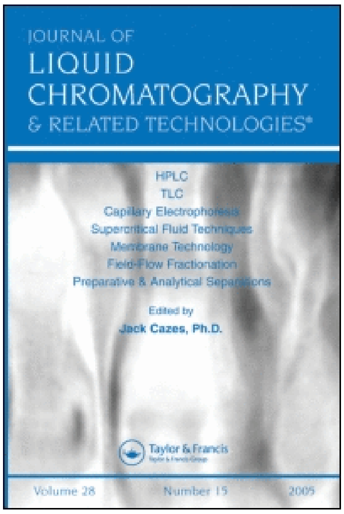

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Use of RP-HPTLC Systems for the Determination of Lipophilicity of 3,5-Dioxo-4-azatricyclo[5.2.2.02,6]undecanes-5-HT$_{1A}$ Antagonists

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Use of RP-HPTLC Systems for the Determination of Lipophilicity of 3,5-Dioxo-4-azatricyclo[5.2.2.02,6]undecanes–5-HT_{1A} Antagonists

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Abstract: The lipophilicities of twelve 3,5-dioxo-4-azatricyclo[5.2.2.02,6]undecanes, potential serotonergic 5-HT_{1A} receptors antagonists, was determined by means of the reversed-phase RP-18W and RP-18 thin-layer chromatography. The use of the linear (Soczewinski-Wachtmeister) and square (Schoenmaker) equation for R_{MW} calculation was evaluated. Due to the rather poor correlation between linear and square R_{MW} values, despite very good correlation coefficients for independent measurements for each solvent system, the results of the RP-18 measurements and use of the linear equation for the R_{MW} calculation were found to be the most reliable. Their reliability was also confirmed by the best values of F and s. The standardization (for six standards of known lipophilicity – log P) allowed calculation of the experimental lipophilicities (log P_{EXP}) for compounds investigated.

Keywords: Reversed-phase thin-layer chromatography, Lipophilicity determination, Soczewinski-Wachtmeister equation, Schoenmaker equation, Experimental lipophilicity

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INTRODUCTION

The interactions in chromatographic systems, consisting of stationary phase, eluent, and solute, are complicated. In reversed phase (RP) systems, where generally silica based alkyl stationary phases are used, several interactions of nonspecific nature (van der Waals interactions, hydrophobic interactions), as well as specific nature, with residue surface silanols between solute and/or solvent molecules and surface active sites should be taken into account. Moreover, interactions resulting from solvation of solute and solvent molecules can play a significant role. There are, however, several theories modelling chromatographic systems–simplifying retention mechanisms in individual chromatographic system. Any model can reflect only some aspects of physicochemical processes and intermolecular interactions significant for the retention of individual molecule in individual chromatographic system. From the models of retention, several equations combining retention-eluent composition have been derived. Among various models of retention are, for example, those proposed by Snyder,[1,2] Soczewiński,[3,4] Scott-Kucera,[5–7] Jaroniec,[8,9] and Kowalska.[10,11]

For RP systems, a few simple relationships between k and the mobile phase composition (e.g., volume fraction φ) are in use. The most commonly used one is given below:

$$\log k = \log k_W - S\varphi \tag{1}$$

The linear dependence between log k (R_M) and the volume fraction of water in the liquid-liquid partition systems was investigated by Soczewiński and Wachtmeister.[12] For the reversed phase systems comprising octadecyl/water + methanol, Equation (1) is also valid, which was published by Snyder et al.[13] The linear relationship between log k and φ holds over a restricted range of eluent compositions.[14] The quadratic relationship between log k and φ has been found by Schoenmakers and coworkers,[14–17] who introduced solubility parameters for the calculation of intramolecular forces involving the Hildebrand's theory of regular solutions:

$$\log k = A\varphi^2 + B\varphi + C \tag{2}$$

A similar equation was derived from the lattice statistical thermodynamic theory,[18,19] which considers the affinity of the solute for the grafted chains. Jandera et al. [20] came to the same results by applying a model that makes use of the intermolecular interactions between solute and mobile phase molecules.

The above equations are useful for the optimization of chromatographic separation conditions of the real mixture and the correct choice of the optimum gradient. The value of intercept in the linear Equation (1) can be obtained by extrapolation of retention eluent composition plots to determine

R_M in the system alkylsilica/water. Similarly, the value of R_{MW} can be determined from the quadratic equation fitted to the experimental retention-eluent composition data. The determination of the lipophilicity by RP techniques can have very high practical importance, especially when high biological activity is also a consideration.

Anti-anxiety drugs, starting with the introduction of Buspirone* to the market, became a very important group of pharmaceutical substances.[21] They help to cure anxiety, which is one of the most abundant and difficult to treat psychotic disorders. From the recognition of the mechanism of their action,[22] especially involvement of the serotonin receptors (5-HT_{1A}, 5-HT_{2A}), the further search for more selective and faster acting drugs started.

The pharmacophor of the Buspiron* activity was then imprinted in many other compounds with high or reasonable activity.[23] The difference in their profile of action can be easily correlated with the changes in distribution of the polar centers (hydrogen bond acceptors) and lipophilicity.[24]

The importance of the lipophilicity in the profile of the Buspirone,* like anti-anxiety drugs action, explain the importance of the research and finding of the best methodology for their lipophilicity measurements.

The chromatographic methods most commonly used recently for lipophilicity measurement are thin layer chromatography (TLC) and high performance TLC (HPTLC). Use of these techniques, unfortunately, has a serious problem. One of the pharmacophor points in the group of Buspiron* analogs is a basic nitrogen atom; usually, compounds can contain from one to three basic nitrogen atoms. This causes one of the most common problems of almost all analytical methods, and especially chromatography, dissociation, and its reversion.

The compounds investigated in this paper are potential antagonists of the 5-HT_{1A} serotonergic receptor and potential anxiolytic drugs (see Figure 1). Their lipophilicities on different RP-18 (TLC or TLCW) precoated plates, in different eluent systems, was determined. The results are compared and correlated.

EXPERIMENTAL

Chemicals

4-Substituted derivatives of the 3,5-dioxo-4-azatricyclo[5.2.2.02,6]undecane (series A–8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.02,6]undecane-8-ene-1-yl acetates and B—1,11-dimethyl-3,5,8-trioxo-4-azatricyclo[5.2.2.02,6] undecanes) were synthesized[25] in the Department of Medicinal Chemistry, Medical University of Warsaw. Their structures and purities were confirmed by TLC and spectral methods: Nuclear magnetic resonance (NMR) and mass spectrometry (MS). The solvents used (methanol, acetone, dioxane) were of analytical grade and were purchased from Polish Chemical

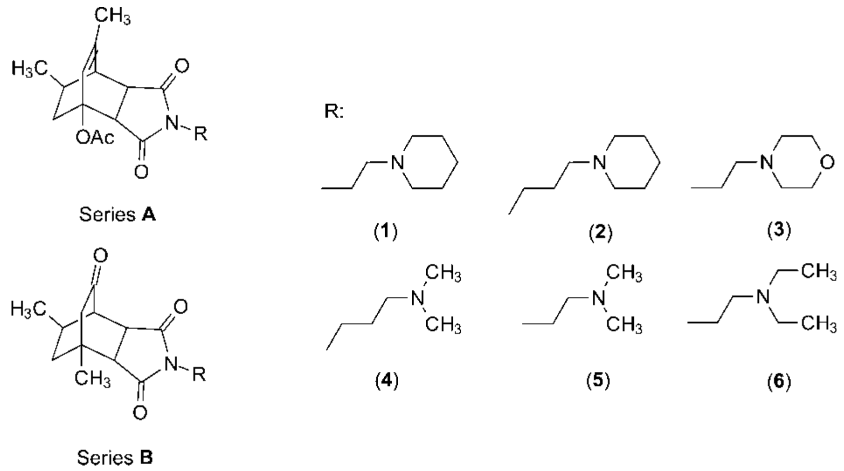

Figure 1. Structures of the compounds investigated.

Reagents, POCH (Gliwice, Poland). Standards of analytical grade were obtained from Fluka (Sigma-Aldrich, Milwaukee, WI) or E. Merck (Darmstad, Germany) and were used without further purification.

Mobile Phase Preparation

The mobile phases were prepared by mixing the respective amounts of water and polar modifier [methanol (MeOH), dioxan (Dx), acetone (Ac)] in the range from 50 to 75 or 90 % (v/v) for RP-TLC or from 40 or 50 to 65 or 75 % (v/v) for RP-TLCW, in 5% increments. The upper value depended on the linearity of the R_M/polar modifier concentration correlation. Because solutes have basic properties and exist in ionized and unionized forms in solutions, they interact with alkyl chains of the surface, as well as with residue surface silanols by ion-exchange forces. This causes broadening of peaks (spots) and inability to determine correct retention data. The addition of an ion-suppressing agent, aqueous ammonia, improves the shapes of the spots. Each eluent contained 2% aqueous ammonia to suppress solute ionization.

RP-TLC Analyses

TLC was performed on 10 × 10 cm glass $\text{RP-18}_{\text{F254}}$ or $\text{RP-18W}_{\text{F254}}$ HPTLC precoated plates (E. Merck) in horizontal Teflon chambers with eluent distributor (DS, Chromdes, Lublin, Poland). Methanolic sample solutions (2 μL, 1% v/v) were spotted onto the adsorbent layer. Plates were developed face-down at 20 ± 1°C untill the eluent reached a distance of 8 cm from the starting line. The final location of the spots was determined with ultraviolet (UV) light (254 nm). Spots were symmetric and no tailing was observed. The R_M values were calculated from R_F values as the mean of three repetitive measurements (standard error did not exceed $\sigma \leq 4 \times 10^{-4}$) using the formula:

$$R_M = \frac{1 - R_F}{R_F}$$

Standardization

The RP-18 TLC results were also standardized by means of recalculation of their R_{MW} values to experimental lipohilicity ($\log P_{EXP}$) with use of the calibration equations. These equations were calculated for six standards of known lipophilicity.[26] 2,3-Dioxoindole (S1), 2,6-dichloroacetanilide (S2), 2,4-dichloroacetanilide (S3), 3,4-dichloroaniline (S4), 2,6-dichloroaniline (S5), and biphenyl (S6) were used. The regression coefficients were very good ($r > 0.95$) for both R_{MW} calculations and calibration equations.

RESULTS AND DISCUSSION

R_F values on RP18W and RP18 plates were determined for the 12 synthesized substances presented in Figure 1. Equations (1) and (2) were fitted to the R_M ($\log k$) values obtained in various modifier concentrations using the least-squares method (linear regression). The regression coefficients (r), statistic (F), and standard error (s) values (see Tables 1–6) were taken into account to verify the goodness of fit. From the presented results, it is seen that, when we have taken into account regression coefficients, the goodness of fit for four-parameter quadratic equations is evidently higher than for the linear ones in all investigated systems. The goodness of fit can be classified according to Jaffe.[27] When we have taken into account experimental data obtained on RP18W layers, it is clearly seen the goodness of fit of quadratic equations to the experimental data is, in 26 cases, excellent and, in 10 cases, good out of 36 cases. The goodness of fit of linear equations to the experimental data is only excellent in 12 cases, in 15 cases good, and in 9 cases fair out of 36 cases (see Tables 1–3). It should be said, however, that the F statistic values for linear equations in all cases are higher than the F critical and, in most cases, are higher than those obtained for quadratic equations, which are, even in three cases, lower than the F critical value. Thus, we cannot definitely say which equation better describes the retention-eluent composition relationships, and which one can be taken to determine intercept values – R_{MW} ($\log k_W$) values, which can show lipophilicities of investigated compounds. It should be emphasised that the best

Table 1. Parameters for the relationships between R_M and modifier concentration (χ or φ) obtained for systems RP-18W – methanol + water (50–75%) calculated from Eqs. (1) and (2) and their statistical evaluation

Solute	S	R_{MW}	r	F	s	n
$R_M = -S\varphi + R_{MW}$						
A1	−3.26	2.64	0.991	108.57	0.07	6
A2	−4.17	3.10	0.976	40.18	0.15	6
A3	−3.76	2.95	0.99	102.82	0.08	6
A4	−2.47	1.60	0.988	83.19	0.06	6
A5	−2.69	1.93	0.996	253.13	0.04	6
A6	−3.42	2.49	0.995	209.49	0.05	6
B1	−2.62	2.11	0.996	233.9	0.04	6
B2	−2.66	1.84	0.997	354.28	0.03	6
B3	−3.34	2.56	0.99	97.58	0.08	6
B4	−1.40	0.70	0.979	46.40	0.05	6
B5	−1.83	0.22	0.999	875.39	0.01	6
B6	−2.62	1.81	0.996	243.40	0.04	6

	A	B	C	r	F	s	n
$R_M = A\varphi^2 + B\varphi + C$							
A1	3.57	−7.90	4.10	0.996	57.21	0.07	6
A2	8.93	−15.78	6.76	0.994	39.51	0.11	6
A3	2.53	−7.05	3.99	0.992	31.68	0.11	6
A4	3.77	−7.36	3.14	0.997	95.03	0.04	6
A5	−2.50	0.55	0.90	0.999	490.00	0.02	6
A6	3.42	−7.86	3.90	0.999	332.05	0.03	6
B1	0.87	−3.75	2.47	0.996	65.27	0.05	6
B2	−1.86	−0.24	1.08	0.999	289.19	0.03	6
B3	−1.01	−2.02	2.15	0.99	25.32	0.11	6
B4	3.24	−5.61	2.03	0.999	1205.97	$6.5 \cdot 10^{-3}$	6
B5	0.13	−2.00	1.27	0.999	222.73	0.0	6
B6	−0.93	−1.42	1.43	0.996	69.32	0.05	6

linearity of retention-eluent composition relationships were obtained in the system methanol-water.

Similar conclusions can be derived from the statistical parameters calculated for the experimental data obtained on RP18 layers. The goodness of fit of quadratic equations to the experimental data is, in 28 cases, excellent and, in 8 cases, good out of 36 cases. The goodness of fit of linear equations to the experimental data is only excellent in 13 cases, in 21 cases good, and in 2 cases fair, out of 36 cases (see Tables 4–6). The F statistic values for linear equations in all cases are higher than F critical and, in most cases, are higher than those obtained for the quadratic equation. The highest r and F statistic values determined on RP18 layers were obtained in the system containing acetone as the modifier.

Table 2. Parameters for the relationships between R_M and modifier concentration (χ or φ) obtained for systems RP-18W – dioxane + water (40–65%) calculated from Eqs. (1) and (2) and their statistical evaluation

Solute	S	R_{MW}	r	F	s	n
$R_M = -S\varphi + R_{MW}$						
A1	−2.44	2.13	0.966	27.87	0.10	6
A2	−4.98	3.18	0.992	80.67	0.11	6
A3	−3.31	2.46	0.984	60.21	0.10	6
A4	−2.24	1.36	0.989	90.86	0.05	6
A5	−2.62	1.87	0.98	49.25	0.08	6
A6	−2.90	2.07	0.985	65.49	0.08	6
B1	−1.56	1.46	0.949	17.99	0.08	6
B2	−2.44	1.62	0.993	141.27	0.05	6
B3	−2.22	1.71	0.972	34.32	0.09	6
B4	−1.35	0.65	0.97	31.52	0.05	6
B5	−1.12	0.85	0.947	17.34	0.06	6
B6	−2.82	1.95	0.927	12.27	0.18	6

	A	B	C	r	F	s	n
$R_M = \mathrm{A}\varphi^2 + \mathrm{B}\varphi + \mathrm{C}$							
A1	5.95	−8.98	3.86	0.989	21.56	0.09	6
A2	−2.07	−2.71	2.58	0.991	26.93	0.15	6
A3	6.63	−10.61	4.38	0.999	460.07	0.03	6
A4	3.69	−6.30	2.43	0.999	1477.05	$9.3 \cdot 10^{-3}$	6
A5	5.19	−8.33	3.38	0.996	56.28	0.06	6
A6	5.17	−8.59	3.57	0.998	100.58	0.05	6
B1	4.42	−6.42	2.74	0.979	11.38	0.08	6
B2	2.29	−4.95	2.28	0.996	70.64	0.05	6
B3	−0.79	−1.35	1.48	0.973	8.74	0.12	6
B4	3.44	−5.13	1.65	0.995	46.48	0.03	6
B5	2.57	−3.94	1.59	0.967	7.10	0.07	6
B6	12.71	−16.80	5.64	0.999	2990.04	$8.8 \cdot 10^{-3}$	6

When the results obtained on RPW and RP plates are compared, it could be noted that, in analogous systems, goodness of fit of equations to experimental data is higher in RPW systems (r values are in most cases higher). But, values of the F statistic are, in most cases, higher for data obtained on RP-18 layers. In that case, for determination of lipophilicity, all experimental data were taken into account. However, the values of R_{MW} (log k_W) obtained in each system were different (Tables 1–6).

Figure 2 shows R_{MW} (log k_W) (intercept of linear equation) obtained on RP18W layers in the system methanol-water with the values obtained in the other systems with dioxane and acetone as modifiers, as well as R_{MW} values obtained in eluent systems with dioxane and acetone. Regression coefficients

Table 3. Parameters for the relationships between R_M and modifier concentration (χ or φ) obtained for systems RP-18W – acetone + water (40–65%) calculated from Eqs. (1) and (2) and their statistical evaluation

Solute	S	R_{MW}	r	F	s	n
$R_M = -S\varphi + R_{MW}$						
A1	−1.76	1.90	0.934	13.77	0.11	6
A2	−2.56	1.95	0.956	21.42	0.12	6
A3	−2.69	2.22	0.919	10.83	0.18	6
A4	−1.64	1.08	0.929	12.60	0.10	6
A5	−1.97	1.61	0.936	14.10	0.12	6
A6	−2.21	1.79	0.938	14.67	0.13	6
B1	−1.33	1.39	0.946	17.09	0.07	6
B2	−1.93	1.48	0.942	15.66	0.09	6
B3	−1.61	1.50	0.957	21.94	0.08	6
B4	−1.93	0.89	0.962	49.93	0.07	6
B5	−1.15	0.90	0.952	19.17	0.06	6
B6	−1.55	1.23	0.969	30.61	0.06	6

	A	B	C	r	F	s	n
$R_M = \mathrm{A}\varphi^2 + \mathrm{B}\varphi + \mathrm{C}$							
A1	6.63	−9.05	3.82	0.986	17.76	0.07	6
A2	8.63	−12.05	4.45	0.999	216.01	0.03	6
A3	12.81	−16.78	5.93	0.998	156.12	0.04	6
A4	5.52	−7.72	2.68	0.97	7.99	0.10	6
A5	7.67	−10.40	3.83	0.991	26.85	0.06	6
A6	8.27	−11.30	4.19	0.989	22.92	0.08	6
B1	5.01	−6.84	2.84	0.998	150.31	0.02	6
B2	5.25	−7.71	3.01	0.969	7.72	0.11	6
B3	3.63	−5.60	2.55	0.977	10.29	0.08	6
B4	5.58	−8.07	2.52	0.998	357.18	0.02	6
B5	2.98	−4.44	1.76	0.977	10.35	0.06	6
B6	3.94	−5.88	2.37	0.994	38.89	0.04	6

of correlations are relatively high $-0.8800 < \mathrm{r} < 0.9000$, when we compare all modifiers. The highest correlation is for systems containing methanol and acetone. The F statistic values are also high. For all correlations compared, $\mathrm{F} > \mathrm{F}_{\mathrm{critical}}$.

The correlations of R_{MW} (log k_W) values obtained from quadratic equations (C) are low $-\mathrm{r} < 0.62$ for all systems investigated. We have also correlated R_{MW} (log k_W) values obtained from both equations (C values from the quadratic equation as a function of intercept from linear equations) for the group of solutes in each investigated system, and the correlation coefficient is high only for acetone as modifier ($\mathrm{r} > 0.8656$).

Table 4. Parameters for the relationships between R_M and modifier concentration (χ or φ) obtained for systems RP-18 – methanol + water (50–90%) calculated from Eqs. (1) and (2) and their statistical evaluation

Solute	S	R_{MW}	$\log P_{EXP}$	r	F	s	n
$R_M = -S\varphi + R_{MW}$							
A1	−2.42	1.98	2.271	0.988	283.25	0.06	9
A2	−3.23	2.73	3.108	0.994	355.65	0.05	9
A3	−3.37	2.84	3.234	0.99	206.95	0.07	9
A4	−2.55	1.94	2.228	0.993	473.75	0.05	9
A5	−2.85	2.27	2.598	0.988	245.96	0.06	9
A6	−2.79	2.29	2.620	0.992	347.35	0.05	9
B1	−1.77	1.30	1.518	0.977	147.08	0.06	9
B2	−2.64	2.11	2.421	0.982	181.45	0.07	9
B3	−2.66	2.10	2.412	0.985	211.90	0.07	9
B4	−1.62	1.05	1.239	0.95	65.55	0.08	9
B5	−1.47	1.01	1.199	0.968	103.55	0.06	9
B6	−1.84	1.38	1.611	0.987	255.44	0.04	9
	A	B	C	r	F	s	n
$R_M = \mathrm{A}\varphi^2 + \mathrm{B}\varphi + \mathrm{C}$							
A1	1.53	−4.55	2.70	0.99	153.93	0.05	9
A2	2.03	−6.07	3.69	0.996	400.36	0.04	9
A3	3.01	−7.58	4.27	0.995	314.42	0.05	9
A4	2.47	−6.01	3.11	0.999	1116.10	0.02	9
A5	3.51	−7.76	3.93	0.998	779.36	0.03	9
A6	2.28	−5.98	3.37	0.996	348.29	0.04	9
B1	3.26	−6.34	2.84	0.998	746.25	0.02	9
B2	4.25	−8.59	4.12	0.998	855.70	0.03	9
B3	3.86	−8.07	3.93	0.998	762.55	0.03	9
B4	4.45	−7.86	3.16	0.995	320.63	0.03	9
B5	2.99	−5.66	2.43	0.993	219.10	0.03	9
B6	1.74	−4.28	2.21	0.992	190.76	0.04	9

Figure 3 shows R_{MW} ($\log k_W$) (intercept of linear equation) obtained on RP18 layers in the system methanol-water with the values obtained in the other systems with dioxane and acetone as modifiers, as well as R_{MW} values obtained in eluent systems with dioxane and acetone. Regression coefficients of correlation lines are high $-0.8800 < \mathrm{r} < 0.9700$, when all modifiers are compared. The highest correlation is for systems containing dioxane and acetone. The F statistic values are also high and, for all correlations compared, are higher than F_{critical}.

The correlations of R_{MW} ($\log k_W$) values obtained from the quadratic equation (C) are low $-0.4300 < \mathrm{r} < 0.7900$, for all systems investigated. We have also correlated R_{MW} ($\log k_W$) values obtained from both equations

Table 5. Parameters for the relationships between R_M and modifier concentration (χ or φ) obtained for systems RP-18 – dioxane + water (50–75%) calculated from Eqs. (1) and (2) and their statistical evaluation

Solute	S	R_{MW}	$\log P_{EXP}$	r	F	s	n
$R_M = -S\varphi + R_{MW}$							
A1	−2.32	1.65	2.461	0.955	41.71	0.08	6
A2	−3.35	2.47	3.302	0.98	97.44	0.07	6
A3	−3.10	2.29	3.114	0.985	133.81	0.06	6
A4	−2.14	1.40	2.203	0.984	125.93	0.04	6
A5	−2.45	1.74	2.547	0.979	90.45	0.05	6
A6	−2.81	2.05	2.864	0.98	99.44	0.06	6
B1	−0.98	0.63	1.408	0.932	26.46	0.04	6
B2	−2.87	1.98	2.8	0.984	118.37	0.06	6
B3	−2.15	1.42	2.226	0.981	100.62	0.04	6
B4	−1.47	0.78	1.568	0.968	59.21	0.04	6
B5	−1.20	0.74	1.527	0.943	32.25	0.04	6
B6	−2.37	1.59	2.392	0.975	76.49	0.06	6
	A	B	C	r	F	s	n
$R_M = A\varphi^2 + B\varphi + C$							
A1	8.14	−12.49	4.77	0.986	52.94	0.05	6
A2	4.90	−9.47	4.35	0.986	51.18	0.07	6
A3	5.83	−10.39	4.52	0.995	138.66	0.04	6
A4	3.88	−6.99	2.89	0.993	106.99	0.03	6
A5	5.17	−8.92	3.72	0.99	74.76	0.04	6
A6	3.59	−7.30	3.42	0.985	48.01	0.06	6
B1	4.65	−6.79	2.41	0.986	52.87	0.02	6
B2	5.53	−9.78	4.10	0.993	109.20	0.04	6
B3	4.37	−7.61	3.10	0.991	86.20	0.03	6
B4	3.66	−6.05	2.19	0.984	44.82	0.03	6
B5	4.72	−7.10	2.55	0.982	39.53	0.03	6
B6	4.42	−7.89	3.28	0.984	45.25	0.05	6

(C values from the quadratic equation as a function of intercept from linear equations) for the group of solutes in each investigated system, and the correlation coefficient is high for acetone as modifier ($r > 0.8000$) and for dioxane ($r > 0.8400$).

The correlations of lipophilicity parameters obtained on RP18 and RP18W layers in analogous eluent systems have also been calculated. The correlations of lipophilicity parameters extrapolated from the quadratic equation are extremely low $-r < 0.4630$. In the case of parameters obtained from linear equations, correlation coefficients are significantly higher $- 0.8300 < r < 0.8600$.

Table 6. Parameters for the relationships between R_M and modifier concentration (χ or φ) obtained for systems RP-18 – acetone + water (50–75%) calculated from Eqs. (1) and (2) and their statistical evaluation

Solute	S	R_{MW}	$\log P_{EXP}$	r	F	s	n
$R_M = -S\varphi + R_{MW}$							
A1	−2.54	1.76	2.068	0.99	208.25	0.04	6
A2	−3.79	2.67	2.920	0.996	471.43	0.04	6
A3	−3.41	2.43	2.696	0.995	383.89	0.04	6
A4	−2.19	1.33	1.660	0.962	48.99	0.07	6
A5	−2.72	1.83	2.129	0.998	1134.3	0.02	6
A6	−3.34	2.29	2.569	0.998	1359.0	0.02	6
B1	−1.94	1.16	1.503	0.998	877.95	0.01	6
B2	−3.39	2.22	2.499	0.998	933.08	0.02	6
B3	−2.86	1.83	2.136	0.999	3737.3	9.8×10^{-3}	6
B4	−2.25	1.14	1.481	0.973	71.10	0.06	6
B5	−1.97	1.10	1.447	0.989	177.12	0.03	6
B6	−2.94	1.82	2.124	0.998	1079.1	0.02	6
	A	B	C	r	F	s	n
$R_M = \mathrm{A}\varphi^2 + \mathrm{B}\varphi + \mathrm{C}$							
A1	1.79	−4.87	2.45	0.992	90.78	0.04	6
A2	1.33	−5.45	3.18	0.996	191.62	0.04	6
A3	3.53	−7.82	3.78	0.998	320.63	0.03	6
A4	8.27	−12.53	4.50	0.997	272.64	0.02	6
A5	0.99	−3.96	2.21	0.999	533.29	0.02	6
A6	1.82	−5.62	2.99	0.999	1107.83	0.01	6
B1	−0.48	−1.34	0.98	0.998	354.33	0.01	6
B2	2.02	−5.91	2.99	0.999	630.23	0.02	6
B3	−0.97	−1.65	1.46	0.999	3251.5	7.4×10^{-3}	6
B4	−0.50	−1.62	0.94	0.973	26.80	0.06	6
B5	2.27	−4.82	1.97	0.992	97.37	0.03	6
B6	0.19	−3.18	1.90	0.998	407.20	0.02	6

For the reason of poor correlations of lipophilicity parameters obtained from the quadratic equations, we decided to reject these values from further consideration. Because linear equations had better goodness of fit to the experimental data obtained on RP18 layers than those obtained on RP18W layers, we decided to consider lipophilicity parameters obtained on RP18 layers. Moreover, correlations of lipopilicity parameters obtained on RP18 layers in all investigated eluent systems were significantly higher.

The retention of standards with known log P values from the literature[26] (listed in Experimental) was examined on RP18 layers in all eluent systems of various modifier concentrations. Extrapolated R_{MW} (log k_W) values were correlated and linear dependencies with high regression coefficients were

Table 7. Parameters for the relationships between R_M [calculated from Eq. (1)] and log P obtained for standards on RP-18 for all eluent systems used (methanol, dioxane, and acetone), their statistical evaluation, and the calibration equations

Standards methanol	$\log P_{EXP} = 1.1122\ R_{MW} + 0.0731$ $r = 0.974$, F $= 12.14$, $s = 0.29$						
	S	R_{MW}	$\log P$	r	F	s	n
S1	1.34	0.576	0.83	0.985	121.98	0.03	6
S2	1.89	1.035	1.32	0.978	87.24	0.04	6
S3	2.98	2.229	2.18	0.994	340.47	0.03	6
S4	3.30	2.492	2.69	0.992	870.53	0.02	6
S5	3.24	2.525	2.82	0.993	258.26	0.04	6
S6	3.55	3.202	4.01	0.998	1456.51	0.02	6

Standards dioxane	$\log P_{EXP} = 1.0272\ R_{MW} + 0.7637$ $r = 0.997$. F $= 11.45$. $s = 0.09$						
	S	R_{MW}	$\log P$	r	F	s	n
S1	0.84	0.090	0.83	0.922	22.78	0.04	6
S2	1.27	0.500	1.32	0.962	49.34	0.04	6
S3	2.17	1.349	2.18	0.981	101.14	0.05	6
S4	2.77	1.830	2.69	0.98	98.77	0.06	6
S5	3.02	2.159	2.82	0.992	256.25	0.04	6
S6	4.09	3.097	4.01	0.99	214.69	0.06	6

Standards acetone	$\log P_{EXP} = 0.9408\ R_{MW} + 0.4109$ $r = 0.991$. F $= 34.58$. $s = 0.17$						
	S	R_{MW}	$\log P$	r	F	s	n
S1	1.61	0.264	0.83	0.983	114.75	0.03	6
S2	2.38	1.042	1.32	0.964	52.75	0.07	6
S3	3.23	2.010	2.18	0.993	280.33	0.04	6
S4	3.89	2.568	2.69	0.999	5379.06	0.01	6
S5	3.80	2.617	2.82	0.996	552.59	0.03	6
S6	4.74	3.600	4.01	0.996	587.67	0.04	6

obtained (see Figure 4 and Table 7). The highest r value was obtained in the system with dioxane as modifier. The experimental lipophilicity values (log P_{EXP}) calculated from these linear equations are presented in Tables 4–6. Obtained log P_{EXP} values seemed to be a better measure of the lipophilicity of the investigated compounds than the R_{MW} values. The deviation of the values (ΔR_{MW} and Δlog P_{EXP}) for the eluent systems used were in the range 0.14–0.68 and 0.11–0.57, respectively, with only three values exceeding 0.4 for log $\mathrm{P_{EXP}}$ in comparison to six for $\mathrm{R_{MW}}$. Besides, the experimental lipophilicity values were always higher than determined on the plates, which seems understandable in view of the compound structures (Figure 1).

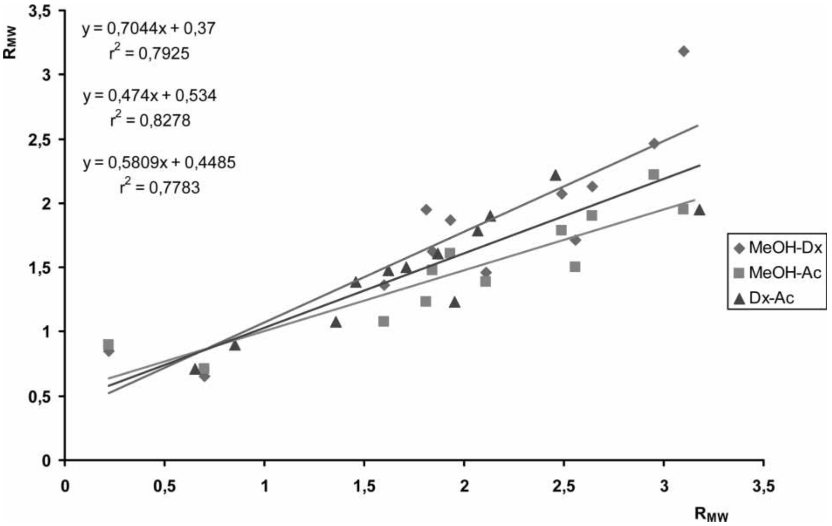

Figure 2. Correlation of the RP-18W lipophilicity calculated by Eq. (1) for the solvents used.

CONCLUSIONS

Retention-eluent composition dependencies for the investigated compounds can be described by linear as well as squared equations.

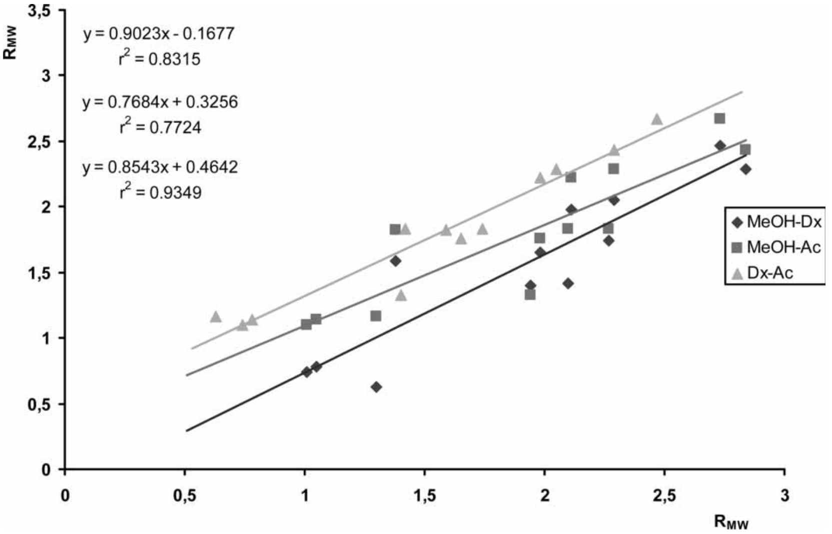

Figure 3. Correlation of the RP-18 lipophilicity calculated by Eq. (1) for the solvents used.

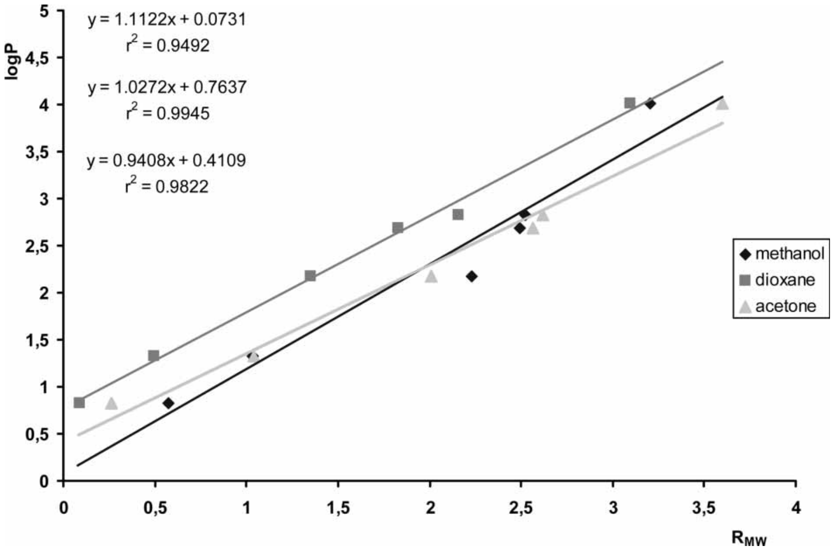

Figure 4. Correlation of the R_{MW} and $\log P$ values for standards in the solvents used.

R_{MW} ($\log k_W$) parameters can be extrapolated from both equations as intercept of linear plots or as free component (C) from squared equations.

R_{MW} ($\log k_W$) obtained in various systems from the linear equations are in better correlation than that obtained from the squared ones.

The results of the standardization ($\log P_{esp}$) obtained for solvents used on RP18 layers are similar, despite the solvent [$\Delta \log P_{esp} \leq 0.57$ (± 0.235)].

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