



## CORRELATION OF SOLVENT STRENGTH PARAMETER WITH TWO MOLECULAR DESCRIPTORS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

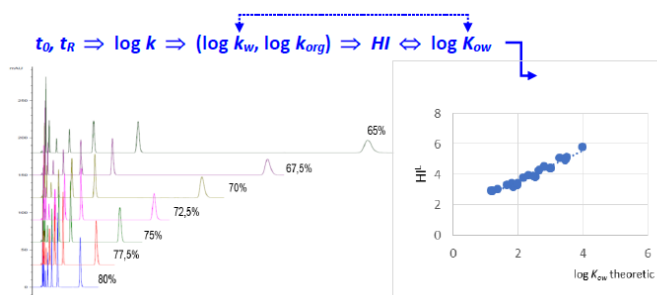
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Received January 4, 2024

A new hydrophobicity index has been previously developed and reported (*Rev. Roum. Chim.*, 2024, 69, 183). The new index is based only on the solvent strength parameter ( $S$ ) that describes the linear dependences ( $\log k = \log k_w - S \cdot \phi$ ) between the logarithm of the retention factor ( $k$ ) and volume fraction of the organic modifier in mobile phase ( $\phi$ ) in an HPLC separation. The correlations between the new hydrophobicity index and octanol-water partition constant ( $\log K_{ow}$ ) were evaluated and reported in present study for a set of 20 solutes by using seven C18 HPLC columns and two organic modifiers, typically utilized in reversed-phase HPLC applications (acetonitrile and methanol). All the correlations were good, showing values above 0.85. Correlations between the extrapolated values of retention factor to zero percent of the organic modifier in mobile phase,  $\log k_w$ , and  $\log K_{ow}$  showed also good determination coefficients  $R^2$ , unlike an older hydrophobicity index based on the ratio between  $\log k_w$  and  $S$ , which was generally characterized by lower  $R^2$  than 0.8 for acetonitrile and 0.7 for methanol.



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### INTRODUCTION

Correlations between the retention behavior in high-performance liquid chromatography (HPLC) with molecular characteristics of eluted compounds (solute) are important from both practical and theoretical points of views. The topic covering this research direction in HPLC is known as quantitative structure-retention relationship (QSRR), which is mainly focused on the most applied retention mechanism, namely the one in the reversed-phase (RP) separation.<sup>1–5</sup> Although

not all the QSRR equations have accurate predictive values for the retention based on structural features of the analytes, they may offer a possibility of differentiating the separation mechanism on different tested stationary phases.<sup>6</sup> Many of the reported studies rely on the logarithm of retention factor  $k$  corresponding to zero percent of the organic modifier in mobile phase, denoted by  $\log k_w$ , as experimental data for being correlated with various molecular descriptors.<sup>7–10</sup> Among the molecular descriptors of studied solutes the hydrophobicity character measured by octanol-

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water partition constant ( $\log K_{ow}$ ) is by far the most used parameter for QSRR correlations.<sup>11–15</sup> This is mainly justified by similarity between the two compared systems, namely the surface of stationary phase in RP-LC and octanol-water non-miscible phases.<sup>16–19</sup> Consequently, the retention process in RP-LC is governed by hydrophobicity of solute measured by  $\log K_{ow}$ , making this analytical technique very useful in many applications in predicting this parameter from various correlations with chromatographic retention.<sup>15,20–23</sup>

A global hydrophobicity index of a RP retention process has to consider the hydrophobic character of solute as well as the hydrophobic characteristics of stationary phase (the length of alkyl chain bonded to the silica surface, its density on it that gives the carbon content of stationary phase, C%). One hydrophobicity index largely utilized in different theoretical studies is  $\log k_w$ , which is almost always determined from extrapolation of  $\log k$  for zero percent of the organic modifier in mobile phase. Another hydrophobicity index has been proposed by Valko *et al.* as a ratio between  $\log k_w$  and the slope of linear dependence between  $\log k$  and the content of organic modifier in mobile phase (denoted by  $S$ , and known as solvent strength parameter)<sup>24,25</sup> that is used in various applications.<sup>26–30</sup> A new hydrophobicity index ( $HI^L$ ) has been recently proposed as a better alternative to Valko's hydrophobicity index (denoted by  $HI^V$ ), which has shown improved correlations with  $\log K_{ow}$  of studied solutes.<sup>31</sup> It is the aim of this study to apply this model to several HPLC columns and for a set of representative solutes, using two organic modifiers in mobile phase composition, and compare the results obtained for these hydrophobicity indexes.

## THEORETICAL BACKGROUND

Generally, the dependence of the retention on the mobile phase composition (retention function) in RP-LC can be described by a linear equation between the ten-base logarithm of the retention factor,  $\log k$ , and the volume fraction ( $\phi$ ) of the organic component in the mobile phase composition:<sup>32</sup>

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

According to this equation, the domain of variation for  $\log k$  in RP-LC is  $[\log k_{org}, \log k_w]$ ,

corresponding to the extreme values of  $\phi$ , *i.e.* [0, 1]. The two parameters of this equation are  $\log k_w$ , representing the extrapolated value of the retention factor for 100% aqueous component when  $\phi = 0$ , and  $S$ , which is the slope of this dependence (solvent strength parameter, *i.e.*  $d(\log k)/d\phi$ ).<sup>33</sup> The value of  $\log k_{org}$  can be calculated from this equation for  $\phi = 1$ :

$$\log k_{org} = \log k_w - S \quad (2)$$

In practice, this equation is valid only on a limited domain of  $\phi$ , and if the measurements are extended to broader interval of  $\phi$  then the dependence  $\log k$  versus  $\phi$  may become a polynomial equation, or other functions.<sup>34–38</sup> The values of  $\log k_w$  are almost always positive, while the values of  $\log k_{org}$  are often negative, exceptions being given by the very hydrophobic solutes.

If a linear regression is applied to the dependence between  $\log k$  and  $\phi$ , written as  $\log k = a + b \phi$ , the parameters  $\log k_w$  and  $S$  are identified by  $a$  and  $b$ , respectively. The other limit of  $\log k$  is  $\log k_{org}$  for  $\phi = 1$ , which can be calculated from eq. 2 as being  $(\log k_w - S)$ , or  $(a - b)$ . According to eq. 2, the expression of  $S$  becomes:

$$S = \log k_w - \log k_{org} = \log \frac{k_w}{k_{org}} \quad (3)$$

According to the literature, the hydrophobicity index  $HI^V$  proposed by Valko *et al.* for RP-LC is suggested to be the ratio:<sup>26,27</sup>

$$HI^V = \frac{\log k_w}{S} \quad (4)$$

However, a careful interpretation of the eq. 1, the ratio  $(\log k_w)/S$  identified as  $HI^V$  accentually represents the value of fraction  $\phi$  for which  $k = 1$ , such that  $\log k = 0$ , or according to the definition of retention factor  $k$ , this is the retention time of studied solute that is twice the value of dead time of separation ( $t_R = 2 t_0$ ). However, in practice, by closing the value of  $\phi$  towards 1 the retention time can be extended between  $t_0$  and  $2 t_0$ , which gives negative values for  $\log k$ . Therefore,  $HI^V$  does not include the entire domain of mobile phase composition [0; 1], which results in the entire domain of  $\log k$ , namely  $[\log k_{org}, \log k_w]$ , as previously demonstrated.<sup>31</sup>

In a previous paper a new hydrophobicity index in RP-LC was derived from the linear retention function given by the eq. 1, denoted by  $HI^L$ , and representing the hypotenuse of the triangle of the geometrical representation of  $\log k$  versus  $\phi$ .<sup>31</sup> This

new hydrophobicity index depends on both extreme values of  $\log k$ , as expressed by eq. 3, and has the formula:

$$HI^L = \sqrt{1+S^2} \quad (5)$$

Unlike  $HI^V$ , which can have values  $> 1$  or  $< 1$  depending on whether  $\log k_w > S$ , or  $\log k_w < S$ , respectively, the values of  $HI^L$  are always  $> 1$ . This descriptor has been correlated with the octanol-water partition constant ( $\log K_{ow}$ ), the correlations being characterized by better determination coefficients  $R^2$  than resulted from correlations between  $HI^V$  and  $\log K_{ow}$ . A more extensive study was undertaken in this work in order to evaluate systematically the theoretical framework of this concept, using several HPLC columns, under various RP experimental separation conditions.

## EXPERIMENTAL DATA

A set of 20 compounds from several classes was systematically studied by RP-LC conditions: benzene ( $\log K_{ow} = 1.99$ ), toluene ( $\log K_{ow} = 2.54$ ), ethylbenzene ( $\log K_{ow} = 3.03$ ), propylbenzene ( $\log K_{ow} = 3.52$ ), butylbenzene ( $\log K_{ow} = 4.01$ ), benzoic acid ( $\log K_{ow} = 1.87$ ) and its four esters from methyl to butyl ( $\log K_{ow} = 1.83$ ; 2.32; 2.81; 3.30, respectively), p-hydroxybenzoic acid ( $\log K_{ow} = 1.39$ ) and its esters from methyl to butyl (known as parabens) ( $\log K_{ow} = 1.99$ ; 2.49; 2.98; 3.47, respectively), p-hydroxybenzaldehyde ( $\log K_{ow} = 1.23$ ), p-hydroxyacetophenone ( $\log K_{ow} = 1.19$ ), p-hydroxypropiophenone ( $\log K_{ow} = 1.68$ ), p-hydroxybutyrophenone ( $\log K_{ow} = 2.17$ ), and p-hydroxyvalerophenone ( $\log K_{ow} = 2.67$ ). All compounds were purchased from Sigma-Aldrich, Steinheim, Germany.

Seven C18 HPLC columns of different proveniences were used in this study, having the following constructive characteristics:

1. Gemini 5 u C18 (Phenomenex): porous; 150 mm length; 4.6 mm inner diameter; 5  $\mu\text{m}$  particle size; 14% carbon; 110  $\text{\AA}$  pore size;
2. Brownlee Choice (Perkin Elmer): porous; 150 mm  $\times$  4.6 mm  $\times$  5  $\mu\text{m}$ ; 27% C; 60  $\text{\AA}$ ;
3. Luna C18(2) (Phenomenex): porous; 150 mm  $\times$  4.6 mm  $\times$  5  $\mu\text{m}$ ; 17.5% C; 100  $\text{\AA}$ ;
4. Brownlee Analytical (Perkin Elmer): porous; 150 mm  $\times$  4.6 mm  $\times$  5  $\mu\text{m}$ ; 13% C; 110  $\text{\AA}$ ;
5. Ultisil XB-C18 (Welch): porous; 150 mm  $\times$  4.6 mm  $\times$  5  $\mu\text{m}$ ; 17% C; 120  $\text{\AA}$ ;
6. SPP (Perkin Elmer): core-shell; 50 mm  $\times$  3 mm  $\times$  2.7  $\mu\text{m}$ ; 90  $\text{\AA}$ ;

7. Boltimate (Welch): core-shell; 50 mm  $\times$  3 mm  $\times$  2.7  $\mu\text{m}$ ; 9% C; 90  $\text{\AA}$ .

HPLC experiments were carried out in separate sessions for each studied analytical column, using a Perkin Elmer LC system, model Flexar (Waltham, USA), with UV detection at 254 nm. Mobile phase compositions were water/organic modifier, with the volume fraction  $\phi$  varying between 0.45–0.60 for methanol, and 0.6–0.75 for acetonitrile, under isocratic conditions. The mobile phase flow rate was kept constant to 1 mL/min, at 25°C. Other experimental conditions are detailed in a previous work.<sup>39</sup>

The experimental values of  $t_R$  and  $t_0$  ( $t_0$  being measured from the retention time of uracil) were used to calculate  $k$  for each studied solute with formula  $(t_R - t_0)/t_0$ . Then linear regressions were applied to the dependences between  $\log k$  and  $\phi$  in order to calculate the regression parameters  $a$  and  $b$  according to eq. 1.

## RESULTS AND DISCUSSION

Acquisition of experimental retention data ( $\log k$ ) for the set of 20 compounds for the seven HPLC columns, under conditions previously described, was followed by calculation of  $\log k_w$  and  $S$  from linear regressions for each compound with eq. 1. Then,  $HI^V$  and  $HI^L$  were calculated for each studied compound, each HPLC column and organic modifier by their presented formula. Thus, 14 tables containing the processed data were developed, containing also the molecular weight  $M_w$  and calculated values of  $\log K_{ow}$  with the aid of Episuite package.<sup>40</sup> The choice of theoretical  $\log K_{ow}$  values instead of their experimental data is justified by the lack of experimental data for two compounds from this set, and a very good correlation between theoretical and experimental  $\log K_{ow}$  values for the rest of 18 compounds.<sup>31</sup> Finally, these data tables were used to investigate linear regressions between the following parameters: 1)  $S$  and  $(M_w)^{0.5}$ , 2)  $\log k_w$  and  $\log K_{ow}$ , 3)  $HI^V$  and  $\log K_{ow}$ , and 4)  $HI^L$  and  $\log K_{ow}$ .

Representations between  $S$  and the square root of molecular weight  $M_w$  showed no trend, on the contrary, for these chosen compounds there was no functional dependence between the two parameters, as suggested in the literature.<sup>41</sup> An example of such random distribution of the experimental data is shown in Fig. 1, where linear trends can be indeed observed, but only for the same homologous series of five studied compounds. The entire data set was characterized by a poor determination coefficient  $R^2$ .

The lack of correlation between  $S$  and  $(M_w)^{0.5}$  can be seen as a result from the different retention behavior for solutes with the same molecular weight. For example, several of the studied solutes have the same  $M_w$ , but very different values of  $S$  for almost all seven of the investigated HPLC columns. Therefore, an

acceptable correlation between the two parameters is possible only for a homologous series of compounds, as noticed among the 20 solutes used in this study. However, the literature reports other examples of solutes with retention in RP-HPLC correlated to the  $M_w$ , such as the case of peptide nucleic acids.<sup>42</sup>

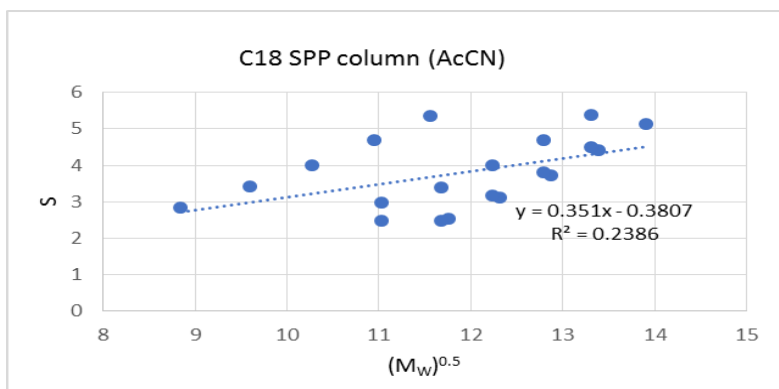


Fig. 1 – Graph representing the linear dependence between  $S$  and  $\sqrt{M_w}$  for C18 SPP PE column, using acetonitrile as organic modifier in mobile phase composition.

Good correlations were noticed for the dependence  $\log k_w$  versus  $\log K_{ow}$  when using methanol as organic modifier. The best example is depicted in Fig. 2 for one of the HPLC column, the determination coefficient  $R^2$  being

0.93. In case of acetonitrile, the correlations were less performant, being better than 0.82 with two exceptions, as can be observed in Table 1 that summarizes all regression data for this dependence.

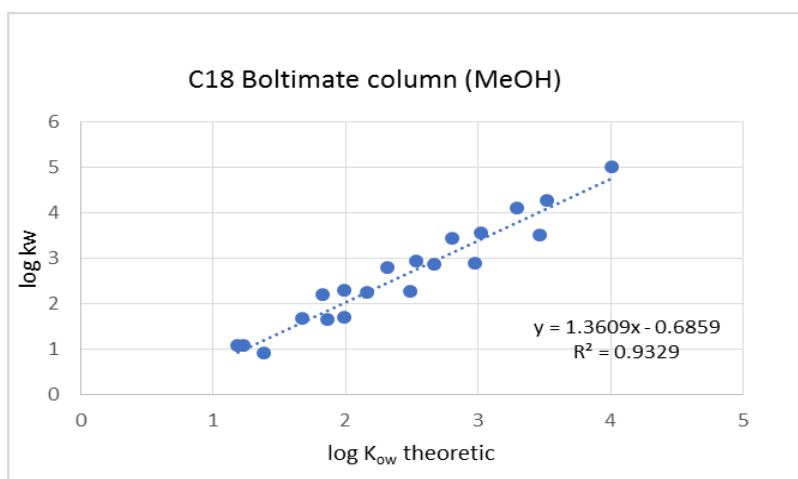


Fig. 2 – Linear regression applied to the dependence between  $\log k_w$  and  $\log K_{ow}$  for C18 Boltimate column (methanol).

Table 1

Correlations between  $\log k_w$  and  $\log K_{ow}$  for the two indicated organic modifiers

Column	Intercept	Slope	$R^2$	Intercept	Slope	$R^2$
	MeOH			AcCN		
Ultisil XB-C18	-0.3242	1.1566	0.9295	-0.4569	0.8745	0.8265
Gemini 5 u C18	-0.2585	1.1489	0.9270	-0.4810	0.8564	0.8306
C18 Choice	-0.1835	1.2228	0.8921	-0.5740	0.9926	0.7987
C18 LUNA	-0.2637	1.1764	0.9297	-0.3553	0.8415	0.8458
C18 Analytical	-0.2900	1.1450	0.9303	-0.7244	0.8985	0.7955
C18 SPP PE	-0.5999	1.3178	0.9268	-0.8227	1.2070	0.8856
C18 Boltimate	-0.6859	1.3069	0.9329	-0.9560	1.2386	0.8857

Generally, the dependences between  $HI^V$  and  $\log K_{ow}$  were characterized by constant determination coefficients, around 0.75 for methanol and 0.65 for acetonitrile. A higher value of  $R^2$  for acetonitrile (0.72) was reported by Valko and colab., but obtained on a larger set of studied solutes (more than 50 unrelated compounds).<sup>27</sup> As a remark of data from Table 2,

the slope and intercept of the applied regressions corresponding to both solvents kept a quite constant value. However, significant differences can be observed between porous stationary phases (first five rows of data) and core-shell stationary phases (the last two HPLC columns). One of the best correlations between the two parameters is depicted in Fig. 3.

Table 2

Correlations between  $HI^V$  and  $\log K_{ow}$  for the two indicated organic modifiers

Column	Intercept	Slope	$R^2$	Intercept	Slope	$R^2$
	MeOH			AcCN		
Ultisil XB-C18	0.3127	0.1723	0.7679	0.1728	0.2035	0.6780
Gemini 5 u C18	0.3271	0.1627	0.7587	0.1471	0.2068	0.6761
C18 Choice	0.3764	0.1742	0.6547	0.1682	0.2279	0.6038
C18 LUNA	0.3322	0.1635	0.7592	0.1975	0.1961	0.6719
C18 Analytical	0.2757	0.1771	0.7579	0.0461	0.2341	0.6251
C18 SPP PE	0.2466	0.1751	0.7340	0.1116	0.1709	0.6385
C18 Boltimate	0.2307	0.1758	0.7454	0.0894	0.1727	0.6761

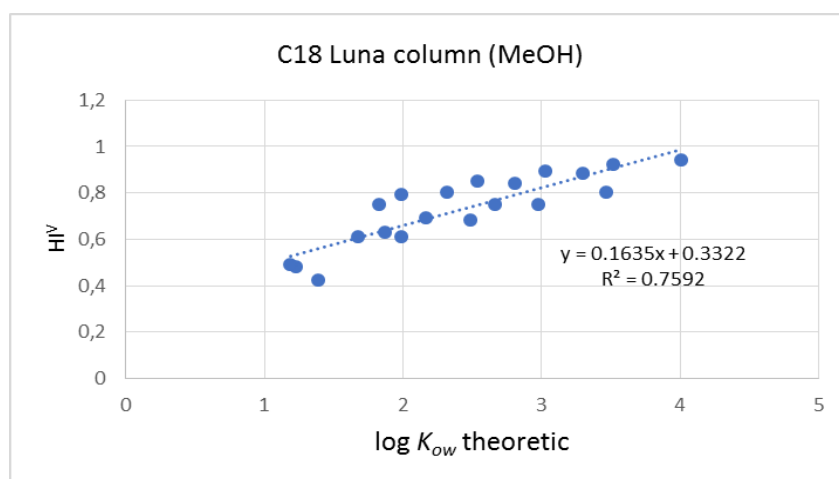


Fig. 3 – Linear regression applied to the dependence between  $HI^V$  and  $\log K_{ow}$  for C18 Luna column (methanol).

Table 3

Correlations between  $HI^L$  and  $\log K_{ow}$  for the two indicated organic modifiers

Column	Intercept	Slope	$R^2$	Intercept	Slope	$R^2$
	MeOH			AcCN		
Ultisil XB-C18	1.5510	0.7756	0.9391	1.2769	0.5384	0.9182
Gemini 5 u C18	1.6357	0.7848	0.9207	1.2547	0.5329	0.9320
C18 Choice	1.6022	0.8036	0.9030	1.1150	0.6093	0.8948
C18 LUNA	1.6338	0.8056	0.9203	1.3744	0.5114	0.9161
C18 Analytical	1.7915	0.7327	0.8932	0.9994	0.5873	0.8446
C18 SPP PE	1.5504	0.9457	0.9478	1.3190	1.0757	0.8780
C18 Boltimate	1.4959	1.0118	0.9617	1.1626	1.1352	0.9435

The best correlations were obtained between  $HI^L$  hydrophobicity index and  $\log K_{ow}$ , for both organic modifiers. This can be observed from data presented in Table 3, and compared to the values from Table 2. Intercept of these correlations did not

vary significantly from column to column, but slope had larger variations between the groups of porous stationary phases and core-shell stationary phases. The determination coefficients  $R^2$  were better than 0.92 for methanol, excepting two

columns, with a maximum value achieved for C18 Boltimate HPLC column ( $R^2 = 0.96$ ), which is illustrated in the graph presented in Fig. 4. In case of acetonitrile as organic modifier, the maximum correlation was obtained for the same HPLC

column,  $R^2$  being 0.94. For comparison of the scattered degree of the two situations, the correlation graph obtained for  $HI^L$  versus  $\log K_{ow}$  with Boltimate C18 column and acetonitrile as organic modifier in mobile phase is given in Fig. 5.

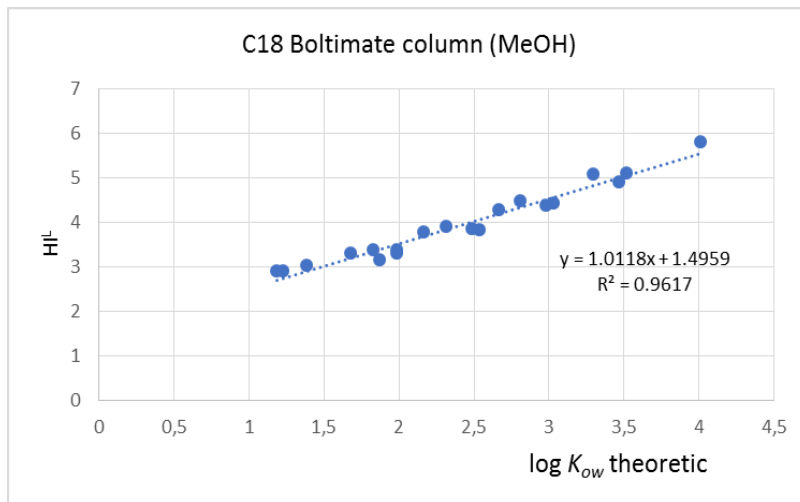


Fig. 4 – Linear regression applied to the dependence between  $HI^L$  and  $\log K_{ow}$  for C18 Boltimate column (methanol).

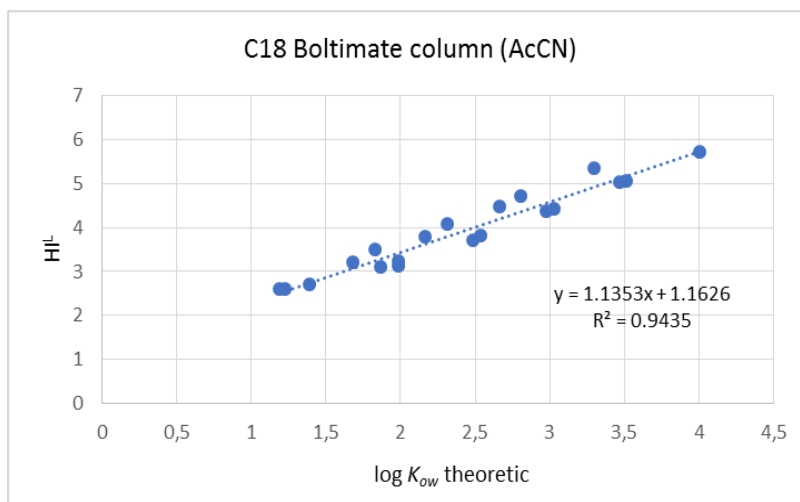


Fig. 5 – Linear regression applied to the dependence between  $HI^L$  and  $\log K_{ow}$  for C18 Boltimate column (acetonitrile).

Some general conclusions resulted from the analysis of data summarized in Tables 1, 2 and 3 are following:

*i)* in all cases the correlations were better for methanol compared to acetonitrile, used as organic modifiers in mobile phase composition;

*ii)*  $HI^L$  correlated better than  $HI^V$  with  $\log K_{ow}$  for all columns:  $R^2$  was higher than 0.9 for  $HI^L$ , excepting one column, while  $R^2$  for  $HI^V$  correlations was less than 0.8;

*iii)* some differences appear between the group of HPLC columns containing porous and core-shell stationary phases;

*iv)* larger variations of slope and intercept

values can be noticed only for the dependences between  $\log k_w$  and  $\log K_{ow}$  for the entire lot of HPLC columns utilized in study.

Limitation of this new descriptor  $HI^L$  is given by the accuracy of determining the value of solvent strength parameter  $S$  and  $\log k_w$ ,<sup>43</sup> which are almost always estimated from extrapolation of eq. 1. Another limitation is due to the deviation from linearity of the dependence between  $\log k$  and  $\phi$ , which has been observed in many situations.<sup>14,37,38,44</sup> However, similarly to the usage of hydrophobicity index proposed by Valko, this new chromatographic index could be convenient in characterization of HPLC columns as well as in

predicting the value of  $\log K_{ow}$  for compounds that are otherwise difficult to be determined by shake-flask method.

## CONCLUSIONS

One may conclude that the new hydrophobicity index, based on solvent strength parameter ( $S$ ), can be considered a better choice for characterizing the hydrophobicity in RP-HPLC than the index based on the ratio between  $\log k_w$  and  $S$ . Diversity of studied compounds and the number of HPLC columns are sufficiently large to draw this conclusion regarding the use of this new index in order to characterize the RP-HPLC separation mechanism. Molecular weight as suggested by some papers from literature appeared not to be correlated with  $S$ , which is in agreement to the experimental observations since two compounds of the same  $M_w$  do not have always the same retention in RP-HPLC. Good correlations between  $\log k_w$  and  $\log K_{ow}$  in the entire study can be considered as a validation of data obtained in this study, being in accordance to other studies reported by the literature.

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