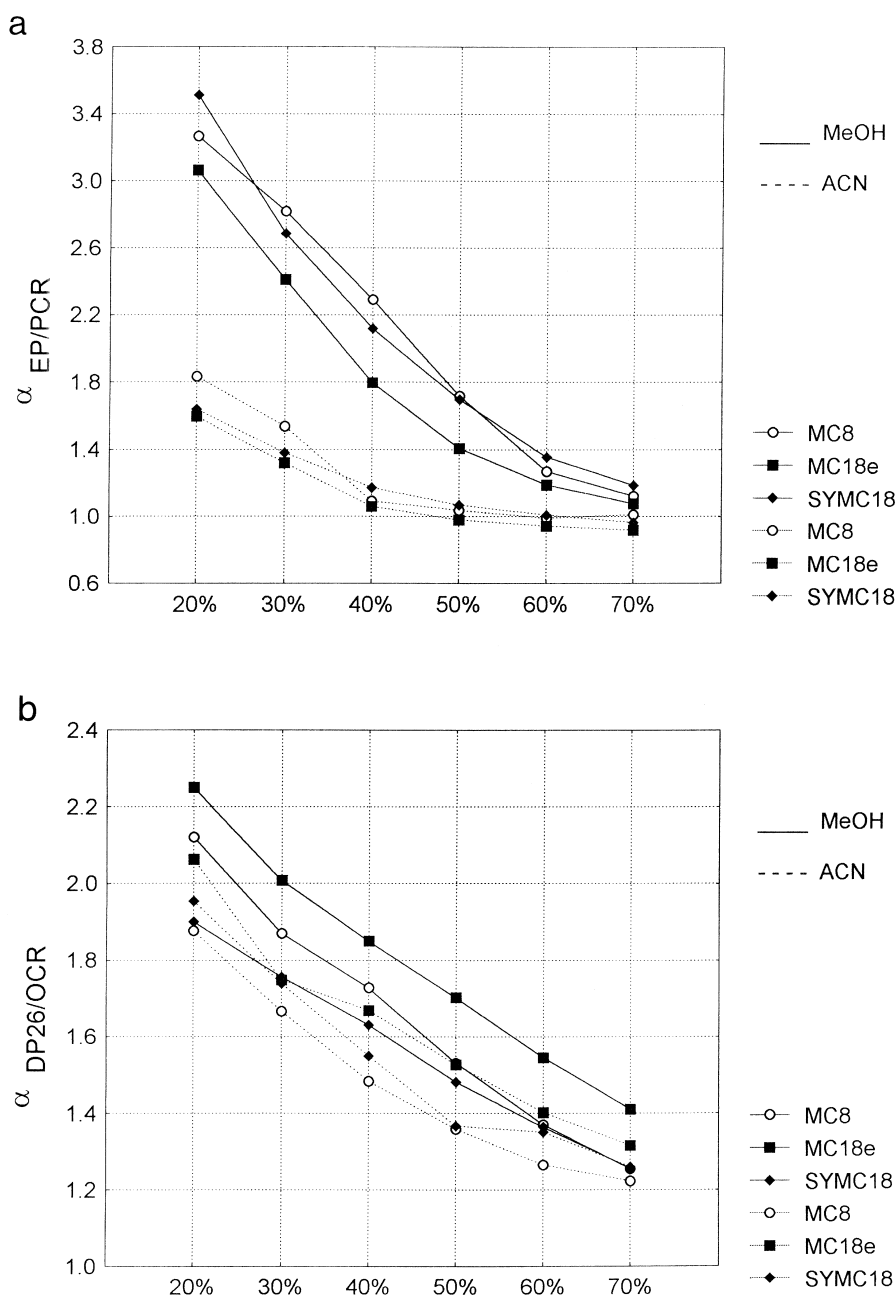


Fig. 3. Relative retention of acidic solutes as a function of the mobile phase composition. (a) Ethylparaben/*p*-cresol; (b) 2,6-dimethylphenol/*o*-cresol.

In Fig. 6a and b,  $\alpha$  relative retentions of basic to acidic solutes, methylbenzoate/methylparaben and dimethylphthalate/methylparaben, are shown as a function of mobile phase composition for the two

organic modifiers investigated. As before, relative retentions increase with increasing water content of the mobile phase. Here acetonitrile seems to give somewhat higher selectivities than methanol. Basic

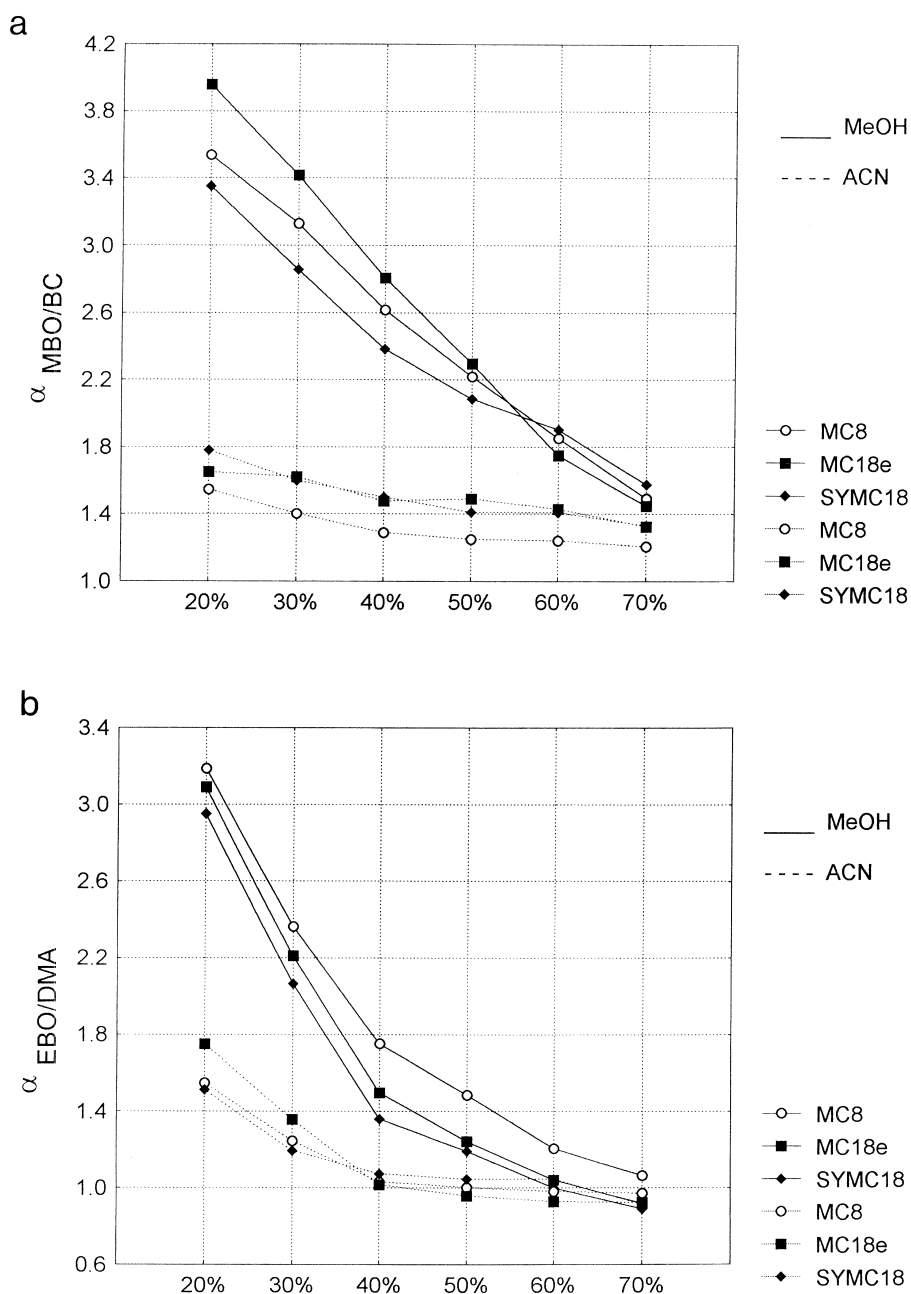


Fig. 4. Relative retention of basic solutes as a function of the mobile phase composition. (a) Methylbenzoate/benzyl cyanide; (b) ethylbenzoate/dimethylaniline.

type (Sym) columns furnish here lower selectivities because of the preferential retention of acidic solutes. However, this effect may give for some solute pairs reversed order of retention resulting even in higher selectivities.

#### 4. Conclusions

We have demonstrated that chromatographic selectivity is a very complex phenomenon depending not only on the phase system, i.e. characteristics of the



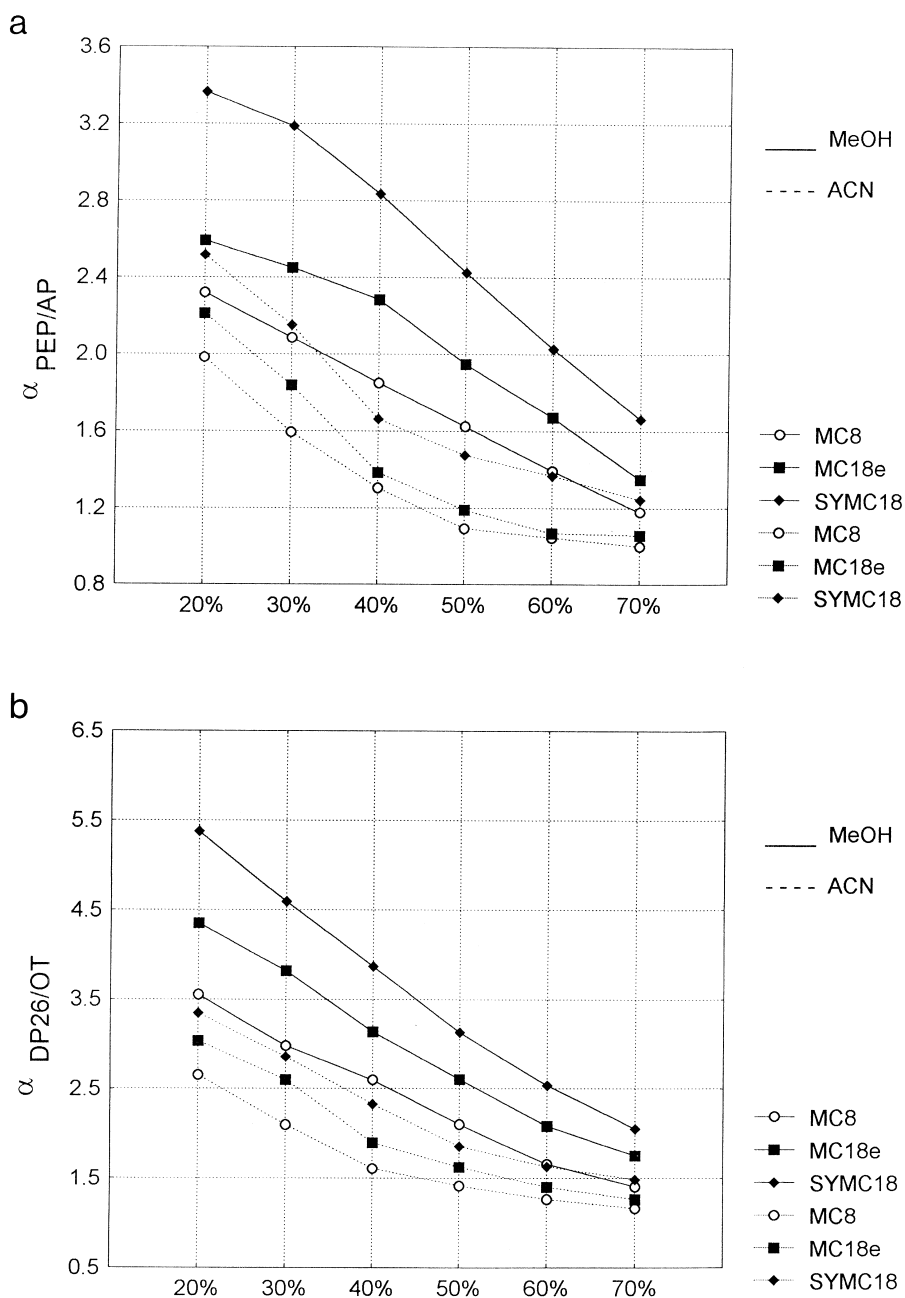


Fig. 5. Relative retention of acidic to basic solutes as a function of the mobile phase composition. (a) *p*-Ethylphenol/acetophenone; (b) 2,6-dimethylphenol/*o*-toluidine.

stationary phase, type of the organic modifier and mobile phase composition, but also on the type and structural characteristics of the compounds to be separated.

Hydrophobic or methylene selectivity depends on

the hydrophobic strength of the column and increases with increasing water content of the mobile phase. Methanol furnishes higher methylene selectivity than acetonitrile.

Polar or chemical selectivity, defined as the rela-

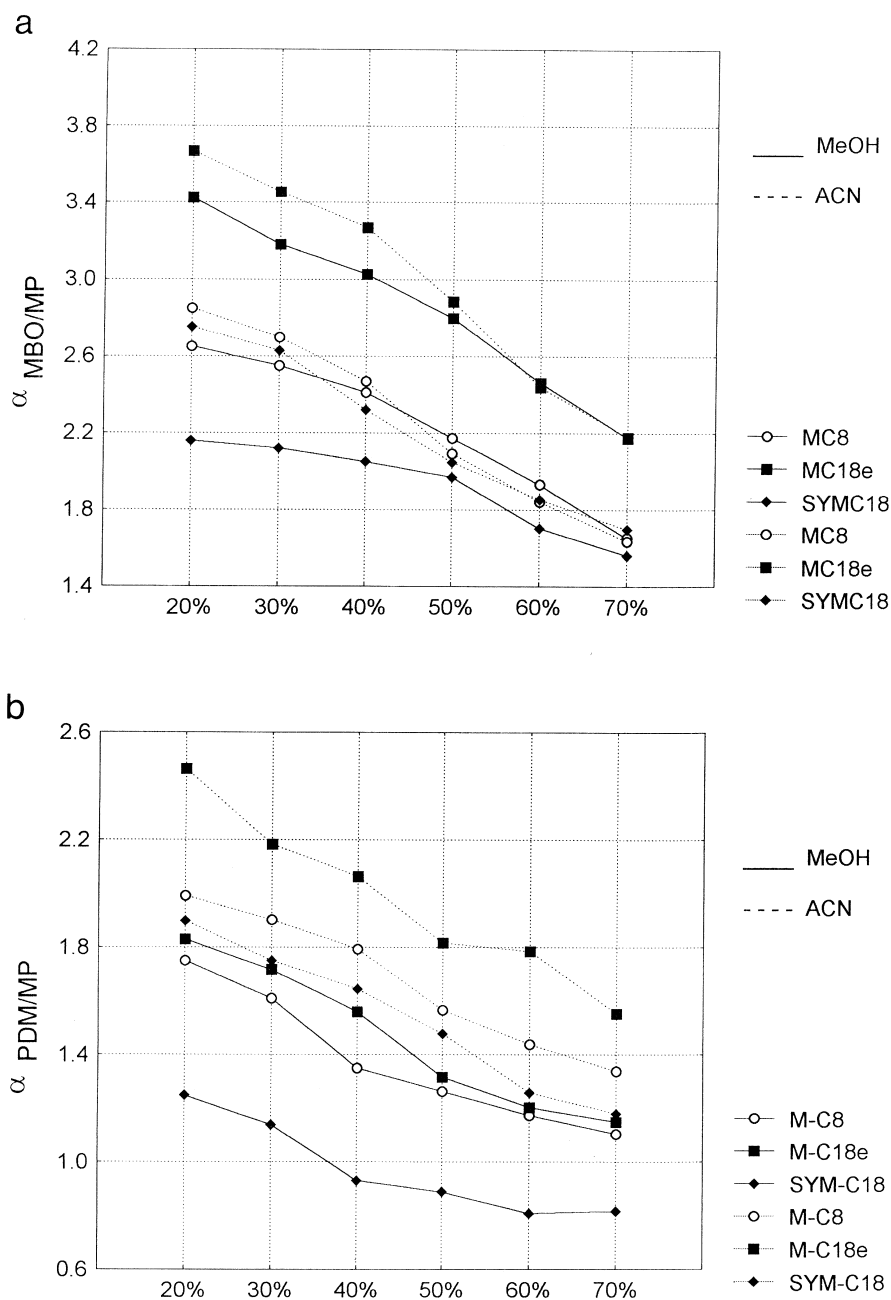


Fig. 6. Relative retention of basic to acidic solutes as a function of the mobile phase composition. (a) Methylbenzoate/methylparaben; (b) dimethylphthalate/methylparaben.

tive retention of polar species to toluene, characterizes the extent of secondary polar interactions between the stationary phase and the solutes. Polar selectivity decreases with increasing water content of the mobile phase and acetonitrile furnishes higher

polar selectivity than methanol. Acidic or basic properties of the column can contribute to higher polar selectivities for basic or acidic solutes because of the enhanced secondary interactions.

Relative retention of acidic solutes, as well as

relative retention of basic solutes, increases with increasing water content of the mobile phase. Methanol furnishes higher selectivities than acetonitrile. The type of column has little influence on selectivities.

Relative retention of acidic to basic solutes also increases with increasing water content of the mobile phase. Methanol furnishes higher selectivities than acetonitrile. Basic type (Sym) columns furnish higher selectivities because of the preferential retention of acidic solutes.

Relative retention of basic to acidic solutes increases with increasing water content of the mobile phase. Here acetonitrile seems to give somewhat higher selectivities than methanol. On basic type (Sym) columns the order of retention may be reversed for some pairs of solutes, resulting even in higher selectivities.

The results of this study have shown that the selectivity of separation of different types of compounds varies considerably and differently depending on the characteristics of columns, the type of the organic modifier and the composition of the mobile phase. By using the above guidelines to select the phase system and the operating conditions, the selectivity of separation of various types of solutes can be modulated in a wide range.

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