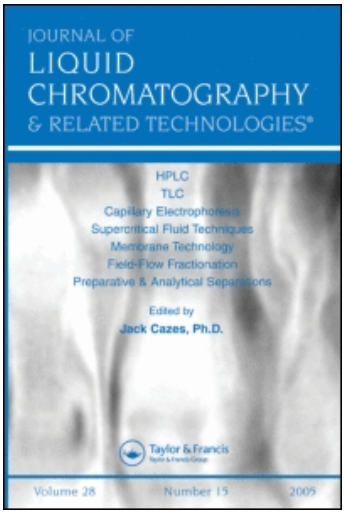

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Characterization of the GEMINI C18™ Column: Lipophilicity Measurement and LSER

Deborah Benhaim[a]; Eli Grushka[a]
[a] Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

To cite this Article Benhaim, Deborah and Grushka, Eli(2008) 'Characterization of the GEMINI C18™ Column: Lipophilicity Measurement and LSER', Journal of Liquid Chromatography & Related Technologies, 31: 15, 2198 — 2218
To link to this Article: DOI: 10.1080/10826070802279202
URL: http://dx.doi.org/10.1080/10826070802279202

Journal of Liquid Chromatography & Related Technologies®, 31: 2198–2218, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802279202

Characterization of the GEMINI C18™ Column: Lipophilicity Measurement and LSER

Deborah Benhaim and Eli Grushka

Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

Abstract: We describe here the use of Gemini C18™ column, a new generation hybrid silica based column, for reversed-phase liquid chromatographic determination of lipophilicity parameters of several structurally different xenobiotics (neutral solutes and ionized drugs). Among the parameters discussed, we show that extrapolated retention factor values $\log k'_w$, as well as retention factor values at 40% methanol ($\log k'_{40}$), obtained on the above column, were well correlated with literature values of $\log P$ (logarithm of the partition coefficient in *n*-octanol/water) of neutral compounds. Also found were linear relationships between measured $\log k'_w$ values and calculated values of the logarithm of the distribution coefficient at pH 7.0 ($\log D^{7.0}$) for ionized acidic and basic drugs. In addition, the Gemini C18™ column was characterized using the linear solvation energy relationship (LSER) model of Abraham. The LSER system constants for the column were compared to the LSER constants of *n*-octanol/water extraction system. For the comparison, the Tanaka radar plots were used. In addition, the LSER system constants of the Gemini C18™ column allow estimating the $\log P$ values of steroid hormones based on calculated $\log k'_w$ values.

Keywords: Lipophilicity determination, Reversed-phase liquid chromatography column, Octanol-water partition coefficients, LSER

Correspondence: Eli Grushka, Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel; E-mail: eliga@chem.ch.huji.ac.il

INTRODUCTION

Lipophilicity is one of the most important physicochemical descriptor; it plays a crucial role in the pharmacological activity of drugs and organic compounds, in particular in the passive transport of xenobiotics through biological membranes.[1–3] Conventionally lipophilicity is expressed by the logarithm of the partition coefficient of a neutral form of a drug in *n*-octanol/water system ($\log P$). For ionized compounds the distribution coefficient D^{pH} (or $\log D^{pH}$), at a given pH, is considered as a structural descriptor for quantitative structure activity relationships (QSAR).[4,5] However, the determination of *n*-octanol/water partition coefficient by the classical shake-flask method has several disadvantages (emulsion problems, large amount of pure compounds required, tedious and time consuming, etc.). Alternative chromatographic techniques were investigated and it was demonstrated that a correct choice of reversed phase HPLC (RP-HPLC) separation system could provide suitable model for estimating *n*-octanol-water partition coefficients.[6] Using a standard set of solutes, a correlation model is constructed between known $\log P$ values and chromatographic retention data, chiefly $\log k'$, of the solutes, obtained for a given mobile phase and stationary phase system:

$$\log P = a + b \log k' \tag{1}$$

The isocratic capacity factor, $\log k'$, or an extrapolated to 100% water capacity factor, $\log k'_w$, are used as chromatographic lipophilicity parameters.[7–9] The $\log k'_w$ parameter is the intercept of the plot of the logarithm of the retention factor ($\log k'$) versus fraction volume of the organic modifier (Φ). The extrapolation is based, among others on Snyder's linear solvent strength (LSS) model, which assumes a linear relationship between $\log k'$ and Φ over a limited range of binary mobile phase composition (Eq. (2)):[10–12]

$$\log k' = \log k'_w - S\Phi \tag{2}$$

where S is the slope of Equation 2. S is solute-dependent solvent strength parameter.

In order to unravel the intermolecular forces that dominate the retention process in reversed-phase liquid chromatography, the linear solvation energy relationship (LSER) of Abraham has widely been applied.[13–17] In its general form the LSER model can be written as:

$$\log k' = c + eE + sS + aA + bB + vV \tag{3}$$

The model is constructed by a sum of products. Each product represents an intermolecular interaction. E, S, A, B and V are the solute descriptors,

each one representing a specific property of the solute: E indicates excess molar refraction, S takes into account dipolarity/polarizability, A represents the effective hydrogen bond acidity, B the effective hydrogen bond basicity and V the Mc Gowan's characteristic volume. The lower case constants $\boldsymbol{e}, \boldsymbol{s}, \boldsymbol{a}, \boldsymbol{b}$ and $\boldsymbol{v}$ are system constants that are complementary to the solutes parameter. The $\boldsymbol{e}$ coefficient is a measure of the difference in the ability of the mobile and the solvated stationary phase to interact with the solute lone pair electrons, the $\boldsymbol{s}$ coefficient relates to the ability to participate to dipole-dipole and dipole-induced dipole interactions, the $\boldsymbol{a}$ and $\boldsymbol{b}$ coefficients refer to the ability to receive a proton and donate a proton in an hydrogen bond formation respectively, $\boldsymbol{v}$ relates to the ability of the solute to create a cavity in the mobile and stationary phases and $\boldsymbol{c}$ is a fitting constant.[18–20] Each system constant represents the difference in a particular property of the mobile phase and the solvated stationary phase.

The system constants are calculated by multiple linear regression analysis for a set of $\log k'$ values of neutral solutes with known solute descriptors. Thus, the system constants for a given system provide information on the relative magnitude of the intermolecular interactions that contribute to the retention process.

A number of stationary phases, in particular octadecyl-bonded silica (ODS, RP18), were used to estimate lipophilicity [*viz.*[21,22] Recently, Kaliszan provided a review[23] on QSRR: Quantitative Structure-(Chromatographic) Retention Relationships where he detailed the most important studies on old and new stationary phases for lipophilicity estimation. It was demonstrated that the choice of methanol as an organic modifier in the binary aqueous mobile phase leads to better correlations between literature $\log P$ values and $\log k'$ or $\log k'_w$.[3,24] The problematic presence of free acidic silanol groups on ODS surfaces has been reviewed.[25] At present, the trend in the new stationary phases is to reduce silanol activity to prevent their interactions with strong hydrogen-bonding and ionized compounds.[26]

The present study examines the possible use of Gemini C18™ column for reversed-phase liquid chromatographic estimation of lipophilic parameters. The Gemini C18™ column is a new generation hybrid silica based column with an extended pH range capability (pH 2 to 12) due to the introduction of saturated hydrocarbons on the surface of the particles.[27] In addition, we aim to characterize the Gemini C18™ stationary phase using LSER to understand which intermolecular interactions are most significant in this mobile-stationary phase system.

EXPERIMENTAL

Instrumentation

The retention data were measured for a Gemini C18™ column (150 mm × 4.6 mm I. D., 5 μm, Phenomenex, Torrance, CA, USA). All measurements were performed with a Hewlett Packard 1090 liquid chromatograph equipped with a diode array detector operated at 254 nm. The flow rate was 1.0 ml/min and the temperature was controlled at 40°C.

Chemicals

HPLC grade Methanol, used as the organic modifier, was purchased from J. T. Baker (USA). The water used throughout was purified and deionized with Seradest SD 2000 system (Germany).

$Na_2HPO_4 \cdot 7H_2O$ was purchased from Merck (Darmstadt, Germany). The buffer for the mobile phase was prepared by adjusting a 0.02 M disodium hydrogen phosphate salt solution with phosphoric acid (J.T. Baker.) to pH 7.0. The mobile phase was a mixture of the above phosphate buffer and methanol, in percentages ranging from 40% to 55%.

The solutes set was separated into two groups: neutral test solutes and basic (local anesthetics, β-blockers), acidic (NSAIDs: Non-steroidal anti inflammatory drugs) and neutral (steroid hormones) drugs. The neutral test solutes, shown in Table 1, were of analytical reagent grade and were obtained from several sources. The steroid hormones hydrocortisone 21-acetate, cortisone 21-acetate, prednisolone, and prednisone were purchased from Sigma. Cortisone, corticosterone and hydrocortisone were obtained from Fluka. The local anesthetics lidocaine, procaine hydrochloride, prilocaine hydrochloride and mepivacaine hydrochloride were obtained from Sigma. The β-blockers atenolol, alprenolol, metoprolol tartrate, nadolol, DL propranolol, acebutolol, pindolol and sotalol were provided by Sigma. Also from Sigma, were the NSAIDs ibuprofen, indoprofen, flurbiprofen, fenbufen and fenoprofen calcium salt hydrate. Naproxen was purchased from Fluka.

Solute solutions were prepared by dissolving the compounds in the mobile phase and filtered through a 0.22 μm filter before injection.

$NaNO_3$, dissolved in the mobile phase, was injected as an unretained solute. The logarithm of the capacity factor, $\log k'$ was used to express the retention data. All measurements were performed in triplicate and the $\log k'$ values reported are the average of three replicates. $\text{Log } k'_w$ values were obtained by extrapolating retention factors measured at

Table 1. LSER Solute descriptors

	Descriptors					
Solute	*V*	*E*	*S*	*A*	*B*	Ref.
acetone	0.547	0.179	0.7	0.04	0.51	[28]
2-butanone	0.6879	0.166	0.7	0	0.51	[29]
3-pentanone	0.829	0.143	0.68	0	0.51	[30]*
2-hexanone	0.968	0.136	0.68	0	0.51	[30]
2-heptanone	1.111	0.055	0.663	0	0.51	[29]
2-octanone	1.252	0.108	0.68	0	0.51	[30]
acetophenone	1.0139	0.767	1.06	0	0.48	[29]
propiophenone	1.155	0.8	0.85	0	0.51	[29]
butyrophenone	1.2957	0.8	0.95	0	0.51	[29]
valerophenone	1.437	0.8	0.95	0	0.5	[29]
phenol	0.7751	0.722	0.736	0.744	0.3	[29]
hydroquinone	0.8338	1.063	1.27	1.06	0.57	[31]
resorcinol	0.8338	0.98	1.11	1.09	0.52	[31]
catechol	0.8338	0.97	1.1	0.88	0.47	[17]
m-aminophenol	0.8747	1.13	1.15	0.65	0.79	[32]
o-aminophenol	0.8747	1.11	1.1	0.6	0.66	[32]
m-nitrophenol	0.9493	1.05	1.57	0.79	0.23	[32]
p-cresol	0.916	0.82	0.87	0.57	0.32	[29]
m-cresol	0.916	0.822	0.88	0.57	0.34	[17]
o-cresol	0.916	0.84	0.86	0.52	0.31	[29]
toluene	0.8573	0.564	0.516	0	0.14	[29]
ethylbenzene	0.9982	0.572	0.511	0	0.15	[29]
nitrobenzene	0.8906	0.871	1.11	0	0.28	[17]
benzonitrile	0.8711	0.779	1.123	0	0.33	[29]
chlorobenzene	0.8388	0.718	0.65	0	0.07	[17]
anisole	0.916	0.71	0.75	0	0.29	[29]
caffeine	1.3632	1.5	1.6	0	1.33	[29]
antipyrine	1.5502	1.32	1.5	0	1.48	[17]
4-chlorophenol	0.8975	0.895	0.745	0.949	0.2	[29]
aniline	0.8162	0.996	0.985	0.254	0.5	[29]
3-chloroaniline	0.939	1.05	1.1	0.3	0.36	[17]
acetaminophen	1.1724	1.06	1.63	1.04	0.86	[33]
m-toluidine	0.957	0.946	0.95	0.23	0.55	[29]
o-toluidine	0.9571	0.966	0.92	0.23	0.59	[29]
o-nitroaniline	0.9904	1.18	1.37	0.3	0.36	[29]
p-nitroaniline	0.9904	1.22	1.83	0.45	0.38	[29]
m-nitroaniline	0.9904	1.2	1.71	0.4	0.35	[17]
quinoline	1.044	1.268	0.97	0	0.51	[29]
3-bromoquinoline	1.2193	1.64	1.23	0	0.42	[33]
2-naphtol	1.1441	1.52	1.08	0.61	0.4	[29]
naphtalene	1.0854	1.34	0.92	0	0.2	[29]

(continued)

Table 1. Continued

Solute	Descriptors					Ref.
	V	*E*	*S*	*A*	*B*	
Steroid hormones						
cortisone-21-acetate	3.05	1.82	3.11	0.21	2.13	[31]
corticosterone	2.74	1.86	3.43	0.4	1.63	[31]
cortisone	2.75	1.96	3.5	0.36	1.87	[31]
hydrocortisone	2.80	2.03	3.49	0.71	1.9	[33]
prednisolone	2.75	2.21	3.1	0.71	1.92	[33]
prednisone	2.71	2.14	3.58	0.36	1.89	[33]
hydrocortisone-21-acetate	3.10	1.89	2.88	0.46	2.16	[33]

*The reference is related to 2-pentanone.

various methanol concentrations (40%, 45%, 50%, 55%) to 100% water using a conventional least square procedure.

Lipophilicity and Structural Parameters

Experimental *n*-octanol-water partition coefficients $\log P$ were taken from Syracuse Research Corporation's PhysProp database. Calculated distribution coefficient $\log D^{7.0}$ were obtained through the American Chemical Society's SciFinder Scholar program.

The solute descriptors for Abraham's linear solvent energy relationship model were obtained from the literature; see Table 1.

Multiple linear regression analysis and the related statistical functions were performed with Microsoft's Excel.

RESULTS AND DISCUSSION

Application of the Linear Solvent Strength Theory Model

One of the important chromatographic parameters relating to lipophilicity is $\log k'_w$, usually obtained by extrapolating $\log k'$ values measured with an organic modifier in the mobile phase to neat aqueous mobile phase. In the present work, we measured k' with mobile phases containing 40, 45, 50 and 55% methanol. Using Eq. (2), which assumes linear relationship between $\log k'$ and the organic content of the mobile phase, $\log k'_w$ (the intercept of the regression equation) and S (the slope of the equation) values were calculated for all the solutes; see Table 2.

Table 2. Lipophilicity and physicochemical parameters of the neutral solutes and steroid hormones

Solute	$\log k'_w$	S	$\log P$
acetone	0.042	1.197	−0.24
2-butanone	0.659	1.790	0.29
3-pentanone	1.200	2.245	0.99
2-hexanone	1.849	2.928	1.38
2-heptanone	2.534	3.677	1.98
2-octanone	3.177	4.377	2.37
acetophenone	1.915	3.127	1.58
propiophenone	2.498	3.663	2.19
butyrophenone	3.076	4.235	2.77
valerophenone	3.661	4.787	3.15
phenol	1.095	2.025	1.46
hydroquinone	0.332	2.025	0.59
resorcinol	0.539	2.015	0.8
catechol	0.677	1.844	0.88
m-aminophenol	0.352	1.921	0.21
o-aminophenol	0.713	2.106	0.62
m-nitrophenol	1.954	3.421	2
p-cresol	1.895	3.077	1.94
m-cresol	1.893	3.081	1.9
o-cresol	1.913	3.044	1.95
toluene	2.768	3.479	2.73
ethylbenzene	3.279	3.899	3.15
nitrobenzene	1.980	2.963	1.85
benzonitrile	1.753	2.935	1.56
chlorobenzene	2.870	3.677	2.84
anisole	2.233	3.100	2.11
caffeine	0.842	2.349	−0.07
antipyrine	1.251	2.825	0.38
4-chlorophenol	2.146	3.255	2.39
aniline	1.054	2.212	0.9
3-chloroaniline	1.929	3.072	1.88
acetaminophen	0.410	1.982	0.51
m-toluidine	1.595	2.737	1.4
o-toluidine	1.517	2.609	1.32
o-nitroaniline	1.838	3.082	1.85
p-nitroaniline	1.390	2.839	1.39
m-nitroaniline	1.517	2.809	1.37
quinoline	2.043	3.299	2.03
3-bromoquinoline	3.003	4.094	3.03
2-naphtol	2.690	4.038	2.7
naphtalene	3.509	4.423	3.37

(continued)

Table 2. Continued

Solute	$\log k'_w$	S	$\log P$
Steroid hormones			
cortisone-21-acetate	4.056	6.139	2.1
corticosterone	3.550	5.285	1.94
cortisone	3.002	4.888	1.47
hydrocortisone	3.079	4.789	1.61
prednisolone	3.079	4.806	1.62
prednisone	2.947	4.861	1.46
hydrocortisone-21-acetate	3.822	5.666	2.19

It is of interest to examine the correlation between S and $\log k'_w$ since a good correlation between these parameters implies that the intermolecular interactions that govern S and $\log k'_w$ are similar. Figure 1(a) and Eq. (4) show a good linear correlation between S and $\log k'_w$ for the neutral solutes, which includes a set of structurally diverse compounds,

$$S = 0.86(\pm 0.03)\log k'_w + 1.44(\pm 0.06)$$
$$n = 41; \quad r^2 = 0.95; \quad s = 0.19; \quad F = 705 \tag{4}$$

For the steroid hormones, a good correlation between S and $\log k'_w$ was also obtained as shown in Figure 1(b) and Eq. (5)

$$S = 1.14(\pm 0.12)\log k'_w + 1.36(\pm 0.39)$$
$$n = 7; \quad r^2 = 0.95; \quad s = 0.13; \quad F = 95 \tag{5}$$

In both equations above, as well as in all the regression equations that will follow, the values in parentheses represented 95% confidence limits, n is the number of the compounds in the regression, r^2 is the squared correlation coefficient, s is the standard deviation and F is the Fischer's test value.

In both sets of solutes, the slope of the correlation between S and $\log k'_w$ is close to unity with a good correlation coefficient, indicating that similar intermolecular interactions are affecting for both parameters.

It should be noted that with conventional reversed phase columns, n-octanol is added to the mobile phase to improve relationships between S and $\log k'_w$ for a set of structurally diverse solutes.[34,35] The role of the n-octanol is, most likely, to modify the underlying silica. The Gemini C18™ column, being a new generation hybrid silica based column, does not require n-octanol to yield good correlation between S and $\log k'_w$.

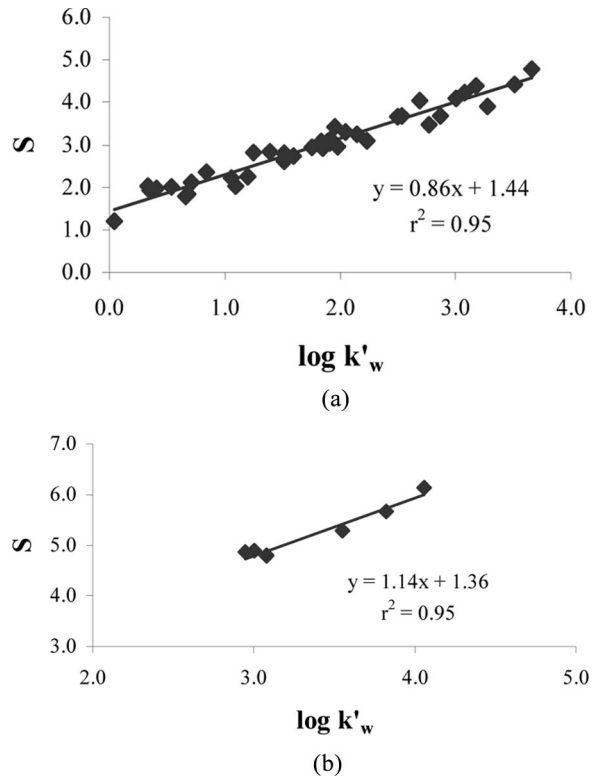

Figure 1. Correlation between S and $\log k'_w$ for (a) neutral test solutes and (b) steroid hormones.

Apparently, the saturated hydrocarbons layer modifies the silica surface sufficiently making the presence of *n*-octanol redundant.

Correlation Between $\log P$ and $\log k'_w$

In order to determine the ability of the Gemini C18™ stationary phase to mimic the partitioning mechanism of the *n*-octanol/water system, we correlated $\log P$ of the neutral solutes and of neutral drugs to their extrapolated capacity factors $\log k'_w$. The correlated data are shown in Figure 2. The regression data are as follows:

(a) for the neutral solutes set:

$$\begin{aligned} \text{Log}\, P &= 0.94(\pm 0.05) \log k'_w - 0.05(\pm 0.10) \\ n &= 41; \quad r^2 = 0.91; \quad s = 0.29; \quad F = 384 \end{aligned} \tag{6}$$

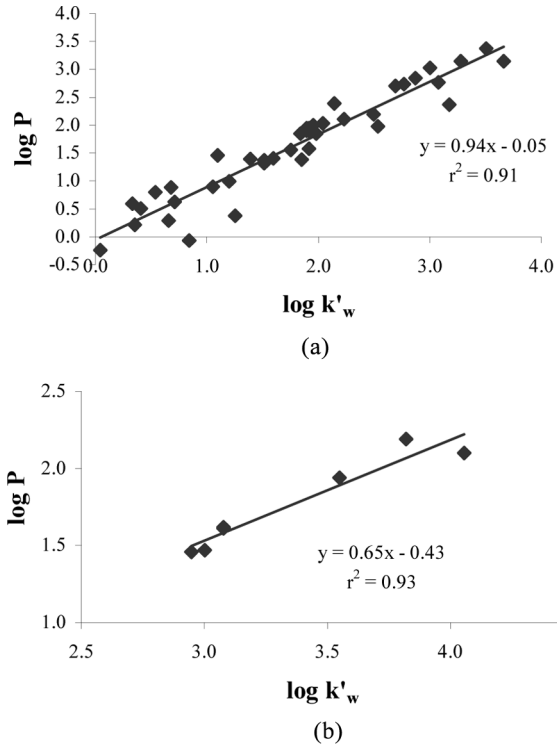

Figure 2. Correlation between $\log P$ and $\log k'_w$ for (a) neutral test solutes and (b) steroid hormones.

(b) for the neutral drugs (steroid hormones) set:

$$\begin{aligned} \text{Log}\, P &= 0.65(\pm 0.08) \log k'_w - 0.43(\pm 0.27) \\ n &= 7; \quad r^2 = 0.93; \quad s = 0.09; \quad F = 65 \end{aligned} \tag{7}$$

For the test solutes and the neutral drugs, good correlations between $\log P$ and $\log k'_w$ values were found. For the neutral solutes (Eq. (6)), the slope is very close to unity and the intercept is nearly zero. According to Minick et al.[36], the correlation between $\log P$ and $\log k'_w$ is a part of linear free-energy relationships and the magnitude of the slope of the correlation is indicative of the similarity of the free energies of the processes investigated. When the slope is close to unity, the two processes are said to be "homoenergetic"; i.e., the free energy changes in both processes are equivalent. Such is the case for the neutral solutes set. However, for the steroid hormones, while the linear correlation is reasonable ($r^2 = 0.93$) the slope of the regression is 0.65 indicating that these two processes are not equivalent; that is, "heteroenergetic".

For ionized drugs, the correlation is established between the distribution coefficient $\log D$ and $\log k'_w$, both obtained at the same pH.[18] In the present work, we verified the relationships between $\log D^{7.0}$ and $\log k'_w$ for basic and acidic drugs. For example, for the β-blockers, the correlation between $\log D^{7.0}$ and $\log k'_w$ is given by Eq. (8) and is shown in Figure 3:

$$\begin{aligned} \text{Log}\, D^{7.0} &= 1.22(\pm 0.11) \log k'_w - 2.15(\pm 0.19) \\ n &= 8; \quad r^2 = 0.95; \quad s = 0.26; \quad F = 114 \end{aligned} \tag{8}$$

Although the distribution coefficient is a calculated parameter, a good linear correlation was obtained for the β-blockers. At pH 7.0, the β-blockers are almost fully protonated; i.e., positively charged. At the same pH, the residual silanols in conventional reversed phase columns are ionized and negatively charged. Thus, strong ion-exchange interactions will make it difficult to obtain a clear correlation between $\log D^{7.0}$ and $\log k'_w$ or $\log k'$.[21] However, with the Gemini C18™ column, the surface of the stationary phase is a specially grafted silica-polymer hybrid that reduces the interactions with free silanol groups, thus yielding a better correlation between $\log D^{7.0}$ and $\log k'_w$.

Although for ionized molecules, the more appropriate lipophilicity parameter is the distribution coefficient $\log D$, we examined the correlation between $\log P$ of the β-blockers and their $\log k'_w$ (Eq. (9) and Figure 3):

$$\begin{aligned} \text{Log}\, P &= 1.08(\pm 0.11) \log k'_w + 0.00(\pm 0.18) \\ n &= 8; \quad r^2 = 0.94; \quad s = 0.26; \quad F = 91 \end{aligned} \tag{9}$$

As can be seen from Eq. (9) and Figure 3, the slope of the correlation is almost unity and the intercept is close to zero. Moreover, as can be seen

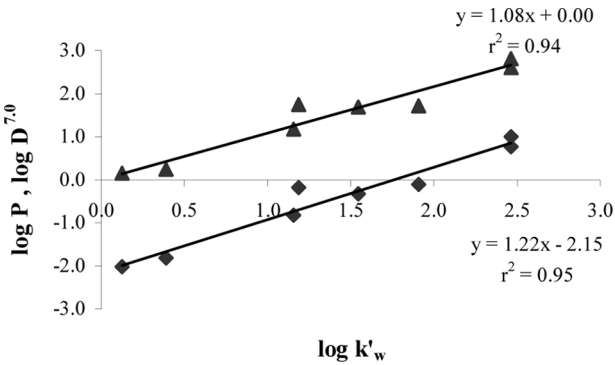

Figure 3. Correlations between $\log P$ (▲), $\log D^{7.0}$(♦) and $\log k'_w$ for β-blockers.

in Figure 3, the lines described by Eqs. (8) and (9) are almost parallel. This parallel behavior of the two lines is understood from the nature of the relationship between $\log D$ and $\log P$. According to Scherrer,[37] for monoprotic bases, $\log D$ is related to $\log P$ by Eq. (10):

$$\log D_{\text{oct}}^{\text{pH}} = \log P_{\text{oct}} + \log \frac{1}{1 + 10^{\text{pKa}-\text{pH}}} \tag{10}$$

Since the pKa values of all the β-blockers studied here are very similar, the $\log D$ values will be shifted from the $\log P$ values by roughly a constant; hence the similar slopes of the two lines in Figure 3. The difference in the intercepts is related to the difference pKa-pH.

The correlation between $\log D^{7.0}$ and $\log k'_w$ can be extended to include several classes of ionized drugs. For example, the correlation for local anesthetics, NSAIDs and β-blockers is shown in Eq. (11):

$$\begin{aligned} \text{Log } D^{7.0} &= 0.98(\pm 0.09) \log k'_w - 1.82(\pm 0.20) \\ n &= 18; \quad r^2 = 0.89; \quad s = 0.34; \quad F = 125 \end{aligned} \tag{11}$$

The correlation line in Eq. (11) is parallel to the correlation of $\log P$ and $\log k'_w$ for neutral solutes as seen in Figure 2. The slope of the correlation in Eq. (11) is almost unity and, as expected, the intercept is negative. The lower correlation coefficient of Eq. (11) is not surprising in light of the wide diversity of the solutes. In general, we found better correlations with a single family of solutes with similar chemical properties.

Correlation Between $\log P$ and Isocratic $\log k'_{40}$

The determination of $\log k'_w$ by extrapolation can be problematic since it may be dependent of the range of organic modifier used for the extrapolation. Therefore, we investigated the possibility of using an isocratic capacity factor, $\log k'_{\%}$ (% represents the percent methanol in the mobile phase), to emulate the partition mechanism of *n*-octanol/water system. Using the neutral solutes set, we examined four methanol contents, 40%, 45%, 50% and 55% for the correlation. Table 3 summarizes the regression results and the relevant statistics.

Table 3. Regression coefficients of the correlation between $\log P$ and $\log k'_{\%}$

	b (=slope)	*a* (=intercept)	r^2	SE	F	*n*
$\text{Log } k'_{40}$	1.42 (±0.07)	0.79 (±0.06)	0.91	0.29	377	41
$\text{Log } k'_{45}$	1.50 (±0.07)	0.95 (±0.05)	0.91	0.28	412	41
$\text{Log } k'_{50}$	1.53 (±0.08)	1.16 (±0.05)	0.90	0.30	355	41
$\text{Log } k'_{55}$	1.55 (±0.09)	1.36 (±0.05)	0.88	0.33	283	41

The results show that lower methanol concentration in the mobile phase yields marginally better correlation between $\log P$ and $\log k'_{\%}$: $r^2 = 0.91$ for 40% methanol versus $r^2 = 0.88$ for 55% methanol.

In all cases, the slope of the correlation line is greater than unity and the intercept is positive. The slope and the intercept of the correlation increase as the percentage of methanol in the mobile phase increases, most likely due to different extent of solvation of the stationary phase by the different concentrations of methanol in the mobile phase.

Similar results are obtained for the steroid hormones; Eq. (12):

$$\begin{aligned} Log\, P &= 1.24(\pm 0.08) \log k'_{40} + 0.18(\pm 0.10) \\ n &= 7; \quad r^2 = 0.98; \quad s = 0.05; \quad F = 238 \end{aligned} \tag{12}$$

In view of our results, it stands to reason that chromatographic data collected at 40% methanol can be used to correlate $\log k'_{40}$ with $\log D^{7.0}$. Eq. (13) shows the correlation data for some β-blockers. Eq. (14) gives the data for a larger set of the ionized drugs from three different families:

$$\begin{aligned} \text{Log}\, D^{7.0} &= 1.71(\pm 0.21) \log k'_{40} - 1.15(\pm 0.15) \\ n &= 8; \quad r^2 = 0.92; \quad s = 0.34; \quad F = 65 \end{aligned} \tag{13}$$

$$\begin{aligned} \text{Log}\, D^{7.0} &= 1.64(\pm 0.15) \log k'_{40} - 1.00(\pm 0.14) \\ n &= 18; \quad r^2 = 0.88; \quad s = 0.35; \quad F = 120 \end{aligned} \tag{14}$$

It should be noted that good correlations are obtained between the lipophilicity parameters and the isocratic capacity factors. For the steroid hormones, the regression coefficient of the linear correlation with $\log k'_{40}$ was better than the value obtained with $\log k'_w$. These results agree with the observations of Pagliara et al.[38] who characterized a Supelcosil LC-ABZ column using a mobile phase with 40% methanol. Based on these results, we propose the use of $\log k'_{40}$ as a rapid and precise method to determine the lipophilicity parameter.

Application of the Linear Solvation Energy Relationship Model for Neutral Compounds

To evaluate the importance of various intermolecular interactions that contribute to the retention of the compounds, we apply the solvation parameter model of Abraham (LSER).[6] The analysis of the LSER equation is based predominantly on two aspects: the magnitude of the coefficients and their sign. The larger the magnitude of the coefficient the greater is the importance of that specific interaction to the retention mechanism. For the chromatographic system constants, a positive sign

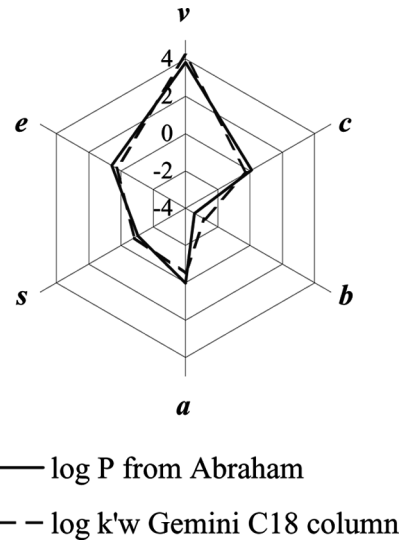

Figure 4. Radar plot of the system constants for the Gemini C18™ column ($\log k'_w$ based) and for the *n*-octanol/water system ($\log P$ from the Abraham LSER model).

of the coefficient means that the interaction favors the stationary phase and consequently leads to an increase in the retention time of the solute. A negative sign of a system constant points out to more favorable interactions with the mobile phase, which decrease the retention times. Similar observations are made in the case of the *n*-octanol/water extraction system. Thus, the LSER model affords a quick comparison of the intermolecular interactions contributing to the retention in reversed-phase separation systems and to partitioning in *n*-octanol/water systems.

Log k'_w values were measured on the Gemini C18™ column for a set of standard solutes with known Abraham descriptors. From these $\log k'_w$ values, the system constants for the Gemini C18™ chromatographic system were calculated using multiple linear regression of Eq. (3). The column constants are compared to available constants for the *n*-octanol/water extraction system.[13] The two sets of system constants were compared using the Tanaka's radar plot.[39] In the radar plot, each axis represents a different system constant. The values of the system constants are scaled to fit the radar plot scale, which is between −4 at the origin and 5 at the highest point. The great advantage of the radar plot is that it affords a rapid visual comparison of the system constants. Figure 4 shows the radar plots for the Gemini C18™ column and the *n*-octanol/water partition system.

The radar plots in Figure 4 show the great similarity in the constants of the two systems; the magnitudes are very close and the sign of each constant is the same in both systems except for the constant ***a*** that is very close to zero (+0.03) in the extraction system and is negative for the chromatographic system. The intermolecular interactions that occur in the Gemini C18™ column and in the shake-flask extraction system are very similar. The similarity between the two systems explains the good correlation between $\log P$ and $\log k'_w$ as seen in Figure 2(a).

For the sake of completion, we also include in Table 4 the values of the system constants for all methanol compositions studied here as well as for $\log k'_w$ and $\log P$. In Figure 5, the behavior of the various system constants as a function of the methanol content is plotted.

From Table 4, we can conclude that system constants ***b*** and ***v*** are the main factors influencing retention or partitioning in the two processes. The large negative values of ***b*** are due to the strong hydrogen bonding capabilities of the aqueous mobile phase in the chromatographic system and of the water phase in the extraction system as compared with the stationary phase and the *n*-octanol phase of the two systems. The more negative ***b*** value in the shake-flask system is due, most likely, to the presence of solvated *n*-octanol in the water phase. Hydrogen bonding also explains the increase in ***b*** with increasing methanol content in the mobile phase; see Table 4. As the amount of methanol extracted into the stationary phase increases, the hydrogen bonding capability of the stationary phase increases and the contribution of this term to the retention increases.

Table 4. System constants on the Gemini C18™ column for different compositions of methanol in the mobile phase and for the *n*-octanol/water system

	System constants						Statistics			
Separation system	***c***	***v***	***e***	***s***	***a***	***b***	r^2	SE	F	***n***
Gemini C18™ column										
Isocratic capacity factors										
$\log k'_{40}$	−0.45	2.58	0.18	−0.63	−0.41	−1.89	0.99	0.08	466	41
$\log k'_{45}$	−0.45	2.36	0.25	−0.66	−0.38	−1.81	0.98	0.09	324	41
$\log k'_{50}$	−0.51	2.22	0.31	−0.70	−0.37	−1.72	0.96	0.12	180	41
$\log k'_{55}$	−0.55	2.06	0.36	−0.73	−0.35	−1.63	0.94	0.15	104	41
Extrapolated capacity factor										
$\log k'_w$	−0.23	4.19	0.27	−0.84	−0.53	−2.84	0.99	0.12	484	41
Octanol-water[13]										
$\log P$	0.09	3.81	0.56	−1.05	0.03	−3.46	0.995	0.12	23162	613

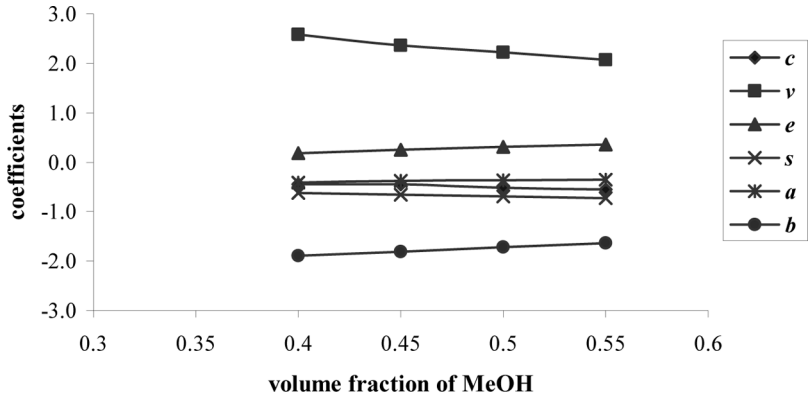

Figure 5. Plot of LSER coefficients vs. volume fraction of methanol in the mobile phase:(♦) coefficient ***c***; (■) coefficient ***v***; (▲) coefficient ***e***; (×) coefficient ***s***; (∗) coefficient ***a***; (●) coefficient ***b***.

The second important constant is ***v***, which measures the relative ability of the solute to create a cavity in the solvated stationary phase and in the mobile phase. The positive sign of ***v*** is due to unfavorable cavity formation in the aqueous mobile phase, which means stronger interactions with the stationary phase. In the shake-flask system the *n*-octanol phase, being able to form hydrogen bonds, is more cohesive than the Gemini C18™ phase and thus the ***v*** coefficient is smaller in this system.

The solvation parameter model provides an understanding of the intermolecular interactions responsible for the retention of neutral solutes on the Gemini C18™ column. From Table 4, we can study the effect of the mobile phase composition on the LSER coefficients of the Gemini C18™ chromatographic system. As the methanol content of the mobile phase increases, the solvated stationary phase becomes less hydrophobic and the two phases become more alike. As a result, system constant ***v*** decreases. The constant ***s***, which relates to dipolarity /polarizability interactions, also decreases as the methanol content of the mobile phase increases. As more methanol is added, the solutes prefer the methanol-rich mobile phase environment to the solvated stationary phase. On the other hand, the extracted methanol in the stationary phase increases the hydrogen bond forming capabilities of that phase causing an increase in system constants ***a*** (hydrogen bond basicity) and ***b*** (hydrogen bond acidity). Similarly, the ***e*** coefficient, which relates to the electron lone pair interactions, increases as more methanol is added to the mobile phase. The increase in ***e*** indicates stronger interactions with the stationary phase, most likely due to the extracted methanol.

Prediction of $\log k'_w$ and $\log P$ of the Steroid Hormones by Using the LSER Equation

The solvation parameter model discussed above can be used for predicting the chromatographic retention of neutral drugs (steroid hormones) as well as for predicting their partition coefficients. The solute descriptors of the neutral drugs are known from the literature[31,33] and are included in Table 1; $\log k'_w$ based system constants were calculated by MLR and are given in Table 4. Log k'_w values for the steroid hormones were obtained experimentally as well as by calculation using the LSER equation. Figure 6 shows a linear correlation between calculated and experimental values of $\log k'_w$. Equation (15) gives the correlation line data:

$$\begin{gathered}\text{Log}\, k'_w(calc) = 1.10(\pm 0.15) \log k'_w(\text{exp}) + 0.02(\pm 0.05) \\ n = 7; \quad r^2 = 0.92; \quad s = 0.16; \quad F = 55\end{gathered} \tag{15}$$

The slope of the linear correlation is close to unity and the intercept is almost zero. Figure 6 demonstrates that the solvation parameter model can be use to predict fairly accurately ($r^2 = 0.92$) the extrapolated capacity factors of neutral drugs (extrapolated to pure aqueous mobile phase). Most importantly, Figure 6 indicates that calculated $\log k'_w$ can be used for estimating $\log P$ values. Equation (16) gives the correlation between these two parameters for the steroid hormones:

$$\begin{gathered}\text{Log}\, P = 0.59(\pm 0.04) \log k'_w(\text{calc}) - 0.39(\pm 0.18) \\ n = 7; \quad r^2 = 0.97; \quad s = 0.06; \quad F = 152\end{gathered} \tag{16}$$

The correlation obtained is very good ($r^2 = 0.97$). Admitingly, the number of solutes studied is small ($n = 7$), however the possibility of

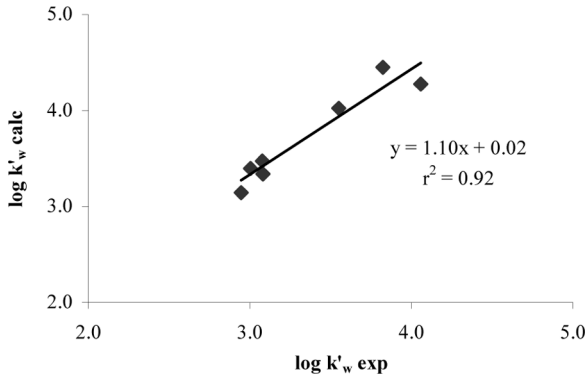

Figure 6. Calculated vs. experimental $\log k'_w$ values.

being able to predict log P values from calculated log k'_w is very attractive and the above approach should be extended to large set of solutes. We are now attempting to increase the number of solutes in the data set.

CONCLUSION

The results obtained in this work show that the novel Gemini C18™ stationary phase is suitable for the measurements of lipophilic parameters that are of interest to the pharmaceutical industry. To begin with, log k' values measured at four different methanol contents in the mobile phase were extrapolated to zero methanol content (log k'_w). Good correlations were found between the slope (S) of the extrapolation and the extrapolated log k'_w for a set of diverse neutral chemical compounds and for seven steroid hormones. Good correlation was established between the extrapolated log k'_w and log P values. Thus, the Gemini C18™ column can be used to estimate accurately n-octanol/water partition coefficients neutral compounds and provide an alternative to the classical shake-flask method. In addition, good correlations were established between log P and log k' measured with 40% methanol in the mobile phase. Moreover, extrapolated log k'_w as well as log k' at 40% methanol can be used to estimate well the n-octanol/water distribution coefficients (log D) of ionized drugs studied here.

Abraham's solvation parameter model (LSER) was used to characterize the Gemini C18™ column and to compare it with the same model for the n-octanol/water system. The Tanaka radar plot was used for easy visual comparison of the two systems. The principal factors which dominate retention as well as the partition are the $\boldsymbol{v}$ and $\boldsymbol{b}$ constants, i.e., the cavity formation energy term and the hydrogen bond acidity of the solvated stationary phase term. The LSER equation obtained for neutral drugs could be use to predict correctly their extrapolated capacity factor log k'_w, which, in turn, can be used to predict log P values of these drugs.

REFERENCES

1. Hartman, T.; Schmitt, J. Lipophilicity – beyond octanol/water: a short comparison of modern technologies. Drug Discovery Today: Technologies **2004**, *1*, 431–439.
2. Poole, C.F.; Gunatilleka, A.D.; Poole, S.K. In search of a chromatographic model for biopartitioning. Adv. Chromatography **2000**, *40*, 159–230.
3. Nasal, A.; Siluk, D.; Kaliszan, R. Chromatographic retention parameters in medicinal chemistry and molecular pharmacology. Curr. Med. Chemistry **2003**, *10*, 381–426.

4. Hansh, C.; Fujita, T. A method for the correlation of biological activity and chemical structure. J. Am. Chem. Soc. **1964**, *86*, 1616–1626.
5. Leo, A.; Hansch, C.; Elkins, D. Partition coefficients and their uses. Chem. Rev. **1971**, *71*, 525–616.
6. Abraham, M.H. Scales of solute hydrogen-bonding: Their construction and application to physicochemical and biochemical processes. Chem. Soc. Rev. **1993**, *22*, 73–83.
7. Dorsey, J.G.; Khaledi, M.G. Hydrophobicity estimations by reversed-phase liquid chromatography: Implications for biological partitioning processes. J. Chromatography A **1993**, **656**, 485–499.
8. Lambert, W.J. Modeling oil-water partitioning and membrane permeation using reversed-phase chromatography. J. Chromatography A **1993**, *656*, 469–484.
9. Kaliszan, R. Quantitative sructure-retention relationships applied to reversed-phase high-performance liquid chromatography. J. Chromatography A **1993**, *656*, 417–435.
10. Snyder, L.R.; Dolan, J.W.; Gant, J.R. Gradient elution in high-performance liquid chromatography I. Theoretical basis for reversed-phase systems. J. Chromatogr. **1979**, *165*, 3–30.
11. Chen, N.; Zhang, Y.K.; Lu, P.C. Effects of molecular structure on the log k'_w index and linear S-log k'_w correlation in reversed-phase high-performance liquid chromatography. J. Chromatography A **1993**, *633*, 31–41.
12. Valko, K.; Snyder, L.R.; Glajch, J.L. Retention in reversed-phase liquid chromatography as a function of mobile-phase composition. J. Chromatography A **1993**, *656*, 501–520.
13. Abraham, M.H.; Chadha, H.S.; Leo, A.J. Hydogen bonding XXXV. Relationship between high-performance liquid chromatography capacity factors and water-octanol partition coefficients. J. Chromatography A **1994**, *685*, 203–211.
14. Sadek, P.C.; Carr, P.W.; Doherty, R.M.; Kamlet, M.J.; Taft, R.W.; Abraham, M.H. Study of retention processes in reversed-phase high-performance liquid chromatography by the use of the solvatochromic comparison method. Anal. Chem. **1985**, *57*, 2971–2978.
15. Abraham, M.H.; Rosés, M.; Poole, C.F.; Poole, S.K. Hydrogen bonding. 42. Characterization of reversed-phase high-performance liquid chromatographic C_{18} stationary phases. J. Phys. Org. Chem. **1997**, *10*, 358–368.
16. Wang, A.; Tan, L.C.; Carr, P.W. Global linear solvation energy relationships for retention prediction in reversed-phase liquid chromatography. J. Chromatography A **1999**, *848*, 21–37.
17. Lázaro, E.; Ràfolds, C.; Rosés, M. Characterization of immobilized artificial membrane (IAM) and XTerra columns by means of chromatographic models. J. Chromatography A **2005**, *1081*, 163–173.
18. Poole, S.K.; Poole, C.F. Separation methods for estimating octanol-water partition coefficients. J. Chromatography B **2003**, *797*, 3–19.
19. Vitha, M.; Carr, P.W. The chemical interpretation and practice of linear solvation energy relationships in chromatography. J. Chromatography A **2006**, *1126*, 143–194.

20. Kiridena, W.; Dekay, C.; Villiere, N.D.; Koziol, W.W.; Poole, C.F. System maps for XTerra MS C_{18}: Effect of solvent type on selectivity in reversed-phase liquid chromatography. Chromatographia **2005**, *61*, 587–593.
21. Stella, C.; Galland, A.; Liu, X.; Testa, B.; Rudaz, S.; Veuthey, J.L.; Carrupt, P.A. Novel RPLC stationary phases for lipophilicity measurement: Solvatochromic analysis of retention mechanisms for neutral and basic compounds. J. Sep. Sci. **2005**, *28*, 2350–2362.
22. Ayouni, L.; Cazorla, G.; Chaillou, D.; Herbreteau, B.; Rudaz, S.; Lantéri, P.; Carrupt, P.A. Fast determination of lipophilicity by HPLC. Chromatographia **2005**, *62*, 251–255.
23. Kaliszan, R. QSRR: Quantitative structure-(chromatographic) retention relationships. Chem. Rev. **2007**, *107*, 3212–3246.
24. Brauman, T. Determination of hydrophobic parameters by reversed-phase liquid chromatography: theory, experimental techniques, and application in studies on quantitative structure-activity relationships. J. Chromatography **1986**, *373*, 191–225.
25. Nawrocki, J. The silanol group and its role in liquid chromatography. J. Chromatography A **1997**, *779*, 29–71.
26. Vervoort, R.J.M.; Debbets, A.J.J.; Claessens, H.A.; Cramers, C.A.; de Jong, G.J. Optimisation and characterisation of silica-based reversed-phase liquid chromatographic systems for the analysis of basic pharmaceuticals. J. Chromatography A **2000**, *897*, 1-22.
27. Samuelsson, J.; Franz, A.; Stanley, B.J.; Fornstedt, T. Thermodynamic characterization of separations on alkaline-stable silica-based C_{18} columns: Why basic solutes may have better capacity and peak performance at higher pH. J. Chromatography A **2007**, *1163*, 177–189.
28. Poole, S.K.; Poole, C.F. A method for estimating the solvent strength parameter in liquid-solid chromatography. Chromatographia **2001**, *53*, S-162–S-166.
29. Poole, C.F.; Kiridena, W.; Dekay, C.; Koziol, W.W.; Rosencrans, R.D. Insights into the retention mechanism on an octadecylsiloxane-bonded silica stationary phase (HyPURITY C_{18}) in reversed-phase liquid chromatography. J. Chromatography A **2006**, *1115*, 133–141.
30. Bolliet, D.; Poole, C.F. Influence of solvent effects on retention for a porous polymer sorbent in reversed phase liquid chromatography. Chromatographia **1997**, *46*, 381–398.
31. Zissimos, A.M.; Abraham, M.H.; Du, C.M.; Valko, K.; Bevan, C.; Reynolds, D.; Wood, J.; Tam, K.Y. Calculation of Abraham descriptors from experimental data from seven HPLC systems; evaluation of five different methods of calculation. Chem. J. Soc. Perkin Trans. **2002**, *2*, 2001–2010.
32. Abraham, M.H.; Rosés, M. Hydrogen bonding. 38. Effect of solute structure and mobile phase composition on reversed-phase high-performance liquid chromatographic capacity factors. J. Phys. Org. Chem. **1994**, *7*, 672–684.
33. Lombardo, F.; Shalaeva, M.Y.; Tupper, K.A.; Gao, F.; Abraham, M.H. A tool for lipophilicity determination in drug discovery. J. Med. Chem. **2000**, *43*, 2922–2928.

34. Liu, X.; Tanaka, H.; Yamauchi, A.; Testa, B.; Chuman, H. Determination of lipophilicity by reversed-phase high-performance liquid chromatography: Influence of 1-octanol in the mobile phase. J. Chromatography A **2005**, *1091*, 51–59.
35. Giaginis, C.; Theocharis, S.; Tsantili-Kakoulidou, A. Octanol/water partitioning simulation by reversed-phase high-performance liquid chromatography for structurally diverse acidic drugs: Effect of n-octanol as mobile phase additive. J. Chromatography A **2007**, *1166*, 116–125.
36. Minick, D.J.; Brent, D.A.; Frenz, J.H. Modeling octanol-water partition coefficients by reversed-phase liquid chromatography. J. Chromatography **1989**, *461*, 177–191.
37. Scherrer, R.A.; Howard, A.M. Use of distribution coefficients in quantitative structure-activity relationships. J. Med. Chem. **1977**, *20*, 53–58.
38. Pagliara, A.; Khamis, E.; Trinh, A.; Carrupt, P.A.; Tsai, R.S.; Testa, B. Structural properties governing retention mechanisms on RP-HPLC stationary phases used for lipophilicity measurements. J. Liq. Chromatogr. **1995**, *18*, 1721–1745.
39. Kimata, K.; Iwaguchi, K.; Onishi, S.; Jinno, K.; Eksteen, R.; Hosoya, K.; Araki, M.; Tanaka, N. Chromatographic characterization of silica C_{18} packing materials—correlation between a preparation method and retention behavior of stationary phase. J. Chrom. Science **1989**, *27*, 721–728.

Manuscript 6312A