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Evaluation of the predictive power of calculation procedure for molecular hydrophobicity of some estradiol derivates

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Abstract

Several calculation procedures for log *P* values based on the fragmental and atomic contributions are compared with experimental reversed-phase liquid chromatography (RPLC) retention of estradiol derivates. The RPLC experiments were performed on HPTLC and HPLC commercially available stationary phases. Binary solvent mixtures of methanol–water and acetonitrile–water were used as mobile phases. The correlation between log *P* and various chromatographically obtained hydrophobicity parameters (R_M^0, log k_w and φ_0) are quantified. The R_M^0, i.e., log k_w were obtained by linear extrapolation of retention to 0% organic modifier. φ_0 values were obtained from the slopes and intercepts of such linear relationship. The mutual relationship between $\varphi_{0,MeOH}$ and $\varphi_{0,ACN}$ values of the compounds were discussed. The obtained statistical results can be summarized in the following order of reliabilities for different log *P* calculation methods: Broto>ACD/logP>Crippen>Rekker>Viswanadhan.

Keywords: Quantitative structure–retention relationships; Hydrophobicity parameters; Estradiol

1. Introduction

Estrogens are important physiologically active substances produced by the ovaries. Among the most important estrogens is estradiol. A direct effect on activity in particular of the binding activity of the estradiol can have some simple chemical modification of the basic structure of steroid. Thus, an understanding of the structure–activity relationships when small conformational changes in estrogens take place is important in the design of new estrogens and antiestrogens [1].

Estrogenic activity may be measured by various methods. For initial chemical screening of activity of newly synthesized estrogens it is recommended first to determine their hydrophobicity since hydrophobic character of a molecule often seems to be the most important physico–chemical parameter in accounting for the variations of biological activity. Usually, the hydrophobicity is quantitatively characterized as log *P* (the logarithm of the ratio of the concentrations of any analyte in a saturated 1-octanol–water system) established by Hansch and co-workers [2,3].

The traditional experimental method for the determination log $P_{o/w}$, is shake flask method. Nowadays liquid chromatography has a tendency to replace tedious and poor interlaboratory reproducible shake flask method for measuring partition coefficients. Among liquid chromatography methods re-

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versed-phase liquid chromatography (RPLC) is an alternative technique that can correlate the hydrophobicity of compounds with the retention parameters [4]. To avoid the practical difficulties that often arise in the direct determination of the partition coefficient, extrapolated retention parameters of various organic–water eluent compositions were used as a measure of hydrophobicity.

The chromatographic retention of a single solute in binary aqueous–organic mobile phases on a RP high-performance liquid chromatography (HPLC) system, can be modeled as liner function of a mobile phase composition within a limited yet useful range of mobile phase compositions:

$$\log k = \log k_w - S\varphi \quad (1)$$

where log k is the solute retention factors at a specific mobile phase composition, φ is the mobile phase composition expressed as the volume fraction of the organic modifier in the eluent. The intercept log k_w corresponds to the retention in pure water as mobile phase and represents the commonly employed chromatographic hydrophobicity parameter. S is a solute-dependent solvent strength specific to the organic modifier on the stationary phase under consideration.

Analogous to log k_w is the R_M^0 parameter from high-performance thin-layer chromatography (HPTLC), so Eq. (1) takes the form:

$$R_M = R_M^0 - S\varphi \quad (2)$$

where R_M expressed the retention of solute in laminar HPTLC chromatography.

Another retention related parameter has recently been introduced, the isocratic chromatographic hydrophobicity index, φ_0 [5,6]. According to Valkó and co-workers the φ_0 value represents the volume fraction of organic solvent in the mobile phase which causes the retention t_0 to equal zero ($\log k = 0$), i.e., the amount of solute in the mobile and stationary phases are equal, and the retention factor is 1:

$$0 = \log k_w - S\varphi_0 \quad (3)$$

From Eq. (3) follows:

$$\varphi_0 = \log k_w / S \quad (4)$$

where φ_0 represents the ratio of the slope and intercept of Eq. (1).

In addition to the experimental method, a number of other methods for calculation of 1-octanol–water partition coefficients have been established [7–10]. Routine application of calculative approaches demands a continuous check of their validity by comparison with the experimental procedure.

This paper will analyze and discuss the correlation between log P data, a hydrophobicity expression, calculated by different procedures, and chromatographic retention parameters of the estradiols (log k_w, R_M^0 and φ_0). In order to get a better insight into the nature of chromatographic measures of hydrophobicity of estradiols, three different chromatographic commercially available stationary phases, with eluents consisting of methanol–water and acetonitrile–water mixtures were used.

2. Experimental

2.1. Thin-layer chromatography

Thin-layer chromatography was performed on 10×10 HPTLC plates (HPTLC Fertigplatten, RP-18 F-254, E. Merck, Darmstadt, Germany) precoated with C_{18} bonded silica gel containing a fluorescent indicator. The chromatograms were developed by ascending technique at room temperature and without previous saturation of the chamber with solvent. Spots were observed under UV light at 254 nm with a Camag UV lamp (Camag, Muttenz, Switzerland).

2.2. High-performance liquid chromatography

The HPLC measurements were made using a Milton Roy (Riviera Beach, FL, USA) liquid chromatograph, consta Metric 3000 pump and a Milton Roy spectro Monitor 3100 variable-wavelength UV–Vis detector set at 254 nm. Samples were injected using a Rheodyne 7125 valve (Cotati, CA, USA) fitted with a 20 μl loop. The columns used were LiChrosorb® RP-18, 250×4 mm I.D., particle size 5 μm (E. Merck) and LiChrospher® RP-8, 150×4.6 mm I.D., particle size 5 μm (E. Merck).

2.3. Chemicals and solutions

Two binary solvent systems, methanol–water and acetonitrile–water, were used as a mobile phase with

a varying content of organic component; methanol 60–95%, and acetonitrile 70–90%; increment 5%. Water used as mobile phase component was twice distilled; methanol and acetonitrile were of HPLC grade (E. Merck). The eluents were pre-filtered through a 0.45-μm filter and degassed in an ultrasonic bath before use. The flow-rate of mobile phase was 1 ml min^{-1} at room temperature (ca. 22°C); the amount of sample solution was 20 μl $injection^{-1}$.

The investigated compounds were estradiol derivates (series A) and 6-ketoestradiol derivates (series B), Table 1, synthesized by original reactions or according to literature methods [11]. Amounts of 2 and 0.2 mg ml^{-1} of each compound were dissolved in methanol for HPTLC and HPLC experiments, respectively. The solutions for HPLC investigations were prefiltered through a 0.2-μm Chromafil filter (Macherey-Nagel, Duren, Germany). Retention values of investigated substances were averages from at least three measurements for each solute–solvent combination.

2.4. Log P calculation

Using atom based Crippen's [12], Viswanadhan's [13], Broto's method [14], and fragmental based Rekker's method [15] and ACD/logP software (Advanced Chemistry, Toronto, Canada), several different log *P* values were calculated for investigated estradiol derivates. Computed log *P* values of investigated compounds are summarized in Table 1.

In both series, hydrophobicity (expressed by log *P*) depended on the characteristics of the substituents in the molecule. Generally, the log *P* values of 6-ketoestradiols (series B) were lower than the log *P* values of estradiols (series A) due to the presence the 6-keto group in the molecules of series B.

Different calculation procedures resulted in different log *P* values. The mutual correlations between calculated log *P* values obtain to various methods [12–15] are different. The Viswanadhan and Broto method for calculation log *P* values show the best intercorrelation (0.9975), while correlation coefficients between Rekker and ACD/logP log *P* values are poor (0.9088). The question arise here is which procedure is suitable for calculation of log *P* values of steroids?

3. Results and discussion

As expected, the experimental data obtained revealed a linear relationship between retention and concentration of organic modifier in eluent. The retention decreases linearly with an increase in concentration of modifier in mobile phase; Eqs. (1) and (2) in HPLC and HPTLC systems, respectively. The parameters of Eqs. (1) and (2) for the methanol–water and acetonitrile–water eluents are given in

Table 1
Steroid nomenclature and its log *P* values calculated by different methods

No.	IUPAC name	Common name	Log *P* calculated by method				
			Atom based			Fragmental based	
			Crippen	Viswanadhan	Broto	Rekker	ACD/logP
1	3,17β-Dihydroxyestra-1,3,5(10)-triene	Estradiol	3.91	4.01	3.79	5.18	4.13
2	17β-Hydroxy-3-metoxyestra-1,3,5(10)-triene	Estradiol-3-methyl ether	4.17	4.04	4.31	5.79	4.78
3	17β-Hydroxy-3-acetoxyestra-1,3,5(10)-triene	Estradiol-3-acetate	3.87	3.79	3.91	5.40	4.20
4	3,17β-Diacetoxyestra-1,3,5(10)-triene	Estradiol diacetate	4.11	3.92	4.84	5.63	5.10
5	17β-Hydroxy-3-propionoxyestra-1,3,5(10)-triene	Estradiol-3-propionate	4.54	4.42	4.37	5.93	4.74
6	3,17β-Dipropinoxyestra-1,3,5(10)-triene	Estradiol dipropionate	5.42	5.18	5.75	6.69	6.16
7	17β-Hydroxy-3-benzyloxyestra-1,3,5(10)-triene	Estradiol-3-benzoyl ether	5.78	5.70	5.20	6.59	6.24
8	3,17β-Dibenzyloxyestra-1,3,5(10)-triene		7.91	7.75	7.42	7.99	9.15
9	17β-Benzylox-3-acetoxyestra-1,3,5(10)-triene		6.01	5.83	6.13	6.81	7.12
10	3,17β-Dihydroxyestra-1,3,5(10)-triene-6-one	6-Ketoestradiol	2.55	2.68	2.38	3.60	3.30
11	17β-Hydroxy-3-metoxyestra-1,3,5(10)-triene-6-one		2.82	2.72	2.90	4.22	3.74
12	3-Hydroxy-17β-propionoxyestra-1,3,5(10)-triene-6-one		3.44	3.44	3.76	4.36	4.73
13	3,17β-Dipropionoxyestra-1,3,5(10)-triene-6-one		4.07	3.85	4.34	5.11	5.12

Table 2
Parameters of the linear correlation between R_M (log k) values of the compounds and the concentration of organic component in the mobile phase

No.	Methanol–water									Acetonitrile–water								
	HPTLC			C_8 (HPLC)			C_{18} (HPLC)			HPTLC			C_8 (HPLC)			C_{18} (HPLC)		
	R_M^0	S	r	Log k_w	S	r	Log k_w	S	r	R_M^0	S	r	Log k_w	S	r	Log k_w	S	r
1	2.832	3.27	0.999	2.654	3.49	0.999	2.293	3.10	0.999	1.071	1.85	0.999	0.925	1.29	0.998	1.251	2.18	0.997
2	4.637	4.65	0.992	5.212	5.82	0.999	4.102	4.50	0.992	2.236	2.44	0.992	1.824	1.93	0.999	2.894	3.35	0.997
3	4.761	5.04	0.996	4.739	5.54	0.992	3.773	4.40	0.996	1.454	1.84	0.996	1.607	1.81	0.999	2.391	3.03	0.998
4	6.270	6.46	0.999	5.674	6.31	0.999	5.153	5.62	0.999	3.000	3.45	0.999	2.709	2.79	0.999	4.066	4.74	0.999
5	–	–		4.852	5.58	0.999	–	–	–	2.477	3.16	0.996	1.856	1.98	0.997	–	–	–
6	7.180	7.20	0.997	6.309	6.83	0.999	6.876	7.25	0.999	3.256	3.47	0.998	3.352	3.308	0.998	4.566	5.00	0.999
7	–	–		6.181	6.81	0.999	5.36	5.60	0.999	2.567	2.77	0.999	2.505	2.55	0.998	3.646	3.98	0.999
8	–	–		8.390	8.75	0.999	–	–	–	4.577	4.58	0.999	4.396	4.15	0.999	–	–	–
9	–	–		6.763	7.24	0.999	–	–	–	4.104	4.27	0.999	3.636	3.56	0.999	–	–	–
10	1.883	2.60	0.998	3.036	4.32	0.999	1.527	2.61	0.997	0.110	0.95	0.997	0.468	1.02	0.989	0.657	1.90	0.999
11	3.584	4.03	0.996	3.162	3.96	0.999	2.758	3.42	0.998	0.853	1.31	0.999	1.012	1.30	0.993	1.359	2.03	0.991
12	4.089	4.57	0.998	–	–	–	3.62	4.44	0.999	2.120	3.02	0.999	–	–	–	2.016	2.83	0.993
13	6.075	6.50	0.999	–	–	–	4.803	5.50	0.999	2.577	3.20		–	–	–	4.077	4.92	0.999

Table 2. From Table 2 it can be observed that the both log k_w (R_M^0) and S values of investigated substances significantly differ between RPLC systems and are evidently higher in the case of data determined with methanol as the eluent modifier than with acetonitrile. Other authors also report a different influence of methanol and acetonitrile on the retention of organic compounds [16,17].

It is supposed that slope value S is related to the hydrophobic surface of the molecule that interacts with the non-polar stationary phase [18]. Lower slope values in the case of the acetonitrile–water system are due to a stronger preferential adsorption of acetonitrile on stationary phase ligand and a higher affinity of analyte to the acetonitrile-solvated than to the methanol-solvated hydrocarbon of the stationary phase. This means that acetonitrile–water mobile phase is less sensitive to the changes in the structure of the studied compounds compared to the methanol–water eluent. Generally, the slope values S are influenced by the presence of a substituent in the molecule and for a given stationary and mobile phase system increase in the following order: 3-OH < 3-$OCOCH_3$ < 3-OCH_3 < 3-$OCOCH_2CH_3$ < 3-$OCOC_6H_5$.

As the log k_w and R_M^0 values characterize the partition of the compound between the non-polar hydrocarbon stationary phase and water they have been used as reliable alternatives to the classical log P to express the lipophilic character of a substance [19]. Correlation between R_M^0 and log k_w values and log P can be expressed by Collander-type equations:

$$\log k_w = a_0 + a_1 \log P \quad (5)$$

$$R_M^0 = a_0' + a_1' \log P \quad (6)$$

where a_0, a_1, a_0', a_1' are constants.

All the log k_w and R_M^0 values obtained with

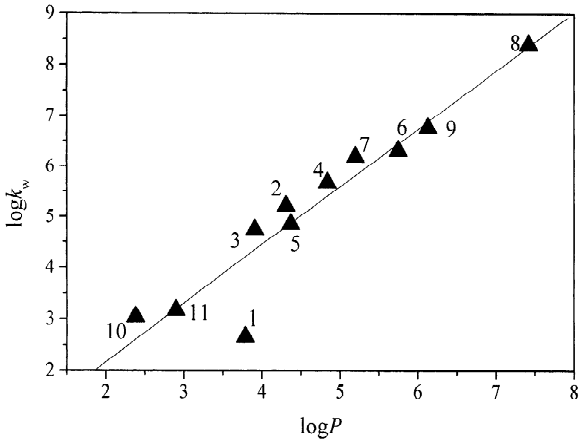

Fig. 1. Plot of log k_w vs. log P_{Broto} for the C_8 HPLC column with methanol–water mobile phase.

Table 3
Results of linear correlation according to the following equations: log $P_{Calc}=a_0+a_1R_M^0$ (HPTLC) and log $P_{Calc}=a_0+a_1$ log k_w (HPLC)

Log P calculated by method	Methanol–water					Acetonitrile–water				
	a_0	a_1	r	n	P	a_0	a_1	r	n	P
HPTLC										
Crippen	−1.892	1.698	0.8296	9	0.0057	−1.006	0.751	0.8624	13	0.0001
Viswanadhan	−1.824	1.717	0.7551	9	0.0186	−0.958	0.757	0.8360	13	0.0004
Broto	−1.681	1.568	0.9118	9	0.0006	−1.629	0.882	0.9404	13	<0.0001
Rekker	−2.966	1.479	0.8053	9	0.0088	−2.676	0.897	0.8524	13	0.0002
ACD/logP	−3.880	1.848	0.9198	9	0.0004	−1.478	0.732	0.9105	13	<0.0001
C_8 HPLC										
Crippen	0.342	1.041	0.9171	11	<0.0001	−1.281	0.748	0.9203	11	<0.0001
Viswanadhan	0.363	1.059	0.8962	11	0.0002	−1.256	0.759	0.8968	11	0.0002
Broto	−0.113	1.142	0.9470	11	<0.0001	−1.723	0.845	0.9788	11	<0.0001
Rekker	−2.453	1.315	0.9247	11	<0.0001	−3.330	0.952	0.9345	11	<0.0001
ACD/logP	0.080	0.956	0.9358	11	<0.0001	−1.505	0.694	0.9480	11	<0.0001
C_{18} HPLC										
Crippen	−1.355	1.340	0.8439	10	0.0021	−1.610	1.072	0.7869	10	0.0069
Viswanadhan	−1.186	1.325	0.7843	10	0.072	−1.406	1.042	0.7192	10	0.0191
Broto	−2.111	1.490	0.9467	10	<0.0001	−2.382	1.232	0.9127	10	0.0002
Rekker	−3.114	1.358	0.8487	10	0.0019	−3.080	1.098	0.8001	10	0.0054
ACD/logP	−3.327	1.548	0.9315	10	<0.0001	−3.294	1.260	0.8844	10	0.0007

methanol–water and acetonitrile–water eluents were regressed versus the calculated log P data. In Fig. 1, as an example, the plot shows the log k_w values obtained on the C_8 HPLC column with methanol–water eluent regressed against the log P calculated by Broto's method. Table 3 compares the results of correlation analysis of experimental R_M^0 or log k_w versus log P calculated values using different algorithms.

The slope and intercept values from Table 3 depend on the nature of the mobile phase and a particular stationary phase. Comparing the calcula-

Table 4
φ_0 values of the estradiols and maximal deviation of calculated φ_0

No.	Methanol–water				Acetonitrile–water			
	$\varphi_{0,MeOH}$			$\varphi_{0,max}$	$\varphi_{0,ACN}$			$\varphi_{0,max}$
	HPTLC	C_8 HPLC	C_{18} HPLC	(%)	HPTLC	C_8 HPLC	C_{18} HPLC	(%)
1	0.866	0.760	0.740	17.0	0.579	0.717	0.574	24.9
2	0.997	0.895	0.911	11.4	0.916	0.945	0.864	9.4
3	0.945	0.855	0.857	10.5	0.790	0.888	0.789	12.5
4	0.970	0.899	0.917	7.9	0.869	0.971	0.858	13.2
5	–	0.869	–	–	0.784	0.937	–	19.5
6	0.997	0.924	0.948	7.9	0.938	1.013	0.913	10.9
7	–	0.908	0.957	5.4	0.927	0.982	0.916	7.2
8	–	0.959	–	–	0.999	1.059	–	6.0
9	–	0.934	–	–	0.961	1.021	–	6.2
10	0.724	0.703	0.585	23.8	0.116	0.459	0.346	295.7
11	0.889	0.798	0.806	11.4	0.651	0.778	0.669	19.5
12	0.895	–	0.815	9.8	0.702	–	0.712	1.4
13	0.935	–	0.873	7.1	0.805	–	0.829	3.0

tive methods, generally, the lowest correlation always gives log $P_{\text{Viswanadhan}}$ data set (with both mobile phases) and the best log P_{Broto}. In Table 3 the statistical P values for the C_8 column are less than 0.01; there is a statistically significant relationship between log k_w values at the 99% confidence level.

From Table 3 eluent acetonitrile–water compared to methanol–water shows slightly better correlation. Actually, one can argue which modifier provides better correlation with calculated log P.

For the slope and intercept of Eqs. (5) and (6) respectively, mean values of 1.0 and 0.0 can be expected [20]. The calculated values of slopes a_1 vary from 0.956 to 1.848 and 0.694–1.260 for methanol–water and acetonitrile–water eluents, respectively. In most cases, calculated a_1 and a_0 values for C_{18} phases (HPTLC and HPLC) differ more than the ones on the C_8 column, emphasizing the different influence of phase ratio and chromatographic response of the stationary phases toward the compounds. Additionally, the correlation coefficients obtained on the C_8 HPLC stationary phase are always the highest, indicating that log k_w obtained from measurements on the C_8 phase slightly better fit the calculated log P_{Calc} data than other systems. Also, highest correlation coefficient between log P_{Broto} and log k_w is observed on the C_8 column.

In order to investigate whether the correlations of the log k_w vs. log P (for HPLC) and R_M^0 vs. log P (for HPTLC) could be further improved, isocratic hydrophobicity index φ_0 was applied as another hydrophobicity parameter (Eq. (4)). Table 4 lists $\varphi_{0,\text{MeOH}}$ and $\varphi_{0,\text{ACN}}$ values. Table 4 also includes $\varphi_{0,\text{max}}$ values, representing the maximum percent deviation in φ_0 for the specific compound between the chromatographic systems applied. $\varphi_{0,\text{max}}$ was calculated according to the following equation:

$$\varphi_{0,\text{max}} = (\varphi_{0,\text{largest}} - \varphi_{0,\text{smallest}})/\varphi_{0,\text{smallest}} \quad (7)$$

φ_0 values in Table 4 reveal that for the eluent methanol–water $\varphi_{0,\text{max}}$ is less than 15% for eight substances. This confirms that in the absence of secondary retention mechanisms φ_0 values for different RPLC systems are rather similar. Greater values for $\varphi_{0,\text{max}}$ of 17.0 and 23.8% are observed for substances 1 and 10, respectively. This is due to the presence of two polar hydroxyl groups in their

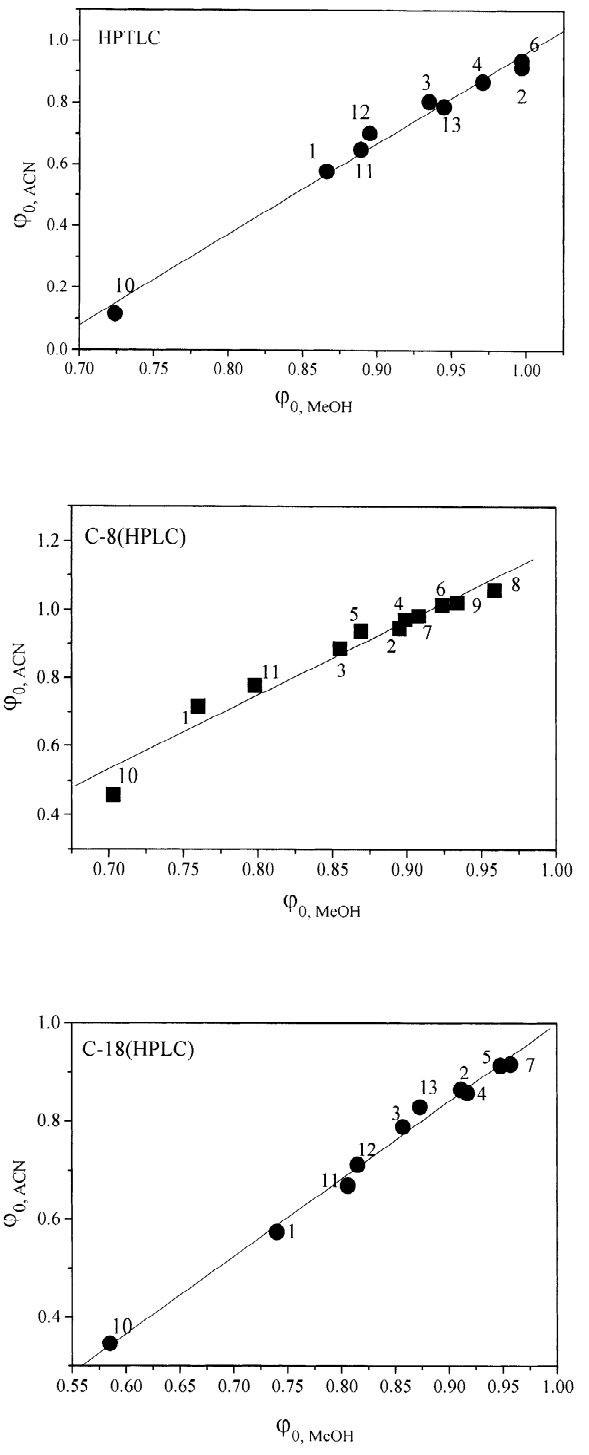

Fig. 2. Plot of $\varphi_{0,\text{MeOH}}$ vs. $\varphi_{0,\text{ACN}}$ of different LC systems.

structure that affected the higher polarity of the molecules.

As characteristics of acetonitrile are far different to methanol [21], $\varphi_{0,max}$ in the case of acetonitrile–water eluent differ compared to the methanol-modified eluents. Hence, $\varphi_{0,max}$ for acetonitrile-containing eluent varies in a wide range (from 1.4–295.7%). There are nine substances with $\varphi_{0,max}$ less than 15% (compounds 2, 3, 4, 6, 7, 8, 9, 12 and 13).

Data from Table 4 demonstrate that the nature of mobile phase in some, but not all cases is decisive for the obtained φ_0 values. Since $\varphi_{0,MeOH}$ and $\varphi_{0,ACN}$ values refer to isoeluotropic eluent mixtures, they both represent a mobile phase composition for which the same retention (log $k=0$) can be obtained. Therefore, it is possible to compare φ_0 values for the two mobile phases. Fig. 2 represents the correlation of the $\varphi_{0,MeOH}$ vs. $\varphi_{0,ACN}$ values on different chromatographic systems. Appropriate correlation coefficients for the lines from Fig. 2 are 0.9933 (HPTLC), 0.9770 (C_8) and 0.9959 (C_{18}).

Fig. 2 shows a high correlation between φ_0 values determined in methanol–water and acetonitrile–water systems. If in calculations for C_8 HPLC data one omits the most polar 6-ketoestradiol (compound 10) a very good correlation coefficient will be obtained; 0.9949. Also, slope and intercept values of C_8 HPLC move towards the values calculated for the C_{18} HPLC system.

Table 5 summarizes the results of regression calculations for $\varphi_{0,MeOH}$ and $\varphi_{0,ACN}$ values against different log P values.

Because of the lower correlation coefficients of φ_0 vs. log P relationships, φ_0 values are less suitable than log k_w or R_M^0 for measuring hydrophobicity compounds investigated. This is also in agreement with the results of other authors [22,23]. Table 5 shows a moderate correlation between the two parameters and suggests that chromatographically derived hydrophobicity scales are not closely similar to log P scales. As matter of fact, the φ_0 vs. log P relationship is better expressed with an exponential function, Fig. 3. However, reliability of estimation of hydrophobicity by φ_0 remains disputable.

We suppose that, as in the case of anilides [24] the addition of correction parameters might lead to the further improvement of correlation. This assumption is supported by the findings of Karajiannis and van de Waterbeemd [25] that traditional log P calculations do not properly account for molecular interac-

Table 5
Results of correlations according to the equation: $\varphi_0 = b_0 + b_1 \log P_{Calc}$

Log P calculated by method	Methanol			Acetonitrile		
	b_0	b_1	r	b_0	b_1	r
HPTLC						
Crippen	0.603	0.081	0.7990	0.243	0.117	0.7278
Viswanadhan	0.602	0.083	0.7387	0.263	0.115	0.6896
Broto	0.624	0.072	0.8464	0.138	0.139	0.8040
Rekker	0.512	0.078	0.8616	−0.126	0.159	0.8186
ACD/logP	0.551	0.079	0.7933	0.214	0.106	0.7114
C_8 HPLC						
Crippen	0.667	0.042	0.8253	0.480	0.088	0.7711
Viswanadhan	0.672	0.042	0.7897	0.492	0.087	0.7333
Broto	0.638	0.049	0.8932	0.415	0.102	0.8437
Rekker	0.524	0.058	0.9096	0.157	0.126	0.8819
ACD/logP	0.661	0.038	0.8218	0.479	0.077	0.7471
C_{18} HPLC						
Crippen	0.4799	0.0899	0.8001	0.181	0.141	0.7899
Viswanadhan	0.4930	0.0884	0.7397	0.204	0.138	0.7268
Broto	0.438	0.098	0.8779	0.110	0.155	0.8745
Rekker	0.333	0.097	0.8534	−0.048	0.151	0.8416
ACD/logP	0.367	0.100	0.8475	−0.001	0.157	0.8433

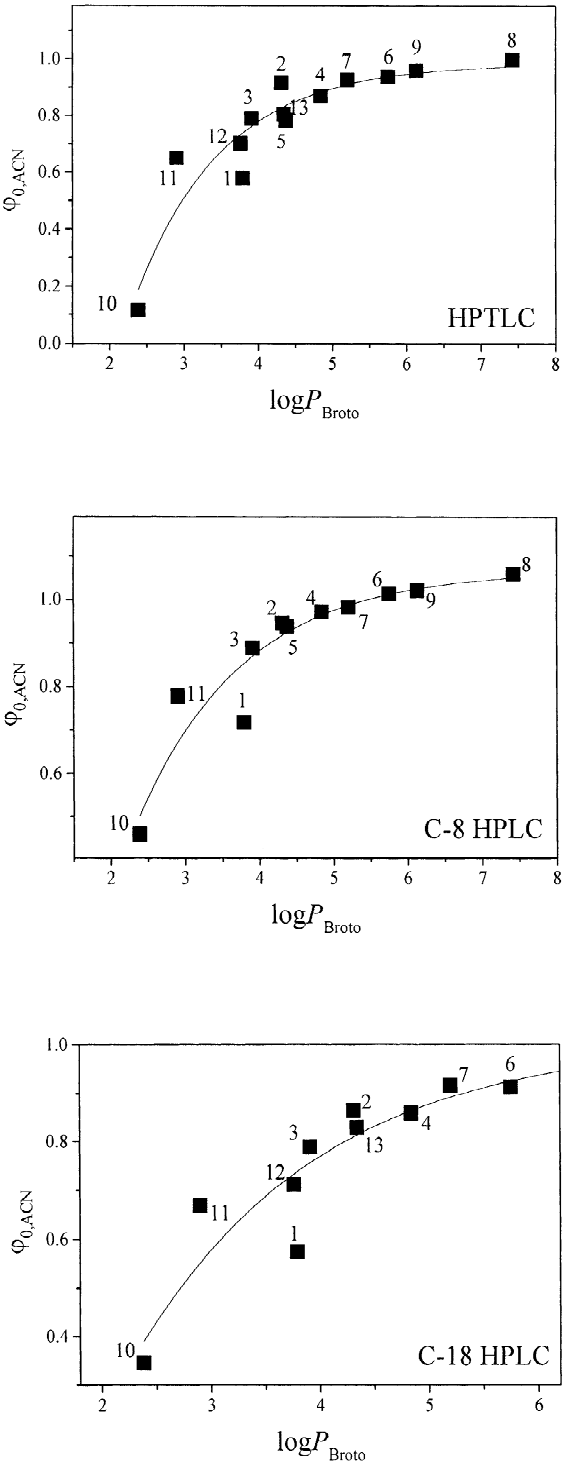

Fig. 3. Plot of $\varphi_{0,ACN}$ vs. log P values calculated by the Broto method on different LC systems.

tions. Hence, as experimental in nature log k_w and R_M^0 should be more reliable and thus could be equally well employed in quantitative structure–activity relationship (QSAR) studies.

4. Conclusions

The presence, number and the location of substituent groups are the main causes of the differences between the hydrophobicity of investigated compounds. The measured and calculated hydrophobicity of the compounds of series B (6-ketoestradiols) was lower than for the series A (estradiols).

Linear regression of log k or R_M vs. volume fraction of organic modifier content in mobile phase provides satisfactory results for both aqueous methanol and aqueous acetonitrile eluents. All investigated HPTLC and HPLC reversed-phases respond chromatographically differently to the test substances. However, all of them show a moderate satisfactory correlation with theoretically calculated log P values. The obtained statistical results can be summarized in the following order of reliabilities for different log P calculation methods: Broto>ACD/logP>Crippen>Rekker>Viswanadhan.

The replacement of R_M^0 and log k_w values with the isocratic hydrophobic index, φ_0, does not improve the linearity of the correlations with the calculated log P values, although the extrapolation to 100% water as the mobile phase was performed from mobile phases with high content of methanol and acetonitrile.

For estradiol derivatives log k_w and R_M^0 are comparable to log P_{Calc} in its ability to describe the hydrophobic nature of bioactive compounds.

The results of this study confirm that the standardization of log P measurement protocols by RPLC with more particularly defined columns to be applied and organic modifier would contribute to an increased reproducibility and reliability of results.

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