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Comparison of retention of aromatic hydrocarbons with polar groups in binary reversed-phase high-performance liquid chromatography systems

Tadeusz H. Dzido[a,*], Tomasz E. Kossowski[a], Dariusz Matosiuk[b]

[a]*Department of Inorganic and Analytical Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland*
[b]*Department of Synthesis and Drug Technology, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland*

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Abstract

The retention of aromatic hydrocarbons with polar groups has been correlated as log k_1 versus log k_2 for reversed-phase high-performance liquid chromatography systems with different binary aqueous mobile phases containing methanol, acetonitrile or tetrahydrofuran as modifiers. Distinct changes in separation selectivity have been observed between tetrahydrofuran and acetonitrile or methanol systems. Methanol and acetonitrile systems show lower diversity of separation selectivity. The changes in retention and selectivity of aromatic hydrocarbons with various polar groups between any two chromatographic systems with binary aqueous eluents (tetrahydrofuran vs. acetonitrile, tetrahydrofuran vs. methanol and methanol vs. acetonitrile) have been interpreted in terms of molecular interactions of the solute with especially one component of the stationary phase region, i.e. extracted modifier, and stationary phase ordering. The ordering of the stationary phase region caused by modifier type influences the chromatographic selectivity of solutes with different molecular shape.

Keywords: Retention behaviour; Separation selectivity; Shape selectivity; Modifier selectivity; Selectivity; Aromatic hydrocarbons, polar

1. Introduction

Investigations concerned with retention mechanism and selectivity changes in reversed-phase high-performance liquid chromatography (RP-HPLC) systems are still in progress in spite of many examples of investigations in the literature. The main reason for this situation is the complexity of the RP-HPLC systems, the properties of which change dynamically with composition of the mobile phase and type of the stationary phase. Some early investigations postulated that retention in RP-HPLC is mainly determined by interactions of the solute in the mobile phase [1–3]. However, more recent investigations take into account the active role of the stationary phase [4–9]. These and many other investigations have brought more useful knowledge to the optimization procedure of RP-HPLC separations. The most popular relationship describing retention of the solutes in reversed-phase systems is the semilogarithmic equation, for the first time applied in partition systems by Soczewiński and Wachtmeister [10] and later applied by Snyder and colleagues [11–14] to

*Corresponding author. Fax: +48-81-5328903.
E-mail address: thdzido@panaceum.am.lublin.pl (T.H. Dzido).

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RP aqueous systems, which represents linear dependence of log k (k is the retention factor) versus composition of the mobile phase. However this relationship often shows curvature in the broad range of mobile phase composition. Better fitting of the experimental values is observed when log k is represented as a quadratic function of the mobile phase composition [15,16]. Other authors have proposed relationships which also satisfactorily describe the dependence of log k versus composition of the mobile phase [8,17–20]. The relationships are successfully used in optimization procedure of separation and some of these are applied in computer simulation of the chromatographic process [21]. Valuable information dealing with retention mechanism has been obtained using linear free energy relationships (LFER) especially the solvation parameter model proposed by Abraham [22–24] which has been demonstrated to provide a satisfactory description of retention of organic compounds in reversed-phase liquid chromatography systems [25–30]. However, a large amount of retention data is necessary to calibrate the chromatographic system in which separation should be optimized and some solutes deviate from the model [31]. It is well established that there is a direct link between the structure of a compound and its retention. The quantitative structure versus retention relationships (QSRR) have been extensively investigated to predict the retention and to explain the retention mechanism in RP-HPLC [32,33]. These examples have led to a better understanding of the retention and selectivity changes in the chromatographic system.

In chromatographic analysis, even small changes in selectivity of the system can lead to substantial enhancement of the resolution. In such instances even subtle differences of the solute properties, which are responsible for retention and selectivity changes, can be very useful to obtain the appropriate resolution. This effect is attained owing to high efficiency of the chromatographic columns available on the contemporary market.

In previous papers, the influence of modifier change in binary aqueous mobile phase on selectivity of separation of benzene derivatives with polar groups in reversed-phase high-performance liquid chromatography was described [34–37]. The main conclusion from the data presented in some of our papers [35,38] is that the binary mobile phase of similar elution strength, with relatively high concentration of water, does not share in selectivity variation, or its contribution to this effect is minimal if the modifier (methanol (MeOH), acetonitrile (ACN) or tetrahydrofuran (THF)) is changed in the system with stationary phase of the C_{18} type. The inference seems to be inconsistent with published papers which have reported the distinct selectivity variations of, for example, aromatic solutes with polar groups separated in RP-HPLC systems with different modifiers [34,36]. In general, the influence of eluent on the selectivity is affected by molecular interactions of the solute with components of the mobile phase and stationary phase as well. The contribution of the former case deals, in general, with properties of water reflected by its cohesive energy, the strongest among conventional eluent components applied in the RP-HPLC systems, which leads to the solvophobic expulsion of the hydrophobic solute from the mobile phase, and also to strong electrostatic interactions with polar solutes. However, the second case (interactions in the stationary phase) depends on the interaction of the solute, at least, with four components of the stationary phase region, i.e. hydrocarbon chains, unreacted silanol groups, water present in the stationary phase and extracted modifier molecules. If stationary phases in systems with different modifiers (methanol, acetonitrile, tetrahydrofuran) in the binary mobile phase of reasonable concentration range are compared, then the composition of the two first components of the stationary phase is constant and the composition of the third component (water) can be judged approximately also as constant in the limited range of modifier concentration. So the fourth component (modifier) is that which is the most responsible for the differentiation of the properties of the stationary phase among systems with various organic solvents.

It means that selectivity changes in reversed-phase liquid chromatography between binary chromatographic systems with different eluents are caused by interactions of the solute with the organic component of the mobile phase extracted into the stationary (brush) phase. The lack of influence (or minute contribution) of the modifier change in the eluent on the selectivity was demonstrated by comparison of partition constants of benzene derivatives in gas–

liquid systems in which liquid solutions were aqueous organic solvents: methanol, acetonitrile, or tetrahydrofuran [38]. The gas–liquid partition constants show very good Collander-type correlations [39]. It means that modifier type does not alter partition selectivity of gas–liquid system or its influence is minor. Then such relationships indicate that the source of the selectivity changes in RP-HPLC systems is caused by different modifiers as follows: the selectivity is not significantly affected by their interactions in the mobile phase when modifier is changed in the eluent, but by interaction in the stationary phase. It is in particular caused by interactions between solute molecules and modifier molecules. Both specific and nonspecific interactions and ordering of the stationary phase, caused by extracted modifier, can lead to retention changes between chromatographic systems with different modifiers [40–42]. The specific interactions of the solute molecule with modifier in the mobile phase are strongly weakened in the presence of relatively high concentrations of water which is the strongest competitor for this interaction against the remaining components of the eluent in the chromatographic system. On the other hand, the molecular interactions of the solute and the modifier in the stationary phase region do not meet such competition from the side of water due to its much smaller concentration.

The above discussion can also be confirmed by applying basic thermodynamic relationships. Retention in liquid chromatography is mostly expressed as retention factor, k or log k. The retention factor is related to the equilibrium partition constant, K, of the solute in the chromatographic system by the relationship:

$$k = K\phi \tag{1}$$

where ϕ is the ratio of the stationary and the mobile phases.

The standard free energy, ΔG°, is expressed as:

$$\Delta G^{\circ} = -RT \ln K \tag{2}$$

The free energy of the solute in the chromatographic system can be determined by differences of its solvation energies [43]:

$$\Delta G = \Delta G_{\mathrm{SLV,S}} - \Delta G_{\mathrm{SLV,M}} \tag{3}$$

$\Delta G_{\mathrm{SLV,S}}$ and $\Delta G_{\mathrm{SLV,M}}$ are the free energies of solvation in the stationary, S, and mobile, M, phases, respectively.

According to Refs. [43–45]:

$$\Delta G_{\mathrm{SLV}} \approx \Delta G_{\mathrm{CAV}} + \Delta G_{\mathrm{INT}} \tag{4}$$

ΔG_{CAV} and ΔG_{INT} are free energy changes due to cavity formation of the molecular size of the solute and interactions with surrounding molecules of the appropriate phase, respectively.

Then:

$$\Delta G = \Delta G_{\mathrm{CAV,S}} + \Delta G_{\mathrm{INT,S}} - \Delta G_{\mathrm{CAV,M}} - \Delta G_{\mathrm{INT,M.}} \tag{5}$$

$$\Delta G_1 = \Delta G_{\mathrm{CAV,S,1}} + \Delta G_{\mathrm{INT,S,1}} - \Delta G_{\mathrm{CAV,M,1}} - \Delta G_{\mathrm{INT,M,1}} \tag{6}$$

$$\Delta G_2 = \Delta G_{\mathrm{CAV,S,2}} + \Delta G_{\mathrm{INT,S,2}} - \Delta G_{\mathrm{CAV,M,2}} - \Delta G_{\mathrm{INT,M,2}} \tag{7}$$

Subscripts 1 and 2 denote two different modifier systems.

After subtraction of Eq. (6) from Eq. (7), we obtain:

$$\Delta(\Delta G) = (\Delta G_{\mathrm{CAV,S,2}} - \Delta G_{\mathrm{CAV,S,1}}) + (\Delta G_{\mathrm{INT,S,2}} - \Delta G_{\mathrm{INT,S,1}}) - (\Delta G_{\mathrm{CAV,M,2}} - \Delta G_{\mathrm{CAV,M,1}}) - (\Delta G_{\mathrm{INT,M,2}} - \Delta G_{\mathrm{INT,M,1}}) \tag{8}$$

Eq. (8) determines the retention change in the solute between two chromatographic systems with different modifiers 1 and 2 according to the equation:

$$\Delta(\Delta G^{\circ}) = \Delta G_2^{\circ} - \Delta G_1^{\circ} = -RT \ln (K_2/K_1) \tag{9}$$

At approximation the term $(\Delta G_{\mathrm{CAV,S,2}} - \Delta G_{\mathrm{CAV,S,1}})$ is cancelled due to similar energies required for cavity formation in the stationary phase in systems 1 and 2. The extraction of the modifier from the binary mobile phase of relatively high concentration of water into the stationary phase increases in the order: methanol < acetonitrile < tetrahydrofuran. Otherwise, the values of cohesive energy density of these pure modifiers increase in opposite order. This can mean that cancellation of the energy values responsible for cavity formation in two stationary phases of the

systems with different modifiers seems to be valid. In addition, the energy required for cavity formation in the hydrocarbonaceous stationary phase is relatively small, much smaller than that of the mobile phase. So it implies minor odds of the energy values for cavity formation in nonpolar stationary phase.

The term $((\Delta G_{CAV,M,2} - \Delta G_{CAV,M,1}) + (\Delta G_{INT,M,2} - \Delta G_{INT,M,1}))$, in general, is approximately equal to zero for a given composition of two binary mobile phases or its value is practically constant in reasonable concentration of water typically applied in reversed-phase liquid chromatography. This assumption is based on the properties of binary water mobile phase of the reversed-phase liquid chromatography systems and on the data for solute partition in gas–liquid (water+modifier) systems of which equilibrium partition constants (log K) show very good correlation with the slope very close to 1.0 [38].

Then, after the simplifications discussed above, the difference in free energies of the solute between two modifier systems can be expressed as:

$$\Delta(\Delta G) \approx \Delta G_{INT,S,2} - \Delta G_{INT,S,1} \quad (10)$$

Then the modifier change in the eluent leads to alteration of the molecular interactions in the stationary phase which can exert its influence on the separation selectivity. This means that selectivity variation can be explained by interactions of the solutes with different modifiers in the stationary phase region taking into account their properties, e.g. from selectivity triangle [46,47] or structural descriptors derived from other experimental data [48–51] or computed theoretically [25,33,52–55]. Likewise, ordering of the stationary phase region, which is dependent on the modifier type, can cause the selectivity changes due to structural differences of the solute molecules what reflects participation of the entropy element in the retention mechanism. Additionally this approach eliminates one of two possible explanations of retention alteration when the modifier is changed in the mobile phase. For example, the relative retention increase in one solute relative to another could be intuitively explained by enhanced interactions in the stationary phase and by reduced interactions in the mobile phase. According to our approach, the relative retention change can be explained by interactions only in one phase (stationary) if one modifier is replaced by another in the eluent maintaining similar elution strength.

In the present paper, we intend to extend the investigation of relative retention changes to the set of aromatic solutes with different groups, especially taking into account the properties which are responsible for the molecular interactions with modifiers in the stationary phase.

2. Experimental

Measurements of retention were performed with an HP 1050 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a 20-μl sample injector (Rheodyne, Cotati, CA, USA) and a variable UV detector (HP-1050) operating at 254 nm. The chromatograms were recorded with a Hewlett-Packard Model 3396 A reporting integrator. The stainless-steel column (10 cm×4.6 mm I.D.) was packed with 5-μm particles of ODS silica gel (C_{18}), carbon content 16 wt%, prepared by Hanai et al. using octadecyltrichlorosilane [56,57]. The column was immersed in a water bath, at 20±0.2 °C. Solvents were of analytical or chromatography grade, water was bidistilled. All eluents contained 0.1% acetic acid (analytical grade) for dissociation, suppressing acidic compounds and residual silanol groups. The dead volume was determined by injection of pure water. Solutes were usually of pro-analysis quality and were from various distributors. Solutes and their numbers are listed in Table 1. The program PC Spartan Pro (v. 1.05, 2000) from Wavefunction Inc. (Irving, CA, USA) was applied for molecular modeling ab initio (HF approximation, 6-31G* basis set). Structural parameters are collected in Table 2.

3. Results and discussion

3.1. Correlation of retention in THF and ACN systems

Tetrahydrofuran and acetonitrile show strong extraction into the stationary phase [58–60]. Due to the almost planar molecular structure and higher molecular volume (and surface) of tetrahydrofuran in comparison with acetonitrile, the stationary phase region

Table 1
List of solutes investigated

Aromatic hydrocarbons	18. 2-Nitrophenol
1. Benzene	19. 3-Nitrophenol
2. Toluene	20. 4-Nitrophenol
Monofunctional compounds	21. 2-Methyl-4-nitrophenol
3. Phenol	22. Methyl 4-hydroxybenzoate
4. 2-Cresol	23. Ethyl 4-hydroxybenzoate
5. 4-Cresol	24. Propyl 4-hydroxybenzoate
6. 2-Naphthol	25. 4-Nitrobenzyl alcohol
7. Methyl phenylacetate	26. 1,2-Dinitrobenzene
8. Ethyl phenylacetate	27. 1,4-Dinitrobenzene
9. Methyl benzoate	28. 3,3′-Dinitrodiphenyl
10. Acetophenone	29. 4-Nitrobenzaldehyde
11. Nitrobenzene	30. 4-Cyanobenzaldehyde
12. Methylbenzyl ketone	31. Dimethyl isophthalate
13. Benzonitrile	32. Methyl benzyl terephthalate
Bifunctional compounds	33. Dimethyl 4,4′-diphenylcarboxylate
14. 1,5-Dihydroxynaphthalene	*Trifunctional compounds*
15. 1,6-Dihydroxynaphthalene	34. 2-Nitro-4-chlorophenol
16. 1,7-Dihydroxynaphthalene	35. 1,3,5-Trinitrobenzene
17. 2-Cyanophenol	36. 1-Chloro-2,4-dinitrobenzene

in its systems is more ordered than for acetonitrile systems. Tetrahydrofuran shows stronger ability to act as a hydrogen bond acceptor (hydrogen bond basicity, β_1 is equal to 0.55 and 0.31, respectively for THF and ACN). The ability of these modifiers to dipolar interactions is similar according to the values of solvatochromic parameters (Table 3) [61]. However, the electric dipole moment of acetonitrile is almost two times greater than that of tetrahydrofuran (3.45 and 1.75 D for acetonitrile and tetrahydrofuran, respectively) [62]. The refraction indices of THF and ACN are equal to 1.405 and 1.342, respectively, which indicates a higher ability of THF than ACN for dispersive interactions.

The results of the investigations are demonstrated in figures as correlations of log k_1 versus log k_2 where k_1 and k_2 are retention factors of the solute in systems 1 and 2, respectively. In Fig. 1, the retention of the solutes in 35% THF against 35% ACN with C_{18} stationary phase is correlated. The solutes with one polar group form two separate lines, one for phenols and the other for benzene derivatives with electron-donor groups. The correlation line for mono-phenols is above the line for solutes with one polar electron-donor group which indicates their relatively higher retention in THF relative to ACN system explained by stronger hydrogen bond interaction of the solutes with tetrahydrofuran than with acetonitrile in the stationary phase region [35]. The two lines show convergence with increase in molecular volume of the solutes. This means that the contribution of hydrogen bond interaction in the stationary phase region to the retention of proton donating solutes relative to other benzene derivatives diminishes with increase in molecular volume. The points for nitrobenzene and benzonitrile are positioned above the line for solutes with one electron-donor group what is also consistent with the previous data [35]. The planar structure of the solutes, in this case especially nitrobenzene (planar configuration of the molecule structure is most probable due to its lowest energy), leads to its higher entropic penetration into the stationary phase region in the system with tetrahydrofuran (more ordered system) than in the system with acetonitrile (less ordered) relative to other solutes of aplanar structure. This can presumably be explained by the almost planar structure of the tetrahydrofuran molecule which leads to greater ordering of the stationary phase. Compare also the thickness of the molecule measured normal to its largest cross-section (Table 2). The value of thickness is the smallest for nitrobenzene and benzonitrile and higher for acetophenone, methylbenzyl ketone, ethyl phenylace-

Table 2
Structural parameters of investigated compounds (1–36)

No.	Surface (Å^2)	Volume (Å^3)	Ovality	Thickness (Å)	Dipole moment			
					Total μ	Partial		
						μ_x	μ_y	μ_z
1	123.14	105.95	1.1375	1.6	0	0	0	0
2	144.84	126.13	1.1911	1.9	0.298	−0.022	0.000	−0.297
3	134.47	116.60	1.1653	1.7	1.188	1.161	0.000	−0.252
4	154.66	136.43	1.2070	1.9	0.893	−0.668	0.000	0.594
5	156.17	136.78	1.2168	1.9	1.265	0.087	−1.126	−0.570
6	183.43	170.11	1.2358	1.7	1.392	1.380	0.000	−0.180
7	206.22	183.18	1.3224	3.8	1.748	−1.302	1.091	0.408
8	227.83	203.83	1.3606	3.9	1.648	−1.279	0.989	0.316
9	180.91	162.08	1.2587	1.9	2.053	−1.666	0.000	1.199
10	167.89	150.73	1.2261	1.9	2.837	2.305	0.000	1.654
11				1.7				
12	193.91	171.95	1.2970	2.9	2.706	−2.138	−1.429	−0.842
13	148.29	130.16	1.1942	1.7	3.704	0.000	0.000	3.704
14	188.20	178.83	1.2264	1.7	0.002	0.000	0.000	−0.002
15	191.45	179.74	1.2433	1.7	2.026	0.162	−0.133	2.016
16	191.51	179.76	1.2436	1.7	0.844	−0.040	0.170	0.826
17	157.66	140.23	1.2081	1.7	2.846	−0.136	0.000	2.842
18	160.92	145.57	1.2028	1.7	4.324	−0.385	0.000	−4.306
19	165.72	147.77	1.2263	1.7	4.298	0.625	0.000	4.252
20	165.69	147.72	1.2264	1.7	5.662	1.136	0.000	−5.547
21	185.01	167.33	1.2602	1.9	5.957	2.075	0.656	5.546
22	192.17	172.68	1.2818	1.9	1.443	−0.473	0.000	1.364
23	214.94	193.34	1.3296	1.9	1.365	−0.798	0.000	1.107
24	237.55	213.82	1.3741	1.9	1.375	0.884	0.000	1.052
25	186.95	168.14	1.2693	2.5	4.757	1.662	−3.174	−3.130
26	186.20	168.75	1.2612	2.3	7.888	0.000	0.000	−7.888
27	185.87	168.42	1.2606	1.7	0	0	0	0
28	269.14	256.00	1.3808	2.5–2.8	3.722	0.000	0.000	3.722
29	180.01	162.26	1.2515	1.7	3.800	1.865	0.000	−3.311
30	173.78	155.24	1.2444	1.7	2.334	1.835	0.000	−1.441
31	238.53	218.17	1.3614	1.9	1.293	0.000	0.000	−1.293
32	329.16	307.60	1.4941	3.1–5.2	0.522	0.389	0.200	0.286
33	324.88	306.54	1.4781	2.3	1.471	−0.668	1.096	−0.718
34	179. 03	163.52	1.2383	1.8	3.696	2.342	0.000	2.860
35				1.7				
36	200.98	185.35	1.2787	1.8	4.997	2.128	4.461	0.736

Table 3
Kamlet–Taft solvatochromic parameters [61]

Solvent	Dipolarity/ polarizability (π_1^*)	Hydrogen bond acidity (α_1)	Hydrogen bond basicity (β_1)
Water	1.09	1.17	0.48
Acetonitrile	0.75	0.19	0.31
Methanol	0.60	0.93	0.62
Tetrahydrofuran	0.58	0.00	0.55

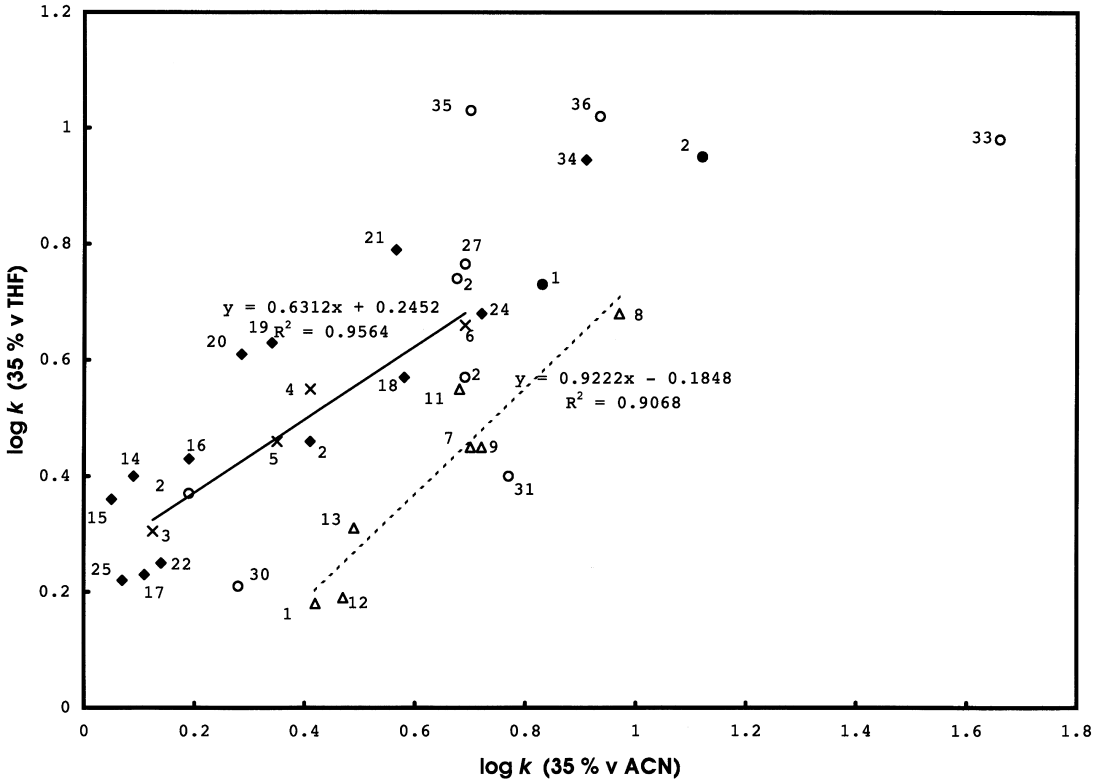

Fig. 1. Log k in 35% v/v THF plotted against log k in 35% v/v ACN. C_{18} stationary phase; ×, monophenols; △, aromatic compounds with one electron-donor group; ●, aromatic hydrocarbons; ♦, aromatic compounds with two polar groups (–OH and electron-donor or two –OH) and 2-nitro-4-chlorophenol; ○, aromatic compounds with two or three electron-donor groups; ——— for monophenols; - - - for aromatic compounds with one electron donor group and solute numbers as in Table 1.

tate, methyl benzoate, and methyl phenylacetate. The selectivity changes in benzene derivatives with polar groups of different shape caused by modifier type are very rarely reported in literature [31,33]. However, the explanation of this shape selectivity of polar groups of benzene derivatives seems to be analogous to changes in retention data obtained for some simple aliphatic solutes. The values of separation factor α ($\alpha = k_2/k_1$) of structural isomer pairs such as propyl acetate and isopropyl acetate, butyl alcohol and isobutyl alcohol, methyl propyl ketone and methyl isopropyl ketone) are equal in methanol and acetonitrile systems (Table 4). However α-values of the same pairs of solutes are higher in the THF system. In particular, it refers to two solute pairs, butyl alcohol–isobutyl alcohol and methyl propyl ketone–methyl isopropyl ketone. In the case of propyl acetate and isopropyl acetate, the effect is partly reduced by the branched structure of the ester group alone in both isomers. This means that retention of *n*-isomers is increased relative to their iso-structures in the tetrahydrofuran system in comparison to the methanol and acetonitrile systems. The effect is presumably due to weaker entropic penetration of the more ordered stationary phase region in the tetrahydrofuran system by molecules of branched structure (isobutyl alcohol and methyl isopropyl ketone) than their *n*-isomers relative to less ordered stationary phase in acetonitrile and methanol systems.

However, the increase in retention of nitrobenzene in the THF system cannot be explained only by its molecular planarity. The nitro group has two bonds which are highly polarized with an electron density deficiency on the nitrogen atom [63]. On the other hand, the tetrahydrofuran molecule has two C–O bonds which are also polarized with higher electron

Table 4
Retention factor (k) and separation factor (α) values of simple aliphatic isomers

Compound (2)	k_2	Compound (1)	k_1	$\alpha = k_2/k_1$
45% methanol in water				
n-Propyl acetate	2.56	Isopropyl acetate	2.27	1.13
n-Butanol	1.22	Isobutanol	1.20	1.02
Methyl propyl ketone	1.35	Methyl isopropyl ketone	1.27	1.06
35% acetonitrile in water				
n-Propyl acetate	2.86	Isopropyl acetate	2.53	1.13
n-Butanol	0.84	Isobutanol	0.82	1.02
Methyl propyl ketone	1.75	Methyl isopropyl ketone	1.66	1.05
30% tetrahydrofuran in water				
n-Propyl acetate	2.29	Isopropyl acetate	1.99	1.15
n-Butanol	0.93	Isobutanol	0.77	1.21
Methyl propyl ketone	1.22	Methyl isopropyl ketone	0.93	1.31

density on the oxygen atom. The strongest electrostatic interactions between nitrobenzene and tetrahydrofuran molecule can take place when the planes of the O–N–O group of nitrobenzene and the C–O–C group of tetrahydrofuran (quadrupoles of these molecules) are in parallel position as demonstrated in Fig. 2 [64,65]. There are also other possible molecule positions which can form mutual interactions between these compounds [64]. But such configuration, presented in Fig. 2 is especially energetically more favorable than typical molecular interactions between two dipoles [65]. This behavior is additionally confirmed by molecular modeling and computer simulation of this interaction with use of the Spartan Pro program. Comparison of the HOMO and LUMO orbital distribution, demonstrated in Fig. 3 shows, when the planes of the nitro and ether groups are parallel positioned (similarly as in Fig. 2), that configuration of the lowest unoccupied molecular orbitals in the nitro group of nitrobenzene is complementary to the highest occupied molecular orbitals in the C–O–C group of the THF molecule.

If the above explanation is valid then an increase in the number of nitro groups in the benzene ring should lead to further retention increase in THF system due to greater probability of interaction of the nitrobenzene derivatives with THF molecules in the stationary phase region. Really it is the fact that benzene derivatives with two or three nitro groups

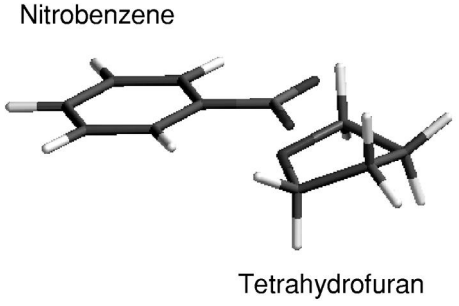

Fig. 2. Respective positioning of the nitrobenzene and tetrahydrofuran molecules favorable for quadrupole interaction.

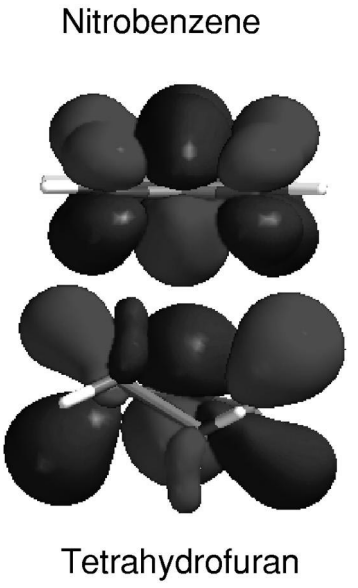

Fig. 3. Complementarity of LUMO orbitals of the nitro group of nitrobenzene and HOMO orbitals of the tetrahydrofuran C–O–C moiety with the parallel configuration of both molecules of the compounds.

demonstrate their point position above the correlation line for monophenols. The explanation of the retention decrease according to the sequence 1,3,5-trinitrobenzene, 1,4-dinitrobenzene and nitrobenzene in THF system is reflected by a decrease in the number of nitro groups in the molecule. It is contrary to the expectations based on the solvophobic expulsion of the solute from the aqueous mobile phase. Then the solute series should be eluted in reverse order due to the effect of the hydrophilic properties of the nitro group. In addition, it should be mentioned that differences between log k values for consecutive members of the nitrobenzene series are similar. For 30% THF system: $\Delta \log k_{(1,4\text{-dinitrobenzene/nitrobenzene})} = 0.21$ and $\Delta \log k_{(1,3,5\text{-trinitrobenzene/1,4-dinitrobenzene})} = 0.24$ and for 35% THF system: $\Delta \log k_{(1,4\text{-dinitrobenzene/nitrobenzene})} = 0.22$ and $\Delta \log k_{(1,3,5\text{-trinitrobenzene/1,4-dinitrobenzene})} = 0.26$. This is a good example of the linear free energy relationship (LFER) where each adjacent nitro group contributes similarly to equilibria in the chromatographic system by interaction of its nitro group with the C–O–C group of tetrahydrofuran.

All nitrobenzene derivatives with two nitro groups: 1,4-dinitrobenzene, 1,2-dinitrobenzene and 3,3′-dinitrodiphenyl show much weaker retention than 1,3,5-trinitrobenzene in the THF system, but much higher than nitrobenzene and almost the same retention in the ACN system. The retention decrease in 1,2-dinitrobenzene relative to 1,4-dinitrobenzene in the THF system can be explained by the difference in planarity of these molecules. The planar structure of 1,2-dinitrobenzene molecule is disturbed by ortho effects of two vicinal nitro groups and this is probably the reason why the ordered stationary phase region with THF is not entropic accessible, in that extent, by this solute molecules in comparison to 1,4-dinitrobenzene. A characteristic behavior is observed for 3,3′-dinitrodiphenyl and 1,4-dinitrobenzene, the molecules with the same number of nitro groups but the molecular volume of the first solute is larger than the second one. Hydrophobic interaction with the stationary phase of the 3,3′-dinitrodiphenyl should then cause a stronger retention and the same conclusion could be drawn taking into account the solvophobic expulsion of the solute from the mobile phase. However, the data in Fig. 1 indicate that 3,3′-dinitrodiphenyl shows slightly weaker retention than 1,4-dinitrobenzene in the THF system and ACN as well. The behavior, especially in the THF system, can probably be explained considering the planarity of the molecule in which each nitrophenyl group can rotate about the C–C bond between the phenyl rings. So this effect can be responsible for a decrease in penetration of the stationary phase region by the molecule and as a consequence, for the retention decrease relative to 1,4-dinitrobenzene. The same explanation can be also applied to the acetonitrile system. In the case of a larger molecule such as 3,3′-dinitrophenyl in which the distinct shape changes can occur, then in spite of weaker ordering of the stationary phase region in acetonitrile system than in THF system the influence of the shape of the molecule on selectivity should also be revealed.

Another example is 4-nitrobenzaldehyde; its point is located on the correlation line for monophenols, and its nitro group can be responsible for increased retention relative to 4-cyanobenzaldehyde in the THF system in comparison to acetonitrile system. The last molecule has also a planar structure but its CN-group is characterized by strong dipolar properties and is capable of dipolar interaction with acetonitrile in the stationary phase region. So this is the reason why 4-cyanobenzaldehyde shows increased retention in the ACN system relative to 4-nitrobenzaldehyde and its point is shifted to the line of solutes with one electron-donor group.

The next distinctive examples of relative retention changes caused by the shape of the molecule are demonstrated for solutes with two electron-donor groups which can have conformations out of the plane of the benzene ring. Dimethyl isophthalate and dimethyl 4,4′-diphenylcarboxylate are more strongly retained in the ACN system than the THF system, their points are positioned below the line for solutes with one electron-donor group (including benzene derivatives with one ester group: methyl phenylacetate, ethyl phenylacetate, methyl benzoate). This effect can be caused by relatively lower entropic accessibility of these solutes into the more ordered stationary phase region of the THF system than that of the ACN system.

Compounds with two phenol groups (1,5-, 1,6- and 1,7-dihydroxynaphthalenes) show stronger retention relative to monophenols in the THF system, their points are above the correlation line for mono-

phenols. This is explained by increased probability to the specific interaction of the bifunctional solutes with THF in the stationary phase region relative to monofunctional ones. This effect is also compatible with the data for phenol derivatives with a polar electron-donor group. 2-Cyanophenol, 2-nitrophenol, methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate show slightly decreased retention relative to monophenols in THF systems in comparison to the ACN system. This behavior can be explained by a decrease in solute ability to the specific interaction in the stationary phase region due to an ortho effect in the case of the first two solutes. However the proton-donor property of 2-nitrophenol is further diminished by an internal H bond. Effective hydrogen bond acidity, α_2^H, of 2-nitrophenol is equal to 0.05 but those of 3-nitrophenol and 4-nitrophenol are 0.79 and 0.82, respectively [48]. However, the nitro group of 2-nitrophenol can interact with tetrahydrofuran in the manner discussed above and additionally an internal H bond causes stabilization of the planar structure of the molecule [66] so both effects lead to retention increase in the THF system relative to the ACN system. The molecular length of the next three solutes is larger than simple phenols which decreases the share of specific interaction in the more ordered stationary phase region (with THF) to their retention relative to phenols. Furthermore, phenols possess smaller molecular thickness than these hydroxyesters which can additionally lead to a relative retention decrease in the more ordered THF system. Other relationships concerned with molecular size are observed for the three esters discussed, which form a homologous series. Their difference of retention relative to benzene derivatives with one electron-donor group is reduced according to the increase in their molecular volume. This can indicate the decrease in participation of specific interaction to the retention in the THF system with an increase in molecular volume of the hydroxyesters. On the other hand, the correlation line of phenols also converge with the points of the hydroxyesters according to the increase in molecular size (propyl 4-hydroxybenzoate is close to the correlation line for monophenols). It means that an increase in the length of the flexible aliphatic chain in the homologous ester molecules does not decrease the contribution to the specific interaction with THF molecules to such an extent as phenols with larger molecular volume, e.g. the rigid naphthol molecule (compare also the discussion in an earlier paper [35]).

Otherwise phenol derivatives with a nitro group in the 3- or 4-position show increased retention relative to monophenols in the THF system than in the ACN system. Their points are above the correlation line for mono-phenols. This effect can also be explained by a stronger specific interaction in the stationary phase region with extracted THF molecules due to their enhanced effective hydrogen bond acidity (see above), in comparison to simple phenols (the α_2^H values are equal to 0.60, 0.52, 0.57 and 0.61, respectively for phenol, 2-methylphenol, 4-methylphenol and 1-naphthol) [48].

However, the point of 2-nitro-4-chlorophenol is positioned above the correlation line for monophenols that indicates stronger retention in the THF system in comparison to the ACN one. If one considers the analogy between 2-nitrophenol and 2-nitro-4-chlorophenol, then such an increase in retention is less probable. But it is well known that the chlorine atom enhances the hydrophobic character of the molecule [36]. The hydrophobic character of the stationary phase region in the THF system is also enhanced relative to that of the ACN system, which is reflected in the behavior of benzene and toluene. Their points are shifted almost onto the correlation line of monophenols. Benzene and toluene molecules cannot specifically interact with components of the stationary phase region in the tetrahydrofuran system, only enhanced contribution of van der Waals interactions can explain this effect. Then a similar behavior of 2-nitro-4-chlorophenol can also be explained.

3.2. Correlation of retention in tetrahydrofuran and methanol systems

Both modifiers can form hydrogen bonds with proton-donor solutes (hydrogen bond basicity, β_1, of methanol is equal to 0.62). However, only methanol as proton donor can form hydrogen bonds with electron donor solutes. Hydrogen bond acidity, α_1, of methanol is equal to 0.93 and tetrahydrofuran to 0. Due to the smallest molecular volume and weakest extraction of methanol, among the modifiers investi-

gated, into the stationary phase [58–60], its influence on the ordering of the stationary phase region and relative retention change due to interaction in the stationary phase should be limited. Taking into account these properties and much stronger sorption of THF than MeOH by C_{18} phase, some relative retention changes can be interpreted.

In Fig. 4, retention data obtained in THF and MeOH systems are correlated. It is worthwhile to mention that the discussion presented in the previous paper [35] is in accordance with the results for the monosubstituted aromatic derivatives in spite of application of stationary phase from another source. In general, the retention increase in solutes with a proton-donor group (solid line) relative to that with a proton-accepting group (dotted line) is observed in the THF system in comparison to the MeOH system. This means that in spite of lower ability of THF than MeOH to form solvates with solutes by hydrogen bonding, in the stationary phase region, the stronger sorption of tetrahydrofuran predominates over this effect leading to an increase in monophenol retention relative to benzene derivatives with one electron donor group in the THF system in comparison to the MeOH system. It is noteworthy that the electron donor solutes show distinct dispersion of points, greater than for correlated systems THF versus ACN (Fig. 1), that indicates presumably various ability of electron donor solutes to interact in the stationary phase region with methanol molecules by hydrogen bonding in spite of lower content of methanol in the stationary phase [48].

Considerable dispersion of points is observed in Fig. 4 for solutes with two or more electron-donor groups, especially for nitrobenzenes. This dispersion is probably caused by different properties of the stationary phase regions of both modifier systems. The retention of nitrobenzenes decreases in the order nitrobenzene > 1,4-dinitrobenzene > 1,3,5-trinitrobenzene in the methanol system but increases in the tetrahydrofuran system. This order in the methanol system could be expected based on solvophobic interactions of the solutes in the mobile phase and hydrophobic interactions in the stationary phase. The retention of benzene derivatives should decrease with the number of hydrophilic nitro groups. The effect supports the hypothesis of smaller influence of methanol molecules on retardation of nitrobenzene molecules by interaction in the stationary phase region especially by H-bond formation due to lower content of the modifier in the stationary phase region. Additional significance of hydrophobic interactions of the methanol system is demonstrated by retention change in 1,4-dinitrobenzene in comparison

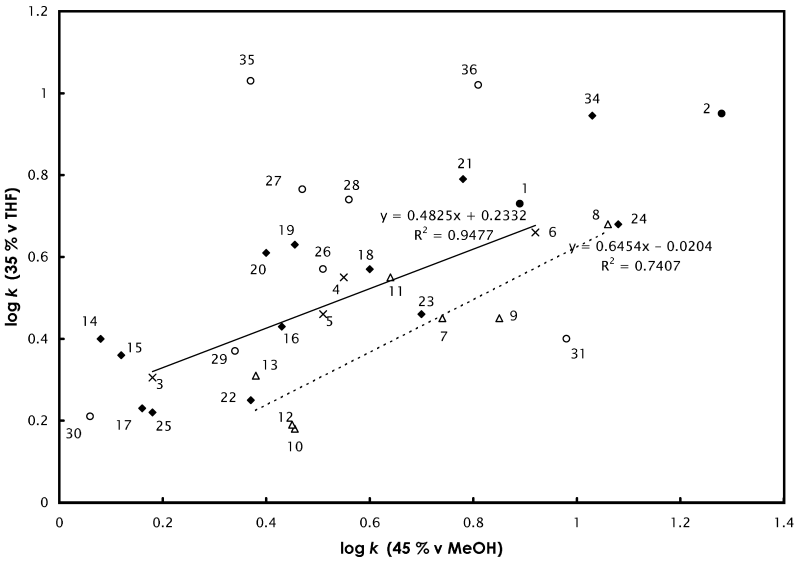

Fig. 4. Log k in 35% v/v THF plotted against log k in 45% v/v MeOH. Notation as in Fig. 1 and solute numbers as in Table 1.

to 3,3′-dinitrodiphenyl. The first solute shows slightly stronger retention in the THF system than the second one, the effect is discussed above (Fig. 1). But these solutes show opposite retention order in the MeOH system which indicates that solvophobic effects in the mobile phase and hydrophobic interactions in the stationary phase (both effects increase the retention of the solute if the volume of its hydrophobic moiety increases) are dominant in the retention mechanism in the methanol system but not in the tetrahydrofuran system.

On the other hand, dimethyl isophthalate (benzene ring with two branched substituents) is located considerably below the correlation line for solutes with one electron-donor group. This means that this solute is characterized by a retention increase in the methanol system relative to the tetrahydrofuran system and relative to solutes with one electron-donor group and other solutes with two electron-donor substituents which are not of the branched shape and show more or less coplanarity with benzene ring, e.g. 4-cyanobenzaldehyde and 4-nitrobenzaldehyde. The points of the two last solutes are located between the correlation lines for monophenols and solutes with one electron-donor group. A similar dependence as for dimethyl isophthalate is found for methyl benzyl terephthalate. The log k values of the solute in 50% MeOH and 30% THF are equal to 1.82 and 1.62, respectively; Δ log k values between the solute and, for example, ethyl phenylacetate in these systems are 0.97 and 0.72, respectively indicating a much stronger retention increase in the methanol system than in the tetrahydrofuran system of the solute with two branched groups in comparison to the solute with one branched group. These behaviors are the next examples which evidence that the shape of the relatively small molecules is an important element influencing selectivity differences between modifier systems.

1,5-Dihydroxynaphthalene and 1,6-dihydroxynaphthalene show increased retention in comparison to monophenols in THF systems, whereas the point of 1,7-dihydroxynaphthalene is positioned on the correlation line for monophenols that indicates its increased retention relative to these dihydroxynaphthalenes in the MeOH system. Probably, for steric reasons, proximity of two hydroxyl groups in 1,7-dihydroxynaphthalene is more favorable to the formation of solvated species in the stationary phase region with self-associated methanol molecules or/and methanol/water complexes [67] than it could be in the case of 1,5- or 1,6-dihydroxynaphthalenes. If one hydroxyl group of 1,5- or 1,6-dihydroxynaphthalene is responsible for formation of solvates with extracted methanol molecule/s then the second hydroxyl group is more probably directed to the mobile phase.

The solutes with one hydroxyl group and one electron-donor group show variable retention changes. Generally, nitrophenols are more strongly retained than monophenols and even dihydroxynaphthalenes in the THF system (their points are located above the correlation line for monophenols) relative to MeOH system. The behavior can be explained in a similar way as discussed above where the retention data for these solutes in THF against ACN systems (Fig. 1) are correlated. Notwithstanding, some solutes show characteristic position of their points on the plots. For instance, the point of 2-nitrophenol is located above the correlation line of monophenols which is contrary to the data presented in Fig. 1. However, in this case, the participation of the interactions with methanol molecules is diminished by lower content of this modifier in the stationary phase region, internal H bond of 2-nitrophenol and its relatively low values of hydrogen bond basicity and hydrogen bond acidity [48]. A characteristic retention change is also shown by 2-nitro-4-chlorophenol the point of which is located high above the correlation line for monophenols indicating the marked relative retention increase in the THF system against the MeOH system is even greater than it was in the case between correlated systems THF versus ACN. The explanation of such an effect is similar as for 2-nitrophenol. However, some additional observations concerned with the data for benzene and toluene presented in Fig. 4 are essential for explanation of this behavior. The points of benzene and toluene are above the correlation line for monophenols which is also contrary to the data presented in Fig. 1. This indicates that the hydrophobic character of the stationary phase region with THF is stronger than for MeOH. So participation of hydrophobic interactions in the stationary phase region modified with THF should increase in comparison to that with MeOH and with ACN. The discussion

indicates that the difference between hydrophobic properties of the stationary phase in THF and ACN systems is smaller than that between THF and MeOH systems. So it should explain the position of the point of 4-chloro-2-nitrophenol on the plot because the chlorine atom usually increases the hydrophobic character of the molecule.

On the other hand, the point of 2-cyanophenol is located below the correlation line for monophenols which indicates the decrease in participation of specific interactions relative to monophenols in the stationary phase modified with THF probably by the shielding effect of the cyano group. According to preferable interaction of methanol with aliphatic alcohols in comparison to phenols, stronger retention of 4-nitrobenzalcohol relative to monophenols is observed in the MeOH system than in the THF system. A similar behaviour was observed for non-substituted phenylaliphatic alcohols [35] (and *n*-alkanols also show similar dependence [68]) which demonstrate even stronger relative retention in comparison to benzene derivatives substituted with one electron-donor group in similar methanol system (THF vs. MeOH) [35]. The effect can be explained by the difference in their ability to stabilize the partial negative charge upon hydrogen bonding [68] with methanol molecules. However, the point of 4-nitrobenzalcohol is located between two correlation lines for mono-substituted aromatics (monophenols and benzene derivatives with one electron-donor group). This means that the nitro group substituted in the benzene ring increases the retention of the solute in the THF system in comparison to ACN or MeOH systems probably due to the planar molecular structure and especially the quadrupole interaction with THF as discussed above. This is the next evidence of positive influence of the nitro group on the retention increase in the tetrahydrofuran system. So this means that the effect of the nitro group of benzene derivatives is the key element causing selectivity variation in the tetrahydrofuran system relative to other modifier systems.

A much stronger retention decrease in phenols substituted with ester groups (methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate) relative to monophenols is observed in the THF system in comparison to the MeOH system. The effect is probably concerned with the branched shape of the ester group and its thickness which decreases the ability of solute molecules to penetrate the solvated region of the stationary phase in THF system in comparison to the less ordered and less solvated methanol system.

3.3. Correlation of retention in acetonitrile and methanol systems

Acetonitrile and methanol differentiate the properties of the stationary phase region by solvation effect. The extraction of ACN to the C_{18} stationary phase is stronger than MeOH. However even similar composition of the mobile phase components in the stationary phase region and in the eluent were reported in systems with MeOH [69]. However, usually a positive excess of methanol sorption by hydrocarbonaceous phases is reported in the concentration range of the bulk phase investigated [58–60,70]. Therefore the stationary phase in the ACN system should be characterized by stronger ordering than in the methanol system and as a consequence the shape selectivity between these systems should be observed to a lesser extent than it is in the tetrahydrofuran system. On the other hand both CH_3OH and CH_3CN molecules are relatively small, of similar molecular volume and both with a small hydrophobic moiety ($-CH_3$). This does not lead to the hydrophobicity increase in the stationary phase by the modifier extraction as takes place in the case of THF.

In Fig. 5, the retention of solutes investigated is correlated as methanol (45%) against acetonitrile (30%) system. The dispersion of points is less pronounced than in the previous two figures discussed (Figs. 1 and 4). The data for some monosubstituted aromatics are also presented and confirm the similarity of the relationship previously obtained [35]. The influence of the second hydroxyl group can be discussed taking into account the data of 1,5-, 1,6- and 1,7-dihydroxynaphthalenes. The first two solutes are characterized by their points positions slightly below the correlation line for monophenols. However, 1,7-dihydroxynaphthalene is located above the correlation line indicating that its retention is more strongly increased in the MeOH system relative to monophenols than is the case for the acetonitrile system (see discussion for Fig. 4) above.

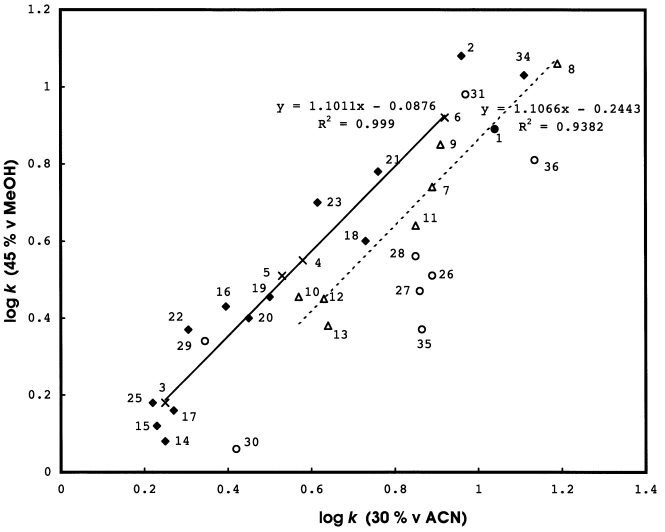

Fig. 5. Log k in 45% v/v MeOH plotted against log k in 30% v/v ACN. Notation as in Fig. 1 and solute numbers as in Table 1.

The presence of the nitro group in the 4- or 3-position of the phenol molecule does not cause retention changes relative to monophenols; their points lay on the correlation line for monophenols. 2-Nitrophenol shows a decreased retention. So the dependence for the nitrophenols indicates that participation of the specific interaction in or on the stationary phase with methanol is important to the selectivity differences in comparison to the acetonitrile system. Probably in this case, the stronger ability to specific interaction of 3- and 4-nitrophenols with methanol in the stationary phase is compensated by dipole–dipole interaction in the stationary phase of the acetonitrile system and perhaps by the higher degree of its ordering.

However, the contribution of the last effect should be considered with regard to the relationships for phenol derivatives with an ester group in the 4-position. Somewhat increased retention of the derivatives relative to monophenols is observed in the methanol system. This can be explained by the presence of stronger restrictions to the penetration of the stationary phase by the solute molecules in the acetonitrile system than can take place in the methanol systems. This can mean that for these systems the influence of molecular shape does play some role in retention and selectivity changes. Nitro or cyano groups in the 2-position relative to the hydroxyl group in phenol molecule apparently decrease the retention of these solutes in comparison to phenols in the methanol system. The effect can be explained by decreased participation of specific interactions in the stationary phase with methanol due to the shielding effect of the –OH group or competitive intramolecular interaction. The position of the point of 4-nitrobenzalcohol is remarkable because it is practically located on the correlation line for monophenols. On the other hand, aliphatic alcohols are more strongly retained than monophenols in the methanol system relative to the acetonitrile system [34,35]. It is remarkable that in the ACN system there is a minor retention difference between nitrocompounds (compare also Figs. 1 and 4). This group of compounds shows much stronger retention than the remaining solutes in the ACN system relative to the MeOH system. This probably means that dipole–dipole interactions between nitrobenzenes and acetonitrile molecules in the stationary phase region play a greater role in the retention than in the methanol system. The driving force for retention of particular nitrobenzenes from the side of the mobile phase in both modifier systems is probably similar but the driving forces from the side of the stationary phase are quite different in acetonitrile and methanol systems. Due to low extraction of methanol its participation to increase the retardation of nitro-

benzenes should not be as strong as the dipolar influence of acetonitrile on this effect. However, in the methanol system the hydrophobic interactions in the stationary phase should be revealed as the dominant effect which differentiate that system from the acetonitrile system. This seems to be confirmed by the retention decrease in nitrobenzenes in the methanol system according to the number of nitro groups in the molecule: nitrobenzene, 1,4-dinitrobenzene, 1,3,5-trinitrobenzene. The hydrophobic properties of the molecules also decrease in the same order. Then the driving forces for retention change in nitrobenzenes in the methanol system from both mobile and stationary phases are congruent. However in the acetonitrile system, the two tendencies: one hydrophilic from the mobile phase, increasing with the number of nitro groups in the molecule and the second dipolar from the stationary phase region act in opposite directions leading to compensation of their influence on retention. So we can see almost similar retention of these nitrobenzenes in the acetonitrile system.

4. Conclusions

Correlation of the retention of aromatic solutes with polar groups in methanol, acetonitrile and tetrahydrofuran systems shows strong differences in selectivity especially for the systems tetrahydrofuran versus acetonitrile and tetrahydrofuran versus methanol. The differences are caused mainly by diverse interactions in the stationary phase region of C_{18} type adsorbent and depend on the type of the modifier. The specific interaction of the solute and modifier in the stationary phase leads to a retention increase relative to the system with modifier which does not display a tendency to this interaction or shows weaker interaction than the first modifier.

Solutes with one proton-donor group, such as phenols, show increased retention relative to solutes with an electron-donor group in tetrahydrofuran system in comparison to methanol or acetonitrile systems. A similar behavior of retention increase in phenols relative to benzene derivatives with one electron-donor group is observed in the methanol system against the acetonitrile system but to a lesser extent.

Larger and rigid molecules show a lower contribution of specific interactions by H-bond formation to relative retention changes in ordered stationary phase in comparison to smaller ones. The increase in the volume of the hydrophobic part of the solute molecule leads to a decrease in the participation of the specific interactions in the stationary phase to the retention.

The benzene derivatives with two proton-donor groups (–OH) can specifically interact with the modifier molecules in the stationary phase; increased retention relative to solutes with one group is observed in comparison to the system with modifier which shows weaker interaction of this type and its extraction into the stationary phase is weaker. The effect depends on the position of the groups in the solute molecule.

The number of nitro groups in the molecule seems to be the key agent influencing selectivity and retention in the THF system relative to acetonitrile and methanol systems.

The shielding effect of the –OH group caused by proton-acceptor group decreases retention in the tetrahydrofuran system relative to the acetonitrile and methanol systems. However, molecules which maintain their planar structure can further show increased retention in the THF system relative to methanol and acetonitrile systems.

Phenol derivatives with a second group which enhances the proton-donor property of the –OH group, show increased retention in the tetrahydrofuran system relative to acetonitrile and methanol systems.

A branched group in the solute molecule decreases retention in the tetrahydrofuran system relative to the methanol and acetonitrile systems.

Phenol derivatives with a second group of branched structure (ester group in the 4-position) have decreased retention in comparison to monophenols in the tetrahydrofuran system relative to acetonitrile and methanol systems and decrease the retention in the acetonitrile system relative to methanol system but to a lesser extent.

Planar structure of the solute molecule increases the retention in the tetrahydrofuran system relative to acetonitrile and especially to methanol system.

The selectivity changes with regard to the shape of the solute molecule are more strongly pronounced

for the THF versus MeOH system and THF versus ACN system than for MeOH versus ACN system.

Ordering of the stationary phase by modifier of the binary water eluent increases in the series: methanol, acetonitrile, tetrahydrofuran.

It should be mentioned that the rules above refer to the liquid chromatography systems which were investigated in the paper (C_{18} stationary phase in system with 35% v/v THF, 30 and 35% v/v ACN and 45% v/v MeOH). The extension of the rules to systems with another stationary phase of the C_{18} type and/or eluents of different concentration ranges can lead, but not necessarily, to other retention and selectivity predictions than those described above.

The conceptual approach to the explanation of selectivity variations presented in the paper seems to be useful for optimization of separation of mixtures in similar chromatographic systems.

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