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## Retention of *ortho*- and *para*-Positional Isomers of Some Model Solutes on Polar Bonded Stationary Phases in Different Eluent Systems by HPTLC

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**Abstract:** The influence of a substituents' position on the retention of isomer pairs on polar bonded stationary phases has been examined. The pairs of *ortho* and *para* substituted phenols and anilines were chromatographed on aminopropyl, diol, and cyanopropyl layers by use of nonaqueous eluents consisting of n-heptane and polar modifier (tetrahydrofuran, ethyl acetate, or 2-propanol) or on cyanopropyl layer in RP systems with aqueous solutions of methanol or acetonitrile as eluents. Results obtained are presented graphically and as parameters of  $R_M$  vs.  $\log X$  (modifier concentration) relationships. The structural effects have been discussed. Stronger retention and higher slope values of *para* derivatives in comparison to *ortho* were ascertained for substituted phenols and anilines, which is typical for silica and magnesium silicate surfaces. Solutes with two strongly polar groups behave differently; in some cases, *ortho* isomers are adsorbed more strongly than *para* isomers on polar bonded stationary phases, in some eluents containing a high concentration of polar modifier in a non-aqueous mobile phase. Similar retention behavior has been observed for *ortho* derivatives, which can interact with active alumina surface centers. The separation selectivity has been analysed by  $\Delta R_M$  values.

**Keywords:** Polar bonded stationary phases, HPTLC, CN-, Diol-, NH<sub>2</sub>-, NP systems, RP systems, Retention behavior, Structural effects, *Ortho* effect

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## INTRODUCTION

The relationship between the chemical structures of compounds and their chromatographic behavior has been considered by many scientists and was first reported for partition chromatography. It was deduced that, for ideal conditions, the partition coefficient of a substance A between two phases is related to the free energy required to transport one mole of A from one phase to a second one. It can be regarded as a sum of partial work connected with a transfer of individual functional groups from phase to phase as an additive value.

Analogously, retention factors in chromatography are also additive. The complex character of the chromatographic process (change of composition and volume proportions of phases), ionization of substances, as well as constitutional effects in molecule–reciprocal interactions of functional groups (internal hydrogen-bond effects, steric, and electromeric effects), cause deviations from the  $R_M$  additivity.<sup>[1]</sup>

For adsorption chromatography, a quantitative adsorption model combining molecular structure and retention coefficient work was carried out by Snyder.<sup>[2]</sup> The following factors influence the adsorption energies of molecules:<sup>[2]</sup> molecular planarity, steric hindrance, chemical interaction of adjacent functional groups (H-bonding), electronic interactions of some functional groups (induction and mesomeric effects), and simultaneous adsorption of two adjacent polar functional groups (“anchorage” effect).

Retention in chromatographic systems can also be combined with the properties of chromatographed compounds and should manifest themselves in Quantitative Structure-Retention Relationships (QSRR) equations.<sup>[3]</sup> At present, the most often reported QSRR analyses of the molecular mechanism of chromatographic reversed-phase process employ the solvatochromic comparison method as a means of correlating retention parameters ( $\log k$ ) with a variety of analytes and chromatographic system properties, such as solute descriptors  $R_2$ —excess molar refraction,  $\pi_2^H$ —solute dipolarity—polarizability,  $\alpha_2^H$ —solute hydrogen bond acidity,  $\beta_2^H$ —solute hydrogen bond basicity,  $V_x$ —solute molar volume,  $\mu$ —total dipole moment of an analyte molecule,  $\delta_{Min}$ —electron excess charge of the most negatively charged atom,  $A_{WAS}$ —water accessible van der Waals surface area of the molecule, and others.

The relationship between the solute structures and the retention presents the possibility of their separation and also presents information about the character of interactions in the chromatographic system.

Comparison of the retention parameters of model solutes and their chemical structures enables the analysis of the structure of different adsorbent surfaces and distribution of their active centers. The special role of the geometry of active centers on the adsorbent surface and their chemical character is performed in adsorption of positional isomers. A large

nonpolar substituent near the polar group disturbs its interaction with the adsorbent active centers and usually causes a decrease of its adsorption energy. However, molecules with two polar groups interact in a different manner on various adsorbent surfaces. On silica, where a significant role is played by the hydroxyl groups (H-bond interactions),<sup>[2]</sup> substances forming internal hydrogen bonds have relatively weak adsorption energy in comparison with *para* isomers. On alumina, the strong adsorption of some *ortho* substituted compounds is caused either by simultaneous interactions of substituents with one active center on the adsorbent surface ( $\text{Al}^{3+}$ ),<sup>[2]</sup> or by the “anchorage” effect of both substituents on the surface.<sup>[4,5]</sup>

Similarities in behaviour of *ortho* and *para* substituted isomers on Florisil and silica demonstrate the comparable distribution of some active centers (e.g., OH groups) on the Florisil (magnesium silicate) surface in comparison with silica.<sup>[6]</sup> Conversely, detailed analysis of the retention behavior of positional isomers on the mixed silica-magnesia adsorbent presents information about strong interactions of molecules substituted with two polar groups in the *ortho* position in comparison to the *para* isomers. This demonstrates similarities of mixed adsorbent with an alumina surface, which is probably due to the presence of  $\text{O}^{2-}$  ions as surface active centers.<sup>[7]</sup>

The aim of this work was to demonstrate the retention of positional isomers on polar bonded stationary phases CN-, Diol-, and  $\text{NH}_2$ -silica in normal-phase systems and, also, on CN-silica in reversed-phase systems. The comparison was performed by the retention–modifier concentration relationships and their parameters. The comparison of retention behavior of positional isomers on polar bonded stationary phases can explain the chemical character of their active centers and also can show the differences and similarities of their surfaces with silica or alumina, the most popular adsorbents applied in TLC.

## EXPERIMENTAL

Thin layer chromatography was performed on  $10 \times 10$  cm glass Diol F<sub>254</sub>, CN F<sub>254</sub>, and  $\text{NH}_2$  F<sub>254</sub> HPTLC precoated plates (E.Merck, Darmstadt, Germany) in horizontal teflon chambers with an eluent distributor (DS, Chromdes, Lublin, Poland). Samples (2  $\mu\text{L}$ ) of 2.5% w/v solutions of the solutes in methanol were spotted to the adsorbent layer. The plates were conditioned for 15 minutes in eluent vapours to eliminate the demixing effect and developed face-down at a distance of 8 cm from the origin at  $20 \pm 1^\circ\text{C}$ . Binary mixtures of polar modifiers: 2-propanol (iPrOH), ethyl acetate (AcOEt), tetrahydrofuran (THF) with n-heptane (H), or acetonitrile (ACN) or methanol (MeOH) with bidistilled water were used as eluents. Solvents were analytical grade from Polish Reagents (POCh, Gliwice, Poland). The location of the spots was determined under UV light ( $\lambda = 254$  nm). In all

cases, the spots were symmetrical and did not exhibit tailing. The investigated compounds are listed in Table 1.

## RESULTS AND DISCUSSION

Table 1 presents  $\Delta R_M$  ( $R_{Mp} - R_{Mo}$ ) values for eight investigated pairs of isomers. The values are, in most cases, positive, which means stronger adsorption of *para* isomers than *ortho* ones on polar bonded stationary phases. The separation selectivity from the values can be also compared. It is seen that, in most cases, the highest separation selectivity was obtained on  $\text{NH}_2$ -silica. The poorest separation selectivity was obtained on CN-silica in RP systems for polar compounds.

The retention, mobile phase composition relationships in the systems of the polar adsorbent/binary eluent can be expressed as the linear plots of  $\log k$  ( $R_M$ ) vs.  $\log X$  ( $X$ , molar fraction of polar modifier in eluent).<sup>[8]</sup> This proves the simple displacement model of the retention of chromatographed substances in normal-phase systems. It is assumed that, in the model displacement of a polar solvent molecule from the monolayer on the adsorbent surface, it is accomplished by the adsorbed molecule. Slopes of  $\log k$  vs.  $\log X$  plots presents information about interactions of chromatographed substances with the adsorbent surface.

The experimental results obtained using HPTLC are demonstrated in Tables 2–5 as parameters of  $R_M$  vs.  $\log X$  relationships for eight pairs of *para* and *ortho* isomers of phenols and anilines chromatographed on polar bonded stationary phases in various eluent systems. Tables 2–4 show parameters for retention–eluent composition plots for investigated compounds separated on polar bonded stationary phases in normal-phase systems. The obtained relationships are linear in accordance with the displacement retention model.<sup>[8]</sup> The linearity of plots is confirmed by regression coefficients, in most cases  $R > 0.98$  and  $F$  being statistically higher than  $F_{\text{critical}}$ . Only in 18 out of 142 cases were regression coefficient values lower than  $0.93 < R < 0.97$ . This permits us to draw conclusions about the retention mechanism from the slopes of  $R_M$  vs.  $\log X$  plots. Unit slopes mean one-point adsorption, and such values are usually obtained for monofunctional compounds. The presence of one methyl group in a molecule usually does not cause any significant change in the slope of the  $R_M$  vs.  $\log C$  lines,<sup>[9]</sup> irrespective of the positions of these groups. In our experiments, slopes near unity are observed for cresols, irrespective of the position of methyl group, for ethylanilines, irrespective of the position of the ethyl group, and also for xylenols (see Tables 2–4). It should be noticed that, for alkyl-substituted phenols and anilines, often the slopes are less than unity, which confirms flat adsorption of the molecule on the surface. Unit slopes obtained for bifunctional solutes presumably indicate complete delocalization of the weaker functional group.

**Table 1.** Values of  $\Delta R_M = R_{Mp} - R_{Mo}$  for pairs of positional isomers chromatographed in investigated systems

Solutes		$\Delta R_M(R_{Mp} - R_{Mo})$										
		NH <sub>2</sub>			DIOL				CN			
Name of compounds	Abbreviation	30% THF/H	30% AcOEt/H	30% iPrOH/H	30% AcOEt/H	30% iPrOH/H	30% THFH	30% AcOEt/H	30% THF/H	30% iPrOH/H	30% ACN/H <sub>2</sub> O	30% MeOH/H <sub>2</sub> O
2-Cresol	2MePh	-0.04	0.00	0.09	0.00	0.02	0.00	0.00	0.02	0.05	0.02	0.05
4-Cresol	4MePh											
Resocriinol	12OHB	0.28	0.49	0.50	0.50	0.63	0.54	0.48	0.36	0.36	0.00	-0.04
Hydroquinonel	14OHB											
2,3-Xylenol	23MePh	0.26	0.25	0.09	0.07	0.39	0.09	0.10	0.05	0.00	0.03	0.06
3,5-Xylenol	35MePh											
1,2-Phenyleno-diamine	12AB	0.24	0.28	0.31	0.19	0.08	0.00	0.38	0.06	-0.35	0.55	0.55
1,4-Phenyleno-diamine	14AB											
1-Naphthol	1OHN	-0.05	-0.06	0.04	0.08	0.03	0.04	0.04	0.07	-0.10	0.02	-0.07
2-Naphthol	2OHN											
2-Ethylaniline	2EtAn	0.22	0.14	0.12	—	0.19	0.18	0.05	0.14	0.07	0.14	0.08
4-Ethylaniline	4EtAn											
2-Aminophenol	2APh	0.94	—	0.22	0.17	0.28	0.57	0.65	0.46	0.18	0.32	0.13
4-Aminophenol	4APh											
2-Nitrophenol	2NtPh	0.83	—	0.73	0.32	-0.12	0.26	0.10	0.11	—	0.16	0.10
4-Nitrophenol	4NtPh											

**Table 2.** Parameters of  $R_M = a + b \log X$  equations obtained for investigated compounds chromatographed on  $\text{NH}_2$ -silica with nonaqueous eluents

	Slope	Intercept	s	F	R	n
$\text{NH}_2$ , THF/H (10–50%)						
2MePh	$-1.22 \pm 0.08$	$-0.08 \pm 0.5$	0.05	195.9	0.99	5
4MePh	$-1.16 \pm 0.04$	$-0.14 \pm 0.02$	0.02	692.9	0.99	5
12OHB	$-0.74 \pm 0.08$	$0.22 \pm 0.04$	0.04	83.6	0.98	5
14OHB	$-2.09 \pm 0.09$	$-0.09 \pm 0.05$	0.05	467.5	0.99	5
23MePh	$-0.97 \pm 0.03$	$-0.14 \pm 0.02$	0.02	1100.5	0.99	5
35MePh	$-1.09 \pm 0.18$	$0.07 \pm 0.09$	0.09	36.0	0.96	5
12AB	$-1.79 \pm 0.10$	$0.14 \pm 0.06$	0.05	303.1	0.99	5
14AB	$-2.03 \pm 0.32$	$0.24 \pm 0.13$	0.09	39.9	0.98	4
1OHN	$-1.23 \pm 0.10$	$0.02 \pm 0.05$	0.05	143.3	0.99	5
2OHN	$-1.09 \pm 0.08$	$0.03 \pm 0.04$	0.04	196.8	0.99	5
2EtAn	$-0.74 \pm 0.06$	$-0.23 \pm 0.03$	0.03	156.6	0.99	5
4EtAn	$-0.84 \pm 0.14$	$-0.10 \pm 0.08$	0.06	37.7	0.97	4
2APh	$-2.16 \pm 0.12$	$-0.16 \pm 0.06$	0.06	338.8	0.99	5
4APh	$-4.29 \pm 0.14$	$-0.16 \pm 0.05$	0.02	918.0	0.99	3
2NtPh	$-1.24 \pm 0.10$	$0.08 \pm 0.05$	0.05	160.6	0.99	5
4NtPh	$-2.02 \pm 0.14$	$0.51 \pm 0.06$	0.04	197.4	0.99	4
$\text{NH}_2$ , AcOEt/H (20–60%)						
2MePh	$-0.99 \pm 0.19$	$-0.04 \pm 0.08$	0.07	26.3	0.95	5
4MePh	$-0.99 \pm 0.19$	$-0.04 \pm 0.08$	0.07	26.3	0.95	5
12OHB	$-0.83 \pm 0.10$	$0.25 \pm 0.04$	0.04	71.6	0.98	5
14OHB	$-2.15 \pm 0.22$	$0.01 \pm 0.09$	0.08	97.1	0.98	5
23MePh	$-0.99 \pm 0.09$	$-0.12 \pm 0.04$	0.03	127.4	0.99	5
35MePh	$-1.80 \pm 0.21$	$-0.17 \pm 0.09$	0.08	72.9	0.98	5
12AB	$-1.99 \pm 0.41$	$0.07 \pm 0.17$	0.15	23.5	0.94	5
14AB	$-2.61 \pm 0.15$	$-0.05 \pm 0.06$	0.05	304.2	0.99	5
1OHN	$-1.06 \pm 0.13$	$0.10 \pm 0.05$	0.05	70.0	0.98	5
2OHN	$-1.18 \pm 0.13$	$0.03 \pm 0.05$	0.05	84.2	0.98	5
2EtAn	$-0.69 \pm 0.06$	$-0.24 \pm 0.02$	0.02	150.6	0.99	5
4EtAn	$-0.94 \pm 0.05$	$-0.23 \pm 0.02$	0.02	336.2	0.99	5
2APh	$-1.94 \pm 0.08$	$-0.09 \pm 0.04$	0.03	529.9	0.99	5
4APh	—	—	—	—	—	—
2NtPh	$-0.79 \pm 0.08$	$0.32 \pm 0.04$	0.03	91.4	0.98	5
4NtPh	—	—	—	—	—	—
$\text{NH}_2$ , iPrOH/H (10–50%)						
2MePh	$-0.85 \pm 0.05$	$-0.26 \pm 0.02$	0.02	359.1	0.99	5
4MePh	$-0.84 \pm 0.03$	$-0.22 \pm 0.02$	0.02	642.2	0.99	5
12OHB	$-0.86 \pm 0.12$	$0.10 \pm 0.06$	0.06	49.0	0.97	5
14OHB	$-1.89 \pm 0.08$	$0.18 \pm 0.04$	0.04	622.2	0.99	5
23MePh	$-0.78 \pm 0.01$	$-0.26 \pm 0.01$	0.005	4698.8	0.99	5

(continued)

Table 2. Continued

	Slope	Intercept	s	F	R	n
35MePh	$-0.96 \pm 0.04$	$-0.26 \pm 0.02$	0.02	632.2	0.99	5
12AB	$-1.06 \pm 0.06$	$0.17 \pm 0.03$	0.03	363.9	0.99	5
14AB	$-1.9 \pm 0.06$	$0.17 \pm 0.03$	0.03	1172.4	0.99	5
1OHN	$-0.81 \pm 0.08$	$-0.11 \pm 0.04$	0.04	104.8	0.99	5
2OHN	$-0.78 \pm 0.06$	$-0.06 \pm 0.03$	0.03	167.3	0.99	5
2EtAn	$-0.46 \pm 0.02$	$-0.25 \pm 0.01$	0.01	352.5	0.99	5
4EtAn	$-0.64 \pm 0.02$	$-0.18 \pm 0.01$	0.01	1036.1	0.99	5
2APh	$-1.56 \pm 0.06$	$0.11 \pm 0.03$	0.03	735.2	0.99	5
4APh	$-1.90 \pm 0.20$	$0.23 \pm 0.10$	0.10	86.5	0.98	5
2NtPh	$-0.88 \pm 0.10$	$0.23 \pm 0.05$	0.05	79.5	0.98	5
4NtPh	$-2.03 \pm 0.80$	$0.54 \pm 0.25$	0.11	5.8	0.92	3

Such a situation occurs when the second functional group is an apolar one, for example, an alkyl chain or fused ring.

The fundamental conditions of two-point adsorption for solutes are sufficient adsorption energies of the two groups and a suitable distance between the groups. The experimental data seem to indicate that, for two-point adsorption on silica, the distance between two functional groups should exceed 5.0 Å; since two-point adsorption is observed for both *para* and *meta* isomers.<sup>[9]</sup> The second important factor which determines two-point adsorption is the adsorption energy of the two groups. In the case of polar bonded stationary phases, it occurs for substances with two strongly polar functional groups, e.g., for hydroquinone (14OHB), for 1,4-phenylenediamine (14AB) 4-aminophenol (4APh), and, in most cases, for 4-nitrophenol (4NtPh). It can be also mentioned that *ortho* substituted solutes behave, on polar bonded stationary phases, like monofunctional solutes. In most cases, *ortho* derivatives have a slope near unity, for example pyrocatechine (12OHB), 2-nitrophenol (2NtPh), and, in some cases, 1,2-phenylenediamine (12AB), whereas the slope values for analogous *para* isomers are much higher. In most cases, slope values for *para* substituted compounds are higher than for *ortho* substituted ones. This is caused by the *ortho* effect, H-bond interactions of two neighbouring polar groups (internally H-bonded groups), which causes their weaker adsorption and one-point interactions with surface active centers (compare slopes of pyrocatechine and hydroquinone, 1,2-phenylenediamine and 1,4-phenylenediamine, 2-aminophenol and 4-aminophenol, and 2-nitrophenol and 4-nitrophenol). It should also be noted that *para* isomers are more strongly retained on the polar bonded stationary phases than *ortho* isomers (see Table 1). However, when one analyzes plots presented in Figures 1 and 2, there are cases of crossing plots for positional isomers, which changes the sequence with the variation of eluent composition. For example, in the



**Table 3.** Parameters of  $R_M = a + b \log X$  equations obtained for investigated compounds chromatographed on Diol-silica with nonaqueous eluents

	Slope	Intercept	s	F	R	n
DIOL, AcOEt/H (20–60%)						
2MePh	$-1.14 \pm 0.09$	$-0.39 \pm 0.04$	0.03	166.9	0.99	5
4MePh	$-1.14 \pm 0.09$	$-0.39 \pm 0.04$	0.03	166.9	0.99	5
12OHB	$-1.09 \pm 0.08$	$-0.08 \pm 0.03$	0.03	200.7	0.99	5
14OHB	$-2.41 \pm 0.09$	$-0.21 \pm 0.04$	0.03	700.2	0.99	5
23MePh	$-0.93 \pm 0.06$	$-0.32 \pm 0.03$	0.02	221.6	0.99	5
35MePh	$-1.09 \pm 0.04$	$-0.32 \pm 0.02$	0.01	926.9	0.99	5
12AB	$-1.84 \pm 0.1$	$0.15 \pm 0.04$	0.03	368.1	0.99	5
14AB	$-2.63 \pm 0.16$	$-0.07 \pm 0.06$	0.03	267.2	0.99	4
1OHN	$-1.08 \pm 0.04$	$-0.30 \pm 0.02$	0.01	927.0	0.99	5
2OHN	$-1.20 \pm 0.04$	$-0.28 \pm 0.02$	0.01	955.0	0.99	5
2EtAn	$-0.87 \pm 0.05$	$-0.26 \pm 0.02$	0.02	283.3	0.99	5
4EtAn	$-1.10 \pm 0.07$	$-0.17 \pm 0.03$	0.03	220.8	0.99	4
2APh	$-1.98 \pm 0.12$	$-0.06 \pm 0.05$	0.04	275.8	0.99	5
4APh	$-1.57 \pm 0.43$	$0.42 \pm 0.15$	0.09	13.2	0.93	4
2NtPh	$-1.14 \pm 0.02$	$-0.19 \pm 0.01$	0.01	2356.2	0.99	5
4NtPh	$-2.05 \pm 0.04$	$-0.29 \pm 0.02$	0.01	2694.2	0.99	5
DIOL, iPrOH/H (10–50%)						
2MePh	$-0.92 \pm 0.06$	$-0.47 \pm 0.03$	0.03	213.2	0.99	5
4MePh	$-0.84 \pm 0.08$	$-0.44 \pm 0.04$	0.04	95.5	0.98	5
12OHB	$-0.94 \pm 0.06$	$-0.21 \pm 0.03$	0.03	232.1	0.99	5
14OHB	$-2.02 \pm 0.32$	$-0.07 \pm 0.12$	0.08	40.8	0.98	4
23MePh	$-0.75 \pm 0.04$	$-0.39 \pm 0.02$	0.02	282.7	0.99	5
35MePh	$-0.85 \pm 0.04$	$-0.44 \pm 0.02$	0.02	389.3	0.99	4
12AB	$-1.29 \pm 0.04$	$0.35 \pm 0.02$	0.02	964.0	0.99	5
14AB	$-2.08 \pm 0.08$	$0.09 \pm 0.03$	0.02	647.9	0.99	4
1OHN	$-0.75 \pm 0.08$	$-0.33 \pm 0.04$	0.04	86.5	0.98	5
2OHN	$-0.79 \pm 0.06$	$-0.30 \pm 0.03$	0.03	149.1	0.99	5
2EtAn	$-0.53 \pm 0.04$	$-0.22 \pm 0.02$	0.02	165.3	0.99	5
4EtAn	$-0.71 \pm 0.04$	$-0.10 \pm 0.02$	0.02	367.4	0.99	5
2APh	$-1.14 \pm 0.07$	$-0.17 \pm 0.03$	0.03	271.9	0.99	5
4APh	$-2.71 \pm 0.12$	$-0.46 \pm 0.06$	0.06	489.0	0.99	5
2NtPh	$-0.90 \pm 0.07$	$-0.28 \pm 0.03$	0.03	168.5	0.99	5
4NtPh	$-1.24 \pm 0.09$	$-0.47 \pm 0.04$	0.04	209.1	0.99	5
DIOL, THF/H (10–50%)						
2MePh	$-1.00 \pm 0.08$	$-0.38 \pm 0.04$	0.04	164.9	0.99	5
4MePh	$-0.97 \pm 0.07$	$-0.35 \pm 0.04$	0.04	188.8	0.99	5
12OHB	$-1.22 \pm 0.15$	$-0.28 \pm 0.08$	0.07	66.8	0.98	5
14OHB	$-2.31 \pm 0.25$	$-0.34 \pm 0.13$	0.12	86.0	0.98	5
23MePh	$-0.83 \pm 0.05$	$-0.34 \pm 0.03$	0.03	234.1	0.99	5

(continued)

Table 3. Continued

	Slope	Intercept	s	F	R	n
35MePh	$-1.1 \pm 0.12$	$-0.37 \pm 0.06$	0.06	85.1	0.98	5
12AB	$-2.43 \pm 0.46$	$0.02 \pm 0.20$	0.12	27.9	0.97	4
14AB	$-2.72 \pm 0.55$	$-0.13 \pm 0.23$	0.14	24.6	0.96	4
1OHN	$-0.85 \pm 0.08$	$-0.24 \pm 0.04$	0.04	117.8	0.99	5
2OHN	$-0.86 \pm 0.07$	$-0.21 \pm 0.04$	0.04	147.4	0.99	5
2EtAn	$-0.68 \pm 0.03$	$-0.23 \pm 0.02$	0.02	421.6	0.99	5
4EtAn	$-1.00 \pm 0.06$	$-0.19 \pm 0.03$	0.03	331.2	0.99	5
2APh	$-2.24 \pm 0.21$	$-0.34 \pm 0.11$	0.11	113.3	0.99	5
4APh	$-2.41 \pm 0.18$	$-0.12 \pm 0.07$	0.05	184.5	0.99	5
2NtPh	$-1.28 \pm 0.11$	$-0.29 \pm 0.06$	0.06	126.1	0.99	5
4NtPh	$-1.57 \pm 0.05$	$-0.17 \pm 0.03$	0.03	893.8	0.99	5

Diol/40% iPrOH and Diol/50% iPrOH system, 1,2-phenylenediamine is more strongly retained than the *para* isomer. It is also remarkable that 2-nitrophenol is more strongly adsorbed on the diol- layer than 4-nitrophenol, when 2-propanol (30, 40, 50%) in n-heptane as eluents are used (see Figure 1). Such behavior of phenylenediamine isomers is observed on the cyanopropyl phase, also, in the 50% THF system in n-heptane (see Figure 2). From the  $\log k$  vs.  $\log X$  parameters presented in Tables 2–4, the analysis of intercept values can also present information about adsorption of *ortho* isomers. The highest intercept ( $\log k$  values in pure modifier) of *ortho* isomers, in comparison to *para*, can be maintained for resorcinol in comparison to hydroquinone on  $\text{NH}_2$ -layer by the use of THF and AcOEt as modifiers, and on Diol layer by the use of all modifiers investigated for 1,2-phenylenediamine, in comparison to 1,4-phenylenediamine on  $\text{NH}_2$ -silica by the use of AcOEt and on Diol and CN layers in all eluent systems investigated for 2-nitrophenol, in comparison to 4-nitrophenol on Diol and CN- layers by the use of AcOEt and iPrOH as eluents. Thus, this means that, on polar bonded stationary phases with eluents of high eluent strength, *ortho* isomers of polar compounds are more strongly retained than *para* isomers. Stronger adsorption of *ortho* derivatives is not typical behavior in comparison to silica and Florisil surfaces, but occurs on alumina, due to anchorage of adjacent polar groups by formation of chelates with  $\text{Al}^{3+}$  ions. For polar bonded stationary phases, such behavior of *ortho* isomers can be explained by simultaneous interaction of neighbouring polar groups with diol-, aminopropyl-, or cyanopropyl- ligands and underivatized surface silanols.

Frequently the description of retention-eluent composition for reversed-phase systems is the semilogarithmic equation;<sup>[10]</sup> however, the equation derived from the displacement model, elaborated primarily for adsorption

**Table 4.** Parameters of  $R_M = a + b \log X$  equations obtained for investigated compounds chromatographed on CN-silica with nonaqueous eluents

	Slope	Intercept	s	F	R	n
CN,AcOEt/H (10–50%)						
2MePh	$-1.37 \pm 0.04$	$-0.46 \pm 0.02$	0.01	1227.6	0.99	5
4MePh	$-1.37 \pm 0.04$	$-0.46 \pm 0.02$	0.01	1227.6	0.99	5
12OHB	$-1.76 \pm 0.07$	$-0.35 \pm 0.03$	0.03	588.9	0.99	5
14OHB	$-2.96 \pm 0.13$	$-0.26 \pm 0.05$	0.05	529.8	0.99	5
23MePh	$-1.08 \pm 0.06$	$-0.40 \pm 0.02$	0.02	380.2	0.99	5
35MePh	$-1.38 \pm 0.09$	$-0.43 \pm 0.04$	0.03	243.4	0.99	5
12AB	$-1.52 \pm 0.17$	$-0.07 \pm 0.09$	0.09	79.6	0.98	5
14AB	$-3.29 \pm 0.23$	$-0.65 \pm 0.22$	0.03	196.5	0.99	4
1OHN	$-1.39 \pm 0.06$	$-0.38 \pm 0.02$	0.02	615.0	0.99	5
2OHN	$-1.44 \pm 0.11$	$-0.34 \pm 0.05$	0.04	159.6	0.99	5
2EtAn	$-0.85 \pm 0.06$	$-0.33 \pm 0.03$	0.02	172.3	0.99	5
4EtAn	$-1.02 \pm 0.12$	$-0.26 \pm 0.05$	0.04	71.2	0.98	5
2APh	$-2.46 \pm 0.42$	$-0.46 \pm 0.18$	0.15	34.8	0.96	5
4APh	$-4.23 \pm 0.09$	$-0.36 \pm 0.03$	0.02	2212.2	0.99	4
2NtPh	$-1.84 \pm 0.09$	$-0.36 \pm 0.04$	0.03	385.7	0.99	5
4NtPh	$-2.47 \pm .20$	$-0.49 \pm 0.08$	0.07	154.0	0.99	5
CN,THF/H (10–50%)						
2MePh	$-0.86 \pm 0.05$	$-0.42 \pm 0.03$	0.03	262.2	0.99	5
4MePh	$-0.82 \pm 0.04$	$-0.39 \pm 0.02$	0.02	328.2	0.99	5
12OHB	$-1.17 \pm 0.03$	$-0.33 \pm 0.02$	0.02	1214.4	0.99	5
14OHB	$-2.03 \pm 0.26$	$-0.29 \pm 0.14$	0.13	62.7	0.98	5
23MePh	$-0.70 \pm 0.07$	$-0.38 \pm 0.04$	0.03	108.8	0.99	5
35MePh	$-0.80 \pm 0.06$	$-0.35 \pm 0.03$	0.03	187.1	0.99	5
12AB	$-1.52 \pm 0.17$	$-0.07 \pm 0.09$	0.09	79.6	0.98	5
14AB	$-2.99 \pm 0.10$	$-0.57 \pm 0.04$	0.03	849.1	0.99	4
1OHN	$-0.86 \pm 0.05$	$-0.33 \pm 0.03$	0.02	325.8	0.99	5
2OHN	$-0.88 \pm 0.05$	$-0.29 \pm 0.03$	0.02	344.6	0.99	5
2EtAn	$-0.65 \pm 0.09$	$-0.30 \pm 0.05$	0.04	57.1	0.97	5
4EtAn	$-0.73 \pm 0.06$	$-0.21 \pm 0.03$	0.03	150.2	0.99	5
2APh	$-1.51 \pm 0.10$	$-0.33 \pm 0.05$	0.05	222.9	0.99	5
4APh	$-1.86 \pm 0.53$	$-0.10 \pm 0.22$	0.14	12.4	0.93	4
2NtPh	$-1.21 \pm 0.07$	$-0.31 \pm 0.04$	0.03	330.1	0.99	5
4NtPh	$-1.32 \pm 0.11$	$-0.29 \pm 0.06$	0.06	134.1	0.99	5
CN,iPrOH/H (5–30%)						
2MePh	$-1.15 \pm 0.06$	$-0.84 \pm 0.05$	0.04	317.4	0.99	5
4MePh	$-1.14 \pm 0.05$	$-0.80 \pm 0.04$	0.03	501.6	0.99	5
12OHB	$-1.45 \pm 0.32$	$-1.11 \pm 0.32$	0.06	20.4	0.98	3
14OHB	$-1.95 \pm 0.14$	$-0.49 \pm 0.10$	0.08	194.2	0.99	4
23MePh	$-1.13 \pm 0.08$	$-0.85 \pm 0.06$	0.04	210.3	0.99	5

(continued)

Table 4. Continued

	Slope	Intercept	s	F	R	n
35MePh	$-1.23 \pm 0.11$	$-0.88 \pm 0.08$	0.06	129.8	0.99	5
12AB	$-2.89 \pm 0.33$	$-0.42 \pm 0.18$	0.03	76.1	0.99	3
14AB	$-2.87 \pm 0.53$	$-0.82 \pm 0.32$	0.16	29.3	0.97	4
1OHN	$-1.23 \pm 0.08$	$-0.76 \pm 0.06$	0.05	224.0	0.99	5
2OHN	$-1.42 \pm 0.13$	$-0.87 \pm 0.10$	0.07	112.7	0.99	5
2EtAn	$-0.79 \pm 0.12$	$-0.58 \pm 0.09$	0.07	45.3	0.97	5
4EtAn	$-0.88 \pm 0.14$	$-0.54 \pm 0.10$	0.08	40.1	0.96	5
2APh	$-1.35 \pm 0.09$	$-0.52 \pm 0.07$	0.05	214.9	0.99	5
4APh	$3.45 \pm 0.50$	$1.24 \pm 0.32$	0.15	46.7	0.99	3
2NtPh	$-0.45 \pm 0.11$	$-0.42 \pm 0.09$	0.06	15.8	0.94	4
4NtPh	$-1.44 \pm 0.30$	$-0.93 \pm 0.19$	0.06	23.9	0.98	3

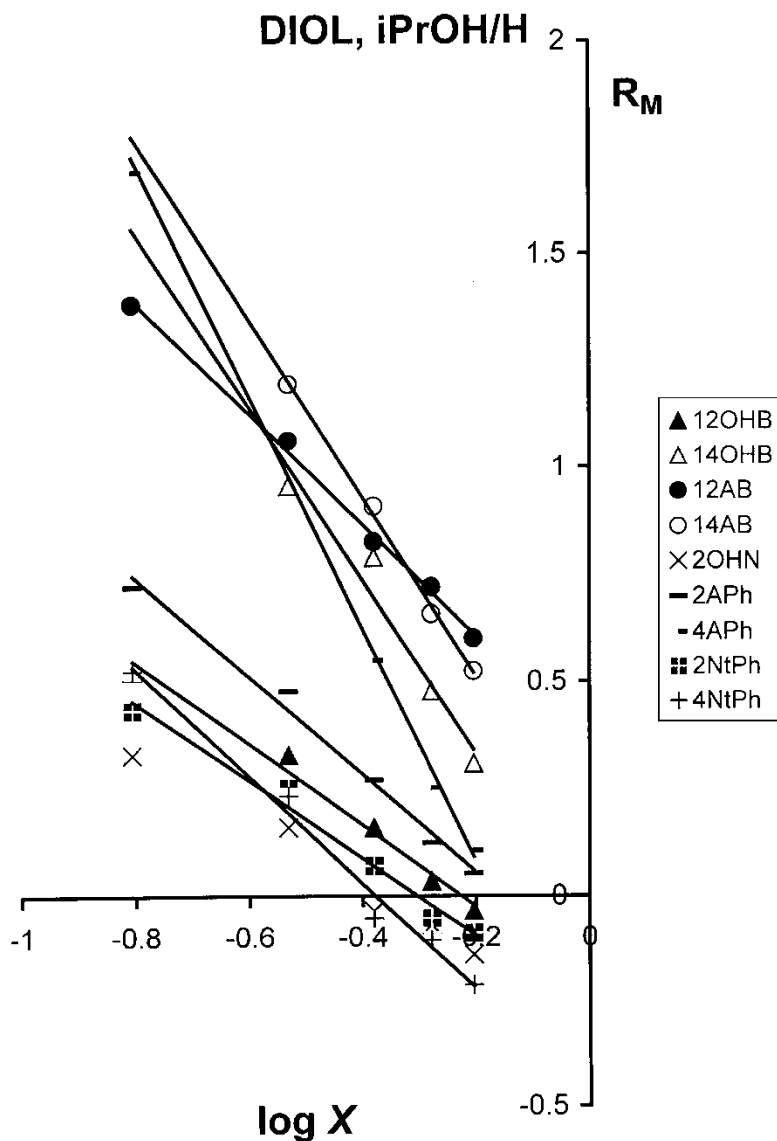
chromatography, has also been used for RP bonded phases.<sup>[11–14]</sup> It has been suggested that the bristles of chemically bonded phases are solvated with a monolayer of the organic solvent molecules in the aqueous organic eluent.<sup>[14]</sup> When the solute molecules are adsorbed into the solvated bristle, some solvent molecules are released. In our experiments, we have also applied logarithmic equations for the retention-eluent composition characteristics of investigated compounds in CN-silica/aqueous eluent systems. The parameters of  $R_M$  vs.  $\log X$  equations are presented in Table 5. From the presented data, it is seen that there are little differences in retentions of nitrophenols and dihydroxybenzene isomers (resorcinol and hydroquinone) and their slope values are low,  $<1$ . This means that, in RP-systems, polar groups are strongly solvated by polar solvents and interact weakly with surface's active centres. In the case of phenylenediamines and aminophenols, slope values are higher for *para* derivatives ( $< 1.5$ ), they are more strongly retained than the *ortho* isomers, similar as in NP systems. This proves the strong interactions of the basic amino group with cyanopropyl ligands. Simultaneously, it can be clearly seen that non-polar substituents (alkyl chain or condensed ring) interact relatively strongly with surface ligands in RP systems; naphthols, xylenols, and ethylanilines, are more strongly retained than the derivatives substituted with the second polar group. Also, the slope values of these moderately polar compounds are high, in most cases  $> 1.5$ .

## CONCLUSIONS

For phenols and anilines substituted with a nonpolar group (alkyl chain, condensed ring) chromatographed on polar bonded stationary phases in NP

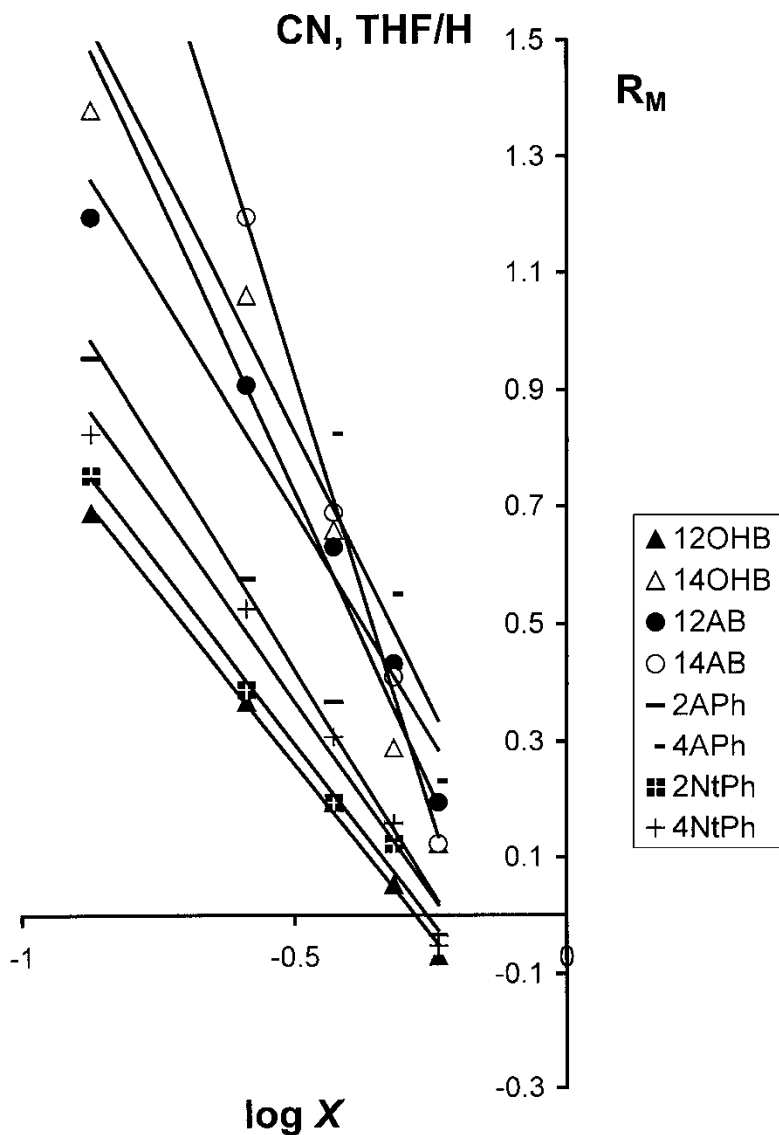
**Table 5.** Parameters of  $R_M = a + b \log X$  equations obtained for investigated compounds chromatographed on CN-silica with aqueous eluents

	CN,ACN/H <sub>2</sub> O (10–50%)						CN,MeOH/H <sub>2</sub> O (20–60%)					
	Slope	Intercept	s	F	R	n	Slope	Intercept	s	F	R	n
2MePh	$-0.76 \pm 0.13$	$-0.16 \pm 0.12$	0.08	34.9	0.96	5	$-1.42 \pm 0.18$	$-0.49 \pm 0.12$	0.08	59.3	0.98	5
4MePh	$-0.78 \pm 0.06$	$-0.19 \pm 0.05$	0.04	183.3	0.99	5	$-1.59 \pm 0.13$	$-0.62 \pm 0.08$	0.06	146.5	0.99	5
12OHB	$-0.42 \pm 0.02$	$-0.45 \pm 0.01$	0.01	637.2	0.99	5	$-0.74 \pm 0.06$	$-0.55 \pm 0.03$	0.03	145.7	0.99	5
14OHB	$-0.43 \pm 0.03$	$-0.46 \pm 0.03$	0.02	216.3	0.99	5	$-0.68 \pm 0.13$	$-0.52 \pm 0.08$	0.06	27.2	0.95	5
23MePh	$-1.30 \pm 0.10$	$-0.36 \pm 0.09$	0.06	179.6	0.99	5	$-1.67 \pm 0.17$	$-0.46 \pm 0.11$	0.08	92.8	0.98	5
35MePh	$-1.64 \pm 0.17$	$-0.57 \pm 0.15$	0.10	92.3	0.98	5	$-1.82 \pm 0.18$	$-0.47 \pm 0.11$	0.08	98.1	0.99	5
12AB	$-0.36 \pm 0.08$	$-0.16 \pm 0.07$	0.05	22.1	0.94	5	$-0.60 \pm 0.03$	$-0.30 \pm 0.02$	0.01	510.0	0.99	5
14AB	$-0.36 \pm 0.04$	$0.43 \pm 0.04$	0.03	75.0	0.98	5	$-0.91 \pm 0.09$	$-0.03 \pm 0.06$	0.04	98.7	0.99	5
1OHN	$-1.64 \pm 0.06$	$-0.21 \pm 0.05$	0.04	736.4	0.99	5	$-2.48 \pm 0.10$	$-0.56 \pm 0.06$	0.05	584.2	0.99	5
2OHN	$-1.70 \pm 0.18$	$-0.35 \pm 0.16$	0.11	90.5	0.98	5	$-2.16 \pm 0.22$	$-0.49 \pm 0.14$	0.10	94.8	0.98	5
2EtAn	$-0.97 \pm 0.10$	$-0.16 \pm 0.09$	0.06	99.4	0.99	5	$-1.53 \pm 0.16$	$-0.44 \pm 0.10$	0.07	94.8	0.98	5
4EtAn	$-0.91 \pm 0.15$	$-0.03 \pm 0.14$	0.10	35.9	0.96	5	$-1.69 \pm 0.11$	$-0.44 \pm 0.07$	0.05	245.2	0.99	5
2APh	$-0.51 \pm 0.07$	$-0.23 \pm 0.07$	0.05	50.0	0.97	5	$-0.84 \pm 0.06$	$-0.35 \pm 0.04$	0.03	219.9	0.99	5
4APh	$-1.26 \pm 0.14$	$-0.59 \pm 0.13$	0.09	80.4	0.98	5	$-1.39 \pm 0.07$	$-0.43 \pm 0.05$	0.03	357.9	0.99	5
2NtPh	$-0.91 \pm 0.11$	$-0.33 \pm 0.10$	0.07	73.6	0.98	5	$-1.51 \pm 0.17$	$-0.58 \pm 0.11$	0.08	76.4	0.98	5
4NtPh	$-0.86 \pm 0.11$	$-0.13 \pm 0.10$	0.07	63.9	0.98	5	$-1.43 \pm 0.24$	$-0.42 \pm 0.15$	0.10	35.3	0.96	5



**Figure 1.** Plots of  $R_M$  vs.  $\log X$  ( $X$ -molar fraction) of 2-propanol obtained for investigated solutes in system: Diol-silica/iPrOH—*n*-heptane.

systems, unit slopes of  $R_M$  vs.  $\log X$  plots are obtained. This presumably indicates complete delocalization of the weaker functional group, irrespective of its position. For alkyl-substituted phenols and anilines, slopes often are less than unity, which confirms flat adsorption of the molecule onto the surface.



**Figure 2.** Plots of  $R_M$  vs.  $\log X$  ( $X$ -molar fraction) of tetrahydrofuran obtained for investigated solutes in system: Diol-silica/THF—*n*-heptane.

Two-point adsorption on polar bonded stationary phases in NP systems occurs for substances with two strongly polar functional groups. However, *ortho* substituted solutes behave on polar bonded stationary phases like monofunctional solutes. In most cases, *ortho* derivatives have slopes

near unity, whereas the slope values for analogous *para* isomers are much higher, which is caused by the *ortho* effect. *Para* isomers are more strongly retained on the polar bonded stationary phases than *ortho* isomers.

There are some cases of stronger adsorption of *ortho* in comparison to *para* isomers, for compounds with two polar substituents (12AB in comparison to 14AB, 12OHB in comparison to 14OHB, 2NtPh in comparison to 4NtPh) on Diol-, CN-, and NH<sub>2</sub>-layers in non-aqueous eluent systems of high eluent strengths.

There are small differences in retention of nitrophenols and dihydroxybenzenes on CN-silica in RP-systems, and their slope values are low, <1. In the case of phenylenediamines and aminophenols, slope values are higher for *para* derivatives (<1.5) and they are more strongly retained than the *ortho* isomers.

Non-polar substituents (alkyl chain or condensed ring) interact strongly with surface ligands in RP systems; naphthols, xylenols, and ethylanilines are more strongly retained on CN-silica than derivatives substituted with a second polar group. Also, the slope values of these moderately polar compounds are relatively high, in most cases >1.5.

The separation selectivity, compared through the  $\Delta R_M$  values indicates that, in most cases, the highest separation selectivity was obtained on NH<sub>2</sub>-silica in NP systems.

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