
**REVERSED-PHASE HIGH PERFORMANCE LIQUID
CHROMATOGRAPHIC BEHAVIOUR OF HOMOLOGOUS SERIES**Bohuslav RITTI^a, Štěpánka RUDOLFOVÁ^b and Pavel JANDERA^c^a *Institute of Systematic and Ecological Biology, 603 00 Brno*^b *Research Institute of Animal Nutrition, 691 23 Pohořelice*^c *Institute of Chemical Technology, 532 10 Pardubice*

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The retention model for reversed-phase high-performance liquid chromatography (RP-HPLC) based on interaction indices was applied to separation of the homologous series members. The validity of the equations describing relationship between retention ($\log k$) and the number of methylene groups in alkyl chain (C_n) and composition of mobile phase (φ) by using statistical methods was verified. The regression coefficients of a quadratic term for relationship $\log k$ vs C_n and $\log k$ vs φ can be neglected. The regression coefficient of interaction term $C_n\varphi$ is statistically significant.

The possibility of predicting the retention of a solute in reversed-phase liquid chromatography has a great importance, as it enables the chromatographer to choose a suitable mobile phase composition for optimal separation. The capacity ratios of the homologous series members have often been used for studying the relationship between the retention of compounds and chromatographic conditions¹⁻⁸.

According to the solvophobic theory^{9,10} the interaction between the solute with surrounding water molecules present in the mobile phase plays a dominant role. The interactions between the solute and the stationary phase are weak and non-selective¹¹. The log of capacity ratio (k) varies linearly with the surface area of hydrocarbon skeleton, and especially in homologous series, with the number of methylene groups in the linear alkyl chain^{9,11}. This dependence has also been observed for series with other structural units than methylene groups¹². In fact stationary phase interactions also influence retention in many cases^{2,3,13-15}.

The purpose of this study was to obtain equations that accurately correlate $\log k$ with both of the number of methylene groups and the composition of the mobile phase.

THEORETICAL

The retention model for reversed-phase liquid chromatography based in interaction indices¹⁶ was applied to the separation of the members of homologous series. Ac-

according to Jandera⁷ the following expression for the $\log k$ values of the members of homologous series in the aliphatic saturated straight chain can be used as follows

$$\log k = A + B C_n + C C_n^2, \quad (1)$$

where C_n is number of methylene groups, A , B , C are constants. According to Schoenmakers et al.¹⁷ the solute retention changes with the composition of mobile phase in a quadratic manner

$$\log k = \alpha - \beta\varphi + \gamma\varphi^2, \quad (2)$$

where $\alpha = \log k_0$, k_0 is the capacity ratio for pure water, β , γ are constants for a given system and φ is the volume fraction of the organic modifier in aqueous eluent. The validity of Eq. (2) is restricted to mobile phases in which $\varphi(\text{water}) < 0.9$ (ref.¹⁷). The relationship between $\log k$ and φ at very high water concentrations is neither linear nor quadratic¹⁸. In the region of low water concentrations the discontinuity of the retention mechanism has also been observed¹¹. A sufficient solvation of the bonded phase is achieved at φ values^{19,20} of 0.1–0.2. Therefore, it is necessary to measure the dependence of $\log k$ on φ over a organic modifier volume fraction range at most 0.1–0.9. According to authors^{7,21–23} the quadratic relationship can be expressed for practical purposes by a linear relationship.

For the binary mobile phase composed of water and one organic solvent Eq. (1) can be expressed as follows

$$\begin{aligned} \log k = & (a_0 - a_1\varphi + a_2\varphi^2) + (b_0 - b_1\varphi + b_2\varphi^2) C_n + \\ & + (c_0 - c_1\varphi + c_2\varphi^2) C_n^2 \end{aligned} \quad (3)$$

thus

$$A = a_0 - a_1\varphi + a_2\varphi^2 \quad (3a)$$

$$B = b_0 - b_1\varphi + b_2\varphi^2 \quad (3b)$$

$$C = c_0 - c_1\varphi + c_2\varphi^2. \quad (3c)$$

If we consider that quadratic terms can be neglected the Eq. (3) is simplified to

$$\log k = a_0 + b_0 C_n - a_1\varphi - b_1 C_n\varphi. \quad (4)$$

The authors consider it useful to verify if the coefficient b_1 of interaction term $C_n\varphi$ can be neglected and so the Eq. (4) simplified to

$$\log k = a_0 + b_0 C_n - a_1\varphi. \quad (5)$$

EXPERIMENTAL

The values of the capacity ratios used in this study were taken from papers published earlier (Table I)⁵, (Table III)⁷, (Table I)²⁴. The values of $\log k$ of fatty acids were taken from paper Koopmans and Rekker²⁵ (Table I), which were derived from d'Amboise and Hanai's graphical representation of $\log k$ vs $\log K_D$ (distribution constant determined in 1-octanol-water system) – ref.²⁶. The regression coefficients of Eqs (1), (4) and (5) were tested on null hypothesis according to procedure presented by Eckschlager et al.²⁷. The coincidence of the coefficients B for different compounds in Table I were tested by procedure published by Rod and Vondráček²⁸. The precision of compared equations was tested by using Fischer-Snedecor criterion F (ref.²⁷).

RESULTS AND DISCUSSION

The Dependence of Retention on the Number of Methylene Groups

The relationship between $\log k$ and C_n , C_n^2 terms (number of methylene groups) were calculated by using regression analyses. The results are given in Table I. The correlation coefficients were very high. There were not great difference in their absolute values. The regression coefficients C for C_n^2 term were small, but in many cases statistically significant. By using F test, it was found out that the precision of calculated equations was influenced by C_n^2 term mostly on phenyl-bonded silica gel packing.

The differences from the behaviour expected according to the Eq. (1) in the retention of the members of homologous series with $C_n = 0$ and $C_n = 1$ were observed. The difference in the retention of phthalimide was caused by the interaction of hydrogen in $-\text{NH}-$ group with unreacted silanol groups¹³⁻¹⁵. The behaviour of N -substituted phthalimides with short alkyl chain is influenced by ineffective shielding of polar $-\text{CO}-\text{N}-\text{CO}-$ group⁵ which can interact both with mobile and stationary phases. For $C_n > 2$, this influence is insignificant (refs^{5,6}).

Because the slope of straight line was calculated for the increment of $-\text{CH}_2-$ group, the deviation for $C = 1$ member is caused by hydrophobicity of the third hydrogen in $\text{CH}_3 -$ group.

From Table II it follows that the elimination of $C_n \leq 2$ members from the tested set had not significant influence on the values of the coefficient B in Eq. (1). The constant B of the $\log k$ vs C_n equation does not depend on the type of the character of the organic solvent in the mobile phase. These results agree with conclusions published by Jandera⁷.

TABLE I
Relationships between $\log k$ and the number of the methylene groups of homologous series C_n

Compound	C_n	A	B	C	n	r resp. I	s	F	System	Ref.
Alcohols	4-16	-0.770**	0.132**P	—	9	0.999	0.017	7.916**	YMC-p, ACN, $\varphi = 0.5$	24
		-0.863	0.154**	$-1.1 \cdot 10^{-3}$ **	9	0.999	0.006	—		
Alkylbenzenes	0-10	0.294**	0.128**P	—	10	0.999	0.008	1.253	LC7-C ₁₈ , ACN, $\varphi = 0.5$	25, 26
		0.288**	0.132**	$4.3 \cdot 10^{-4}$	10	0.999	0.007	—		
Fatty acids	6-18	-0.966	0.101**P	—	7	0.999	0.007	1.490	YMC-C ₁₈ , ACN, $\varphi = 0.9$	—
		-0.928	0.094**	$3.0 \cdot 10^{-4}$	7	0.999	0.006	—		
Alcohols	4-16	-1.956**	0.131**R	—	9	0.999	0.004	1.028	YMC-C ₁₈ , THF, $\varphi = 0.6$	—
		-1.199**	0.132**	$-4.6 \cdot 10^{-5}$	9	0.999	0.004	—		
Alkylbenzenes	0-10	-0.272	0.124**R	—	10	0.999	0.010	4.961*	YMC-p, THF, $\varphi = 0.6$	—
		-0.254**	0.115**	$9.0 \cdot 10^{-4}$ **	10	0.999	0.004	—		
Alcohols	4-16	-0.330**	0.047**S	—	9	0.994	0.022	22.347**	YMC-C ₁₈ , THF, $\varphi = 0.6$	—
		-0.449	0.075**	$-1.4 \cdot 10^{-3}$ **	9	0.999	0.104	—		
Alkylbenzenes	0-10	-0.373**	0.069**S	—	9	0.998	0.019	3.395	YMC-p, THF, $\varphi = 0.6$	—
		-0.465	0.091**	$-1.1 \cdot 10^{-3}$ **	9	0.999	0.010	—		
Alkylbenzenes	0-10	0.178**	0.045**S	—	10	0.998	0.010	17.500**	YMC-p, THF, $\varphi = 0.6$	—
		0.164**	0.055**	$1.0 \cdot 10^{-3}$ **	10	0.999	0.002	—		
N-Phthalimides	1-11	-0.384**	0.070**S	—	10	0.999	0.014	1.029	YMC-C ₁₈ , THF, $\varphi = 0.6$	5
		-0.392**	0.065**	$6.0 \cdot 10^{-4}$	10	0.999	0.014	—		
N-Phthalimides	1-11	-0.879**	0.195**	—	11	0.999	0.037	3.719*	LiChrosorb C ₁₈ , MeOH, $\varphi = 0.8$	—
		-0.964**	0.234**	$-3.3 \cdot 10^{-3}$ **	11	0.999	0.019	—		

C_n Number of methylene groups in the solute molecule alkyl chain, A, B, C coefficients of regression equation $\log k = A + B C_n + C C_n^2$ (the coefficients were tested on hypothesis A, B, C = 0), n number of compounds in the set, r correlation coefficient, I index of correlation, s standard deviation, F Fischer-Snedecor criterion, MeOH methanol, ACN acetonitrile, THF tetrahydrofuran, φ volume fraction of the organic modifier, p phenyl-bonded silica, Indices: P, R, S the coefficients with the same index are statistically coincident ($\alpha = 0.01$), * statistically significant differences ($\alpha = 0.05$), ** statistically highly significant differences ($\alpha = 0.01$).

TABLE II
Influence of the number of the methylene groups of homologous series on the values of the regression coefficients of Eq. $\log k = A + B C_n$

Compounds	C_n	A	B	n	r	s	$L_{b, 0.05}$	System	Ref.
Alkylbenzenes	0-10	0.294**	0.128**	10	0.999	0.008	0.002	YMC-p, ACN, $\varphi = 0.5$	24
	1-10	0.293**	0.128**	9	0.999	0.007	0.002		
	2-10	0.296**	0.128**	8	0.999	0.008	0.002		
N-Phthalimides	0-11	-1.069**	0.220**	12	0.979	0.175	0.033	LiChrosorb C_{18} , MeOH, $\varphi = 0.8$	5
	1-11	-0.879**	0.195**	11	0.999	0.037	0.008		
	2-11	-0.834**	0.189**	10	0.999	0.016	0.004		
	3-11	-0.815**	0.187**	9	0.999	0.012	0.003		

$L_{b, 0.05}$ confidence interval of coefficient B of regression equation $\log k = A + B C_n$ and $\alpha = 0.05$. The meaning of other symbols is the same as in Table I.

TABLE III
Relationship between $\log k$ and the number of the methylene groups of homologous series and the volume fraction of organic modifier

Compounds	C_n	a_0	b_0	a_1	b_1	n	I	s	F	System	Ref.
Alcohols	4-16	0.986**	0.090**	-2.766**	—	36	0.978	0.148	2.128*	YMC-P, ACN	24
		-0.427	0.245**	-0.591	-0.239**	36	0.990	0.102	—	$\varphi = 0.5-0.8$	
N-Phthalimides	1-11	2.069**	0.224**	-3.862**	—	31	0.974	0.144	4.842**	LiChrosorb	5
		0.652**	0.614**	-1.890**	-0.529**	31	0.995	0.065	—	C ₁₈ , MeOH $\varphi = 0.5-0.8$	
3,4-Dinitrobenzoates of alcohols	1-6	2.942**	0.167**	-4.338**	—	24	0.995	0.060	25.272**	Siliasorb.	7
		1.731**	0.513**	-2.799**	-0.440**	24	0.999	0.012	—	C ₁₈ , MeOH $\varphi = 0.7-0.9$	
		4.635**	0.178**	-6.109**	—	24	0.973	0.197	1.260	Siliasorb	
		2.648**	0.746**	-3.587**	-0.721*	24	0.980	0.175	—	C ₁₈ , Di $\varphi = 0.5-0.75$	
		2.161**	0.161**	-3.270**	—	24	0.998	0.023	3.352**	Siliasorb	
		1.876**	0.231**	-2.958**	-0.089*	23	0.999	0.012	—	C ₁₈ , ACN $\varphi = 0.6-0.8$	
4-Bromphenacyl esters of saturated carboxylic acids	1-7	2.863**	0.114**	-4.120**	—	23	0.961	0.119	1.132	Siliasorb	7
		1.922**	0.404*	-2.904**	-0.376	23	0.968	0.112	—	C ₁₈ , MeOH $\varphi = 0.7-0.9$	
		4.147**	0.181**	-5.632**	—	23	0.944	0.211	1.432	Siliasorb	
		1.730	0.925**	-2.507*	-0.966**	23	0.963	0.176	—	C ₁₈ , Di $\varphi = 0.5-0.75$	
		2.092**	0.125**	-3.051**	—	23	0.980	0.075	1.017	Siliasorb	
		1.714**	0.241*	-2.564**	-0.151	23	0.981	0.074	—	C ₁₈ , ACN $\varphi = 0.6-0.8$	

a_0, b_0, a_1, b_1 Coefficients of regression equation $\log k = a_0 + b_0 C_n - a_1 \varphi - b_1 C_n \varphi$ (the coefficients were tested on hypothesis $a_0, b_0, a_1, b_1 = 0$), Di-1,4-dioxane. The meaning of other symbols is the same as in Table I.

The Dependence of Retention on the Composition of the Mobile Phase

It is known from literature^{7,17,18} that the dependence $\log k$ on the concentration of the organic modifier in the mobile phase is approximately linear for mobile phases composed of methanol–water and 1,4-dioxane–water. Composition range, in which the simplified linear equation $\log k$ vs φ can be used, was discussed in Theoretical. The results evaluated according to Eqs (4) and (5) are given in Table III. The coefficient b_1 of interaction term $C_n\varphi$ in Eq. (4) is in many cases significant and cannot be neglected. Its influence on precision of Eq. (4) is also in many cases important. The authors cannot explain the reason why coefficient b_1 can be neglected in the rest of equations. It may be noted that the model of interaction indices (Eq. (13) in ref.¹²) predicts that the term b_1 should diminish with increasing polarity and decreasing size of the molecular rest (end group) in a given homologous series. In agreement with this theory, the term b_1 is higher for the mobile phases containing dioxane than for those containing methanol or acetonitrile. Further, in aqueous-methanolic mobile phases, alcohols show lower values of b_1 than other series tested here with bulkier and less-polar end groups.

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