K16 Structural properties governing retention mechanisms on RP-HPLC stationary phases used for lipophilicity measurement

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INTRODUCTION

The lipophilicity of solutes, traditionally expressed by their partition coefficients in the 1-octanol/water system (noted log P_{oct}), is an extremely important parameter in QSAR and ADME predictions [1-5]. The reference procedure to measure log P_{oct} is the shake-flask method, which however is time-consuming and limited in range (ca. $-3 < \log P < 4$). Beyond these limits, log P values measured by the shake-flask method become unreliable. The RP-HPLC method is a promising alternative to the shake-flask method, having such advantages as a higher throughput, an insensitivity to impurities, and a broader lipophilicity range [6]. In RP-HPLC method, lipophilicity indices are derived from the capacity factor log k, which is calculated by Eq. 1

$$k = (t_r - t_0) / t_0 \qquad [1]$$

where t_r and t_0 are the retention times of the solute and of an unretained compound, respectively. Some workers have used isocratic log k values measured in an appropriate mobile phase as a lipophilicity parameter [7, 8]. However, many more investigators used capacity factors extrapolated to 100% water (log k_w) to eliminate organic solvent effects [9-12], and they have indeed demonstrated the usefulness of the log k_w parameter when investigating series of solutes covering a broad lipophilicity range. Generally, the extrapolation to 100% water is based on a quadratic relationship between the isocratic capacity factor log k and the volume fraction of organic solvent in the mobile phase, φ [13]. When methanol is used as the organic modifier, a linear relationship (Eq. 2) is often obtained for neutral solutes [14]:

$$\log k = -S\varphi + \log k_w \qquad [2]$$

where $-S$ is the slope and log k_w the intercept of the regression curve.

The key of the RP-HPLC method to measure log P_{oct} is that the retention mechanism of the solutes on a stationary phase should be the same as the partitioning mechanism in 1-octanol/water. A highly informative interpretation of retention mechanisms on RP-HPLC stationary phases can be obtained by linear solvation free-energy relationships (LSERs) based on the solvatochromic parameters [15]. This method has also been used to evaluate partitioning mechanisms of solutes in various organic/aqueous biphasic systems [16]. LSERs can be expressed by Eq. 3

$$S_p = v \bullet V_w + p \bullet \pi^* + a \bullet \alpha + b \bullet \beta + c \qquad [3]$$

where S_p is a given molecular property of a neutral organic solute, here log k_w or log P_{oct}. The four structural parameters are the van der Waals volume V_w

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which accounts for hydrophobic and dispersive forces, and polar terms known as solvatochromic parameters (dipolarity/polarizability π^*, H-bond donor acidity α, and H-bond acceptor basicity β) which account for polar interactions between solutes and solvents. The regression coefficients *v*, *p*, *a* and *b* reflect the relative contribution of each solute parameter to S_p.

The objective of this study was to assess and compare the mechanisms of retention of two recent stationary phases of interest in lipophilicity measurement, namely the silica based Discovery RP Amide C16 phase and the polymer-based ODP-50 4B phase. A wide range of noncongeneric solutes were selected. The LSERs approach was applied to unravel the retention mechanisms of the solutes on the two stationary phases and to compare them with the partitioning mechanism in 1-octanol/water.

METHODS

A set of 41 compounds with log P_{oct} values from -0.69 to 4.80 were selected in this study. This set consists of model compounds and complex drugs with a broad range of parameter spaces in terms of V_w, π^*, β and α as demonstrated in Figure 1.

In this study, the extrapolated capacity factor log k_w was used as the lipophilicity parameter. The mobile phase consisted of 0.02 M phosphate buffer and methanol in varying proportions from 80 to 10% *v/v*. The phosphate buffer was adjusted to pH 7 for all nonionizable compounds and to a pH value (pH 3, 4 or 7) where the neutral form was in large excess for the ionizable compounds. The retention times were measured at ambient temperature by the UV/Vis detector under the detection wavelength λ_{max} of the analytes. On Discovery RP Amide C16 stationary phase, the measurements were carried out at a flow rate 1.0 mL/min for the compounds with log P_{oct} values higher than 1 and 0.5 mL/min for the compounds with log P_{oct} values below 1. Since the highest pressure limit of ODP-50 4B column used in this study is much lower (about 730 psi) compared to that of silica-based columns (4000 psi), a low flow

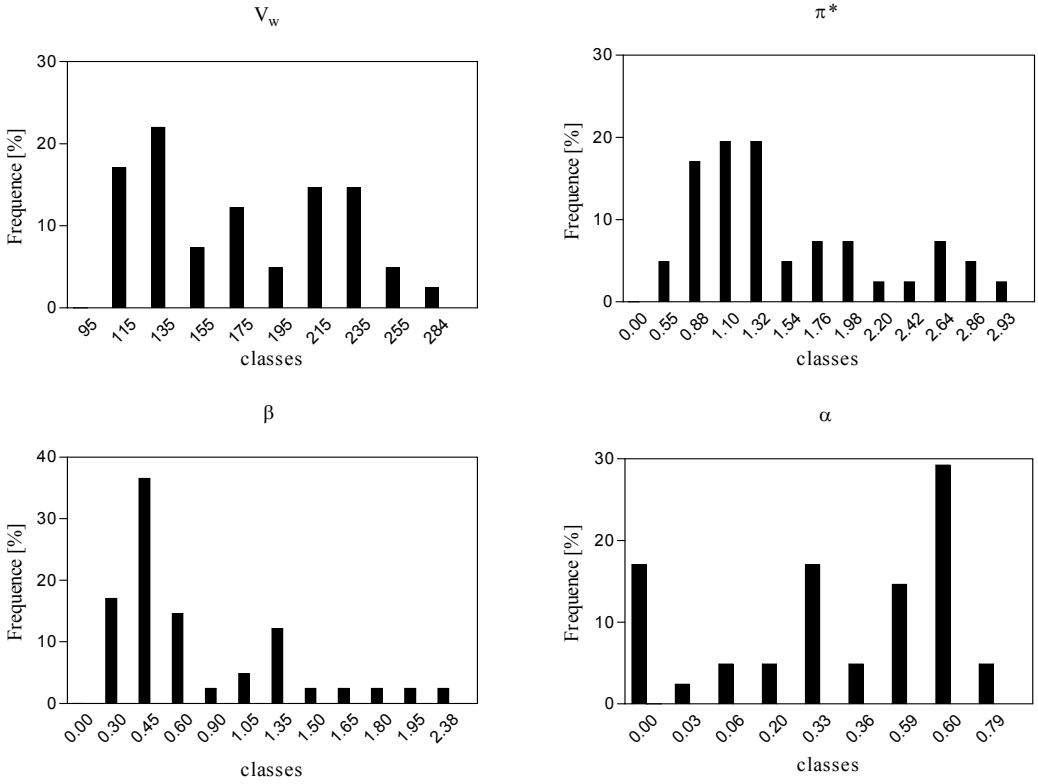

Figure 1: Distribution of the investigated compounds in the parameter spaces

rate (0.5 mL/min) on ODP-50 4B stationary phase was used in order to maintain the column life. In all cases, three isocratic log k were measured with different percent methanol in the eluent. Methanol concentrations were adapted to the log P_{oct} values of the solutes as described in the table below:

log P_{oct} range	% MeOH (Discovery RP Amide C16)	% MeOH (ODP-50 4B)
> 3	60, 65, 70	70, 75, 80
1-3	40, 45, 50	60, 65, 70
< 1	10, 20, 25	20, 30, 40

The log k_w values were then extrapolated to 100% water using equation 2.

The regression analyses were performed via the JMP statistical software package (Version 5.1.1, Japanese Edition, SAS Institute Inc).

RESULTS AND DISCUSSION

The log k_w values obtained with the two stationary phases were analyzed by LSERs, yielding statistically significant equations describing the structural properties governing the retention mechanisms.

- Discovery RP Amide C16 phase:

$$\log k_w = 2.72\bullet10^{-2}(\pm0.44\bullet10^{-2})\bullet V_w - 0.48(\pm0.44)\bullet\pi^* - 2.62(\pm0.57)\bullet\beta - 0.24(\pm0.63) \quad [4]$$

$n = 41$; $q^2 = 0.87$; $r^2 = 0.88$; $s = 0.50$; $F = 87$

- ODP-50 4B phase:

$$\log k_w = 2.12\bullet10^{-2}(\pm0.34\bullet10^{-2})\bullet V_w - 2.27(\pm0.32)\bullet\beta + 0.63(\pm0.46) \quad [5]$$

$n = 41$; $q^2 = 0.85$; $r^2 = 0.86$; $s = 0.40$; $F = 114$

Eq. 4 shows that the main factors governing retention on the Discovery RP Amide C16 phase are the solute's van der Waals volume (V_w) and H-bond acceptor basicity (β), while the importance of dipolarity/polarizability (π^*) is smaller and the H-bond donor acidity (α) is not significant. Eq. 5 reflects the different balance of structural parameters controlling log k_w on the ODP-50 4B phase, for which V_w and β are important parameters, whereas π^* and α are not significant.

To allow a comparison, the log P_{oct} values were also analyzed by LSERs, yielding Eq. 6:

$$\log P_{oct} = 2.41\bullet10^{-2}(\pm0.38\bullet10^{-2})\bullet V_w - 0.42(\pm0.40)\bullet\pi^* - 2.41(\pm0.51)\bullet\beta + 0.41\ (\pm0.56) \quad [6]$$

n = 41; $q^2 = 0.87$; $r^2 = 0.88$; $s = 0.45$; $F = 92$

The ratios of the normalized regression coefficient in Eqs. 4 and 6 are nearly identical (details not shown), meaning that the same balance of intermolecular forces is encoded by log P_{oct} and log k_w measured on the Discovery RP Amide C16 phase.

Due to the same mechanism of retention as the partitioning in 1-octanol/water, the log k_w values derived from Discovery RP Amide C16 gives a much higher quality of correlation with log P_{oct} for the compounds investigated as shown in Eqs. 7 and 8 and Figure 2.

- Discovery RP Amide C16 phase:

$$\log P_{oct} = 0.89\ (\pm0.06)\ \log k_w + 0.56\ (\pm0.12) \quad [7]$$

$n = 41$; $q^2 = 0.96$; $r^2 = 0.96$; $s = 0.24$; $F = 1054$

- ODP-50 4B phase:

$$\log P_{oct} = 1.14\ (\pm0.12)\ \log k_w - 0.80\ (\pm0.32) \quad [8]$$

$n = 41$; $q^2 = 0.91$; $r^2 = 0.91$; $s = 0.38$; $F = 396$

From the above results, it can be concluded that the silica-based Discovery RP Amide C16 phase is a better choice than the polymer based ODP-50 4B

phase to derive a lipophilicity index log k_w correlated with log P_{oct}.

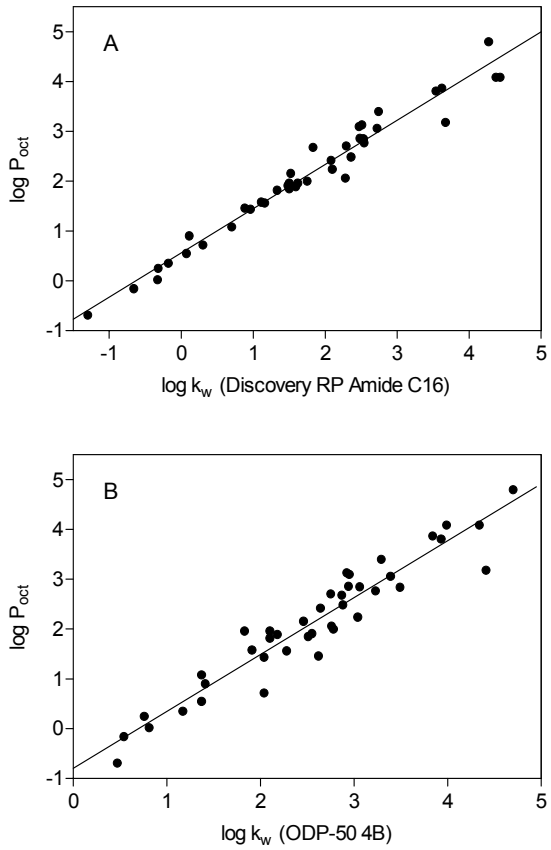

Figure 2: Relationships between log P_{oct} and log k_w. A: on Discovery RP Amide C16 stationary phase. B: on ODP-50 4B stationary phase.

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REFERENCES

[1] H. van de Waterbeemd, B. Testa, in 'Advances in Drug Research'; Ed. B.Testa, Academic Press, London, 1987; Vol. 16, p. 87-227.

[2] C. Hansch, A. Leo, 'Substituent Constants for Correlation Analysis in Chemistry and Biology'; John Wiley and Sons, New York, 1979.

[3] I. Komiya, J. Y. Park, A. Kamani, N. F. H. Ho, W. I. Higuchi, *Int. J. Pharm.* **1980,** *4*, 249.

[4] D. C. Taylor, R. Pownall, W. Burke, *J. Pharm. Pharmacol.* **1985,** *37*, 280.

[5] V. A. Levin, *J. Med. Chem.* **1980,** *23,* 682.

[6] H. van de Waterbeemd, M. Kansy, B. Wagner, H. Fischer, In 'Lipophilicity in Drug Action and Toxicology', Eds. V. Pliska, B. Testa, H. van de Waterbeemd, VCH Publishers: Weinheim, 1996; p. 73.

[7] C. Yamagami, M. Yokota, N. Takao, *Chem. Pharm. Bull.* **1994,** *42,* 907.

[8] W. Klein, W. Kördel, M. Weiss, H. J. Poremski, *Chemosphere* **1988,** *17,* 361.

[9] N. El Tayar, H. van de Waterbeemd, B. Testa, *J. Chromatogr.* **1985,** *320,* 305.

[10] X. Liu, H. Tanaka, A. Yamauchi, B. Testa, H. Chuman, *Helv. Chim. Acta,* in press.

[11] N. El Tayar, H. van de Waterbeemd, B. Testa, *Quant. Struct. -Act. Relat.* **1985,** *4,* 69.

[12] F. Lombardo, M. Y. Shalaeva, K. A. Tupper, F. Gao, M. H. Abraham, *J. Med. Chem.* **2000,** *43,* 2922.

[13] P. J. Schoenmaker, H. A. H. Billiet, L. De Galan, *J. Chromatogr.* **1979,** *185,* 179.

[14] T. Braumann, G. Weber, L. H. Grimme, *J. Chromatogr.* **1983,** *263,* 329.

[15] M. H. Abraham, M. Rosés, C. F. Poole, S. K. Poole, *J. Phys. Org. Chem.* **1997,** *10,* 358.

[16] X. Liu, G. Bouchard, N. Muller, A. Galland, H. H. Girault, B. Testa, P. A. Carrupt, *Helv. Chim. Acta,* **2003,** *86,* 3533.