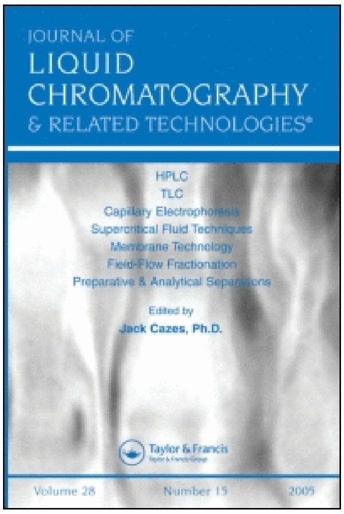

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ESTIMATION OF THE RP-HPLC LIPOPHILICITY PARAMETERS LOG K', AND LOG K_W, A COMPARISON WITH THE HYDROPHOBICITY INDEX φ_0[†]

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ABSTRACT

Chromatographic retention parameters k' and k_w were determined by RP-HPLC for a set of standard non-congeneric compounds and the correlation with log $P_{o/w}$ established. Then, log $P_{o/w}$ was calculated for a series of antimicrobial hydrazides, as a test set. A comparison of the chromatographic parameters log k' and log k_w with the hydrophobicity index φ_0 were also made. It seems that for the study compounds, log k_w describes the partitioning better than the parameter φ_0.

INTRODUCTION

Chromatographic retention parameters have been used in quantitative structure-retention relationships (QSRRs) as numerical descriptors of physicochemical parameters. Many such descriptors have been described - log k', log k'_w, φ_0.[1-5] Due to the advantages of the chromatographic technique over the classical "shake-flask" method, attempts have been made to correlate the chromatographic retention parameters with log $P_{o/w}$.[6,7] Therefore, the literature is full of examples of these relationships.[4,8] However, many of them rely upon determination of retention time ignoring the models employed.[9,10]

As far as the octanol/water model is concerned, the RP-HPLC is believed to mimic not only the biological membrane but also the "shake-flask".[1, 4] However, there is no agreement regarding which stationary phase should be used. Also, the use of either the isocratic or the polycratic mobile phase constitutes a problem. Thus, columns ranging from C_4 to C_{18}, including polymeric and, more recently, immobilised artificial membrane have been used.[1] The mobile phase has been used usually with methanol as the modifier in the polycratic method.[8,11]

Despite the disagreement, chromatographic retention parameters have been correlated to log P through a "Colander type equation":[13]

$$\log k' = a \log P + b \qquad (1)$$

Provided that a equals 1 and b equals zero, equation (1) could be regarded as the descriptor of the partitioning mechanism itself. However, this is far from being the case, and much of the data in the literature, including some reported in this paper, deviate substantially from the ideal behaviour.

It is important to notice that the retention process that operates in the RP-HPLC is very complex and although hydrogen bonding and solvophobic interactions seem to play the predominant role, other interactions[14-16] do not allow a well behaved relationship between log k' and log P.

Minick et al.[8] have shown that the coefficients of equation (1) are satisfied when a small portion of octanol is added into the mobile phase with n-decilamine. These reagents act as a silanol suppresser.

The use of water alone, as a mobile phase, would be desired for the measurement of retention time used as a hydrophobic index. However, this causes very long retention time. Also, the retention mechanism with chemically bonded stationary phases, changes with high water content in the mobile phase. Thus, the use of a co-solvent is needed. The use of a co-solvent diminishes the distinction between the two phases because the solute "drags" not only water molecules into the stationary phase, but also the co-solvent.[17] In trying to overcome these problems, polycratic measurements have been used. The capacity factor k_w can be obtained by extrapolating the values of k' measured to the value of 0% organic modifier in the mobile phase.

$$\log k' = \log k'_w - S\varphi \qquad (2)$$

where φ is the volume fraction of organic modifier.

The relationship is not always linear:

$$\log k' = a\varphi + b \qquad (3)$$

It can sometimes be described as a relationship of quadratic dependence of φ:[12]

$$\log k' = a\varphi^2 + b\varphi + c \qquad (4)$$

However, the linear portion has been used, mainly when methanol is the modifier agent.

Log k' can not be used as a universal scale because log k' is dependent on chromatographic conditions, i.e., it depends on the mobile and stationary phases used. K_w has been suggested as a better descriptor than k', as it is independent of any effect of the organic modifier and relies on the stationary phase alone.[18] However, one of major problems with bonded stationary phases is that, although nowadays they show good reproducibility by being prepared by the same manufacturer, big differences in behaviour are observed with bonded stationary phases prepared from different makers. This is evident if one takes into consideration the differences of the silica used as support in the preparation of those phases.[18]

Bearing this in mind, Valks and Sligel[2] introduced a new chromatographic hydrophobicity index, φ_0, based on the slope and intercept of the log k' versus organic modifier concentration plot. The parameter φ_0 is set up by taking log k' = 0 instead of $\varphi = 0$. Thus, this parameter is independent of the RP column type and length, flow-rate and also the mobile phase composition.

As an on-going program in lipophilicity, a series of antimicrobial hydrazides was studied. Log k', log k_w and φ_0 were determined and their correlation with log P was examined. The attempt to apply this new parameter φ_0 to our work was based on the fact that this parameter, as it is claimed,[2] could be used as an universal scale while k' and k_w cannot.

MATERIALS AND METHODS

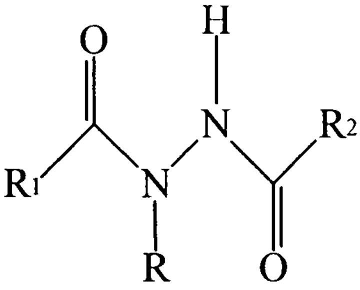
The HPLC system consisted of a Shimadzu LC-10AD pump, a SPD-6AV UV detector operated at 210 or 254 nm, and a LC-R6A chromatopac recorder. A Waters 510 pump with a LC-481 UV detector and a Shimadzu CR-4A recorder were also used. A Rheodyne 7125 injector fitted with a 20 μL loop was used in both cases. The column used was a LiChrosorb RP-8, 5m, 25x0.4 cm id, obtained from Merck (Darmstard, Germany).

The retention time of sodium nitrate, detected at 210 nm, was used as the column dead-time. Methanol was also used for the determination of the dead-time. The solvents used were of Merck HPLC-grade. They were filtered through a nylon membrane (4.7 Å) and degassed prior to use. The mobile phase consisted of methanol: 0.1M ammonium acetate buffer, pH = 4.6 (65:35v/v) for the isocractic measurements (system I). When octanol was used it was added at 0.25%v/v (system II). For the polycratic measurements the composition of the mobile phase was varied from 75% to 25% methanol in the 0.1M ammonium acetate buffer, pH_{app} = 4.6. The samples were run in triplicate and the capacity factor, k', were calculated as usual:

$$k' = t_r\text{-}t_0/t_0.$$

All test compounds were newly synthesised and their purity checked by HPLC. Provided that the retention times of the test compounds are allocated correctly this procedure is not necessary.

The compounds used as standard for the scale of hydrophobicity were: (I) acetanilide, (II) benzaldehyde, (III) benzophenone, (IV) pyridine, (V) toluene, (VI) formamide, (VII) benzyl alcohol, (VIII) acetophenone, (IX) 2-naftol, (X) ethyleneglicol, (XI) quinoline, (XII) ethyl acetate and (XIII) octanol. Measured octanol-water partition coefficients were obtained from Leo et al.[6] Partition coefficients of the study hydrazides, Figure 1, were calculated by employing the Hansch/Leo. approach.[6]



Hydrazides	R	R^1	R^2
1	R = C_6H_5	R^1 = CH_3	R^2 = CH_3
2	R = C_6H_5	R^1 = CH_2CH_3	R^2 = CH_2CH_3
3	R = C_6H_5	R^1 = C_6H_5	R^2 = C_6H_5
4	R = C_6H_5	R^1 = $O_2NC_6H_4$	R^2 = $O_2NC_6H_4$
5	R = C_6H_5	R^1 =BrC_6H_4	R^2 = BrC_6H_4
6	R = C_6H_5	R^1 = $MeOC_6H_4$	R^2 = $MeOC_6H_4$
7	R = C_6H_5	R^1 = ClC_6H_4	R^2 = ClC_6H_4
8	R = H	R^1 = C_6H_5	R^2 = C_6H_5
9	R = H	R^1 = $MeOC_6H_4$	R^2 = $MeOC_6H_4$
10	R = H	R^1 = $O_2NC_6H_4$	R^2 = $O_2NC_6H_4$
11	R = H	R^1 = MeC_6H_4	R^2= C_6H_5
12	R = H	R^1 =$MeOC_6H_4$,	R^2= C_6H_5
13	R = CH_3	R^1 = $O_2NC_6H_4$	R^1 = $O_2NC_6H_4$

Figure 1. Structures of study hydrazides 1-13.

RESULTS AND DISCUSSION

Table 1 shows log k' values obtained with the standard compounds used in the calibration scale. The correlation between log k' versus log $P_{o/w}$ for those dissimilar compounds (donors, acceptors and amphiprotics) using system I resulted in equation 5:

$$\log k' = 0.242(\pm 0.08)\log P_{o/w} - 0.452(\pm 0.16) \qquad (5)$$
$$(n = 13,\ r = 0.902,\ r^2 = 0.814,\ s = 0.191,\ F = 48.14,\ r^2_{cv} = 0.720)$$

When the proton donors (except pyridine) compounds IV, IX, X and XIII were excluded equation 6 was obtained:

$$\log k' = 0.336(\pm\ 0.04)\log P_{o/w} - 0.556(\pm\ 0.7) \qquad (6)$$
$$(n = 9,\ r = 0.992,\ r^2 = 0.984,\ s = 0.060,\ F = 453.26,\ r^2_{cv} = 0.950)$$

Table 1

Log K' and Log $P_{o/w}$ for the Standard Compounds Composing the Chromatographic Scale

Compounds		Classif.	log k'(i)	log k'(ii)	log $P_{o/w}$
I.	Acetanilide	m, f	-0.24	-0.274	1.16
II.	Benzaldehyde	a	-0.018	-0.148	1.48
III.	Benzophenone	a	0.573	0.407	3.18
IV.	Pyridine	a	-0.054	---	0.65
V.	Toluene	m, f	0.415	0.069	2.80
VI.	Formamide	m, f	-0.991	-1.172	-1.51
VII.	Benzyl Alcohol	d, f	-0.272	-0.516	1.10
VIII.	Acetophenone	a	-0.053	-0.046	1.58
IX.	2-Naftol	d, f	0.014	---	2.70
X.	Ethyleneglicol	d, ff	-0.799	---	-1.79
XI.	Quinoline	a	0.128	0.162	2.03
XII	Ethyl Acetate	a	-0.332	-0.253	0.73
XIII.	Octanol	d, f	-0.067	---	3.15

(i) System with no added octanol: (ii) system with octanol (see text for explanation); (iii) Data from reference [6]. a = acceptor, d = donor, m = mixed, f = amphiprotic, ff = double amphiprotic. (-) not measured

Using system II, the correlation observed was that shown in equation 7:

$$\log k' = 0.321(\pm 0.09)\log P_{o/w} - 0.644(\pm 0.16) \qquad (7)$$
$$(n = 9,\ r = 0.955,\ r^2 = 0.911,\ s = 0.144,\ F = 72.68,\ r^2_{cv} = 0.865)$$

The weakening in the statistical significance of equation 7, when compared to equation 6, can probably be explained according to Beezer's[19] remark that the amount of octanol added to the mobile phase was too high.

Equations 6 and 7 have similar coefficients which suggests that the same mechanism operates in both systems. Equation 6 was then used to predict the log $P_{o/w}$ for the hydrazides showed in Fig. 1.

Table 2 shows log k' obtained for the study compounds and equation 8 shows the regression analysis:

Table 2

Experimental Log K' and Calculated Log $P_{o/w}$ for Some Study Hydrizides

Compounds	log k' (I)	log k' (II)	log P (III)
1	-0.47	-0.58	-0.52
2	-0.18	-0.29	0.48
3	0.09	0.13	3.68
4	0.23	---	3.12
5	0.85	0.82	5.40
6	0.39	0.26	3.64
7	0.83	0.75	5.10
8	-0.35	-0.61	1.92
9	-0.24	-0.45	1.88
10	-0.86	-0.97	1.36

See Figure 1 for structures. (i) System with no octanol; (ii) system with octanol (see text for explanation); (iii) Data calculated as from reference 6.

$$\log k' = 0.252(\pm 0.12)\log P_{o/w} - 0.627(\pm 0.37) \quad (8)$$
$$(n = 10,\ r = 0.871,\ r^2 = 0.759,\ s= 0.290,\ F = 25.14,\ r^2_{cv} = 0.608)$$

It is noticeable that equation (8) also has similar regression coefficients, which contribute to a good inference to the "Collander's type equation".

Excluding compounds 1 and 2 (Figure 1), the equation (8) improves to (9):

$$\log k' = 0.380(\pm 0.11)\log P_{o/w} - 1.122(\pm 0.38) \quad (9)$$
$$(n = 8,\ r = 0.962,\ r^2 = 0.925,\ s= 0.173,\ F = 74.93,\ r^2_{cv} = 0.863)$$

When system II was used equation 10 came out:

$$\log k' = 0.421(\pm 0.07)\log P_{o/w} - 1.394(\pm 0.27) \quad (10)$$
$$n = 7,\ r = 0.988,\ r^2 = 0.976,\ s= 0.114,\ F = 213.52,\ r^2_{cv} = 0.952)$$

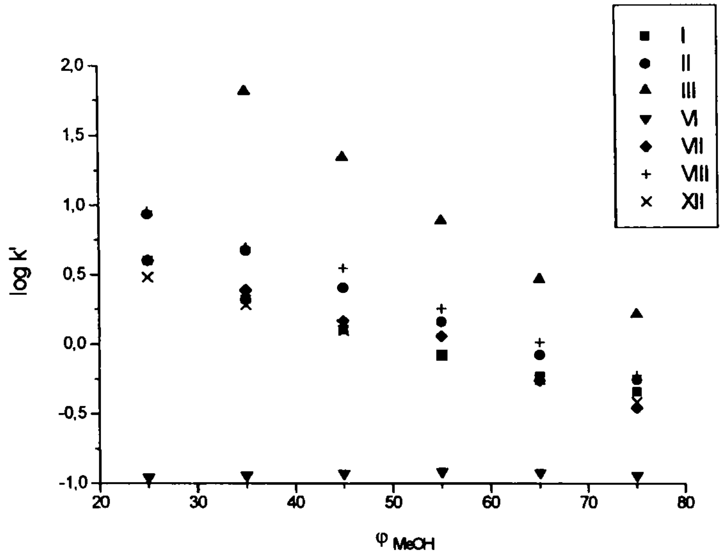


Figure 2. log k′ versus $P_{o/w}$ for the standards compounds.

Table 3

Log K' Versus φ_{Meoh} and Log K_w Obtained Through the Equation: Log K' = $-S\varphi_{Meoh}$ + Log K_w, for Scaling Standards Compounds

Compounds	φ_{MEOH} (%)/log k′ 25	35	45	55	65	75	log k_W
I.	0.599	0.322	0.100	-0.079	-0.231	-0.340	0.995
II.	0.936	0.671	0.405	0.158	-0.077	-0.257	1.514
III.	---	1.810	1.343	0.883	0.464	0.210	3.185
VI.	-0.957	-0.942	-0.931	-0.916	-0.920	-0.939	-0.990
VII.	0.606	0.390	0.166	0.058	-0.258	-0.458	1.137
VIII.	0.959	0.694	0.546	0.256	0.011	-0.223	1.552
XII.	0.482	0.280	0.098	-0.082	-0.253	-0.414	0.913

See text for structures. (-) Peak too broad to be measured.

It is noteworthy that the use of octanol (system II) in this case results in a better correlation than the one obtained with system I, as can been seen by equation 10.

Based on these observations, it was concluded that either system I or II could be used to evaluate log $P_{o/w}$ for the amphiprotic compounds studied. However, one should notice that the retention mechanism operating on these systems is not a pure partitioning one, as shown by the coefficients in all equations given above.[14,15]

Because of this, log k'_w was then obtained for the following compounds: (I) acetanilide, (II) benzaldehyde, (III) benzophenone, (VI) formamide, (VII) benzyl alcohol, (VIII) acetophenone and (XII) ethyl acetate. Table 3 shows log k_w for seven standards and Figure 2 shows the results.

All regression equations obtained showed correlation coefficients in the range 0.988-0.999 and F = 162-2947. However, formamide had a different slope which means that the retention mechanism operating for this solute was also different. Thus, this solute was excluded from the calibration scale. Figure 3 shows the plot log k_w versus log $P_{o/w}$ and equation (11) is shown below.

$$\log k_w = 0.974(\pm 0.18)\log P_{o/w} + 0.054(\pm 0.31) \qquad (11)$$
$$(n = 6,\ r = 0.991,\ r^2 = 0.982,\ s = 0.123,\ F = 230.18,\ r^2_{cv} = 0.932)$$

From the above equation, the log k_w and log P_{app} have been estimated for some of the hydrazides given in Fig. 1. Table 4 shows these results. All the regression coefficients are in the range of 0.996-1.000, F = 386-5640.

The above results agree with the assumption that k_w is independent of the effects of the modifier, yet it is not free from the effects of the stationary phase used.

Proceeding in this way, the new chromatographic hydrophobicity index,[2] φ_0, was examined for the same standards as derived in equation (11). However, the correlation obtained was not better than the one obtained using log k_w, as can be seen by equation (12).

$$\varphi_{0,MeOH,298,4.6} = 11.38(\pm 4.75)\log P_{o/w} + 42.93(\pm 8.21) \qquad (12)$$
$$(n = 6,\ r = 0.958,\ r^2 = 0.918,\ s = 3.289,\ F = 44.21,\ r^2_{cv} = 0.498)$$

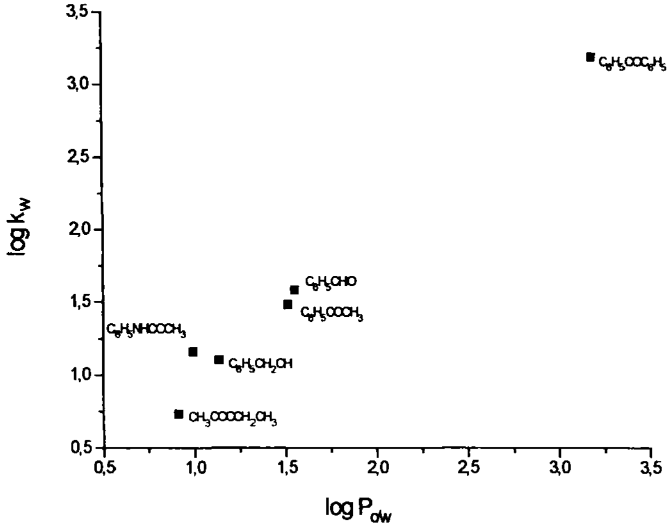


Figure 3. log k_w versus log $P_{o/w}$ for some standard compounds.

Table 4

Log K' and Log P_{app} for Some Study Hydrazides

Compounds	φ_{MEOH} (%)/log k' 25	35	45	55	65	75	log k_W	log P_{app}
3.	0.599	0.322	0.100	-0.079	-0.231	-0.340	0.995	3.319
4.	0.935	0.671	0.405	0.158	-0.077	-0.257	1.514	4.182
9.	---	1.810	1.343	0.883	0.464	0.210	3.185	1.552
10.	-0.957	-0.942	-0.931	-0.916	-0.920	-0.939	-0.990	2.073
11.	0.606	0.390	0.166	0.058	-0.258	-0.458	1.137	1.877
12.	0.959	0.694	0.546	0.256	0.011	-0.223	1.552	1.599

See Figure 1 for Structures.

In this case, log P could not be calculated as accurately as with equation 11. We believe this result suggests that it is inappropriate to model partition systems for structurally unrelated compounds, through φ_0. Nevertheless, we have tried the same calculations for the study hydrazides and, equation 13 shows the results.

$$\varphi_{0,\mathrm{MeOH},298,4.6} = 6.15(\pm\ 2.39)\log P_{app} + 44.74(\pm 6.26) \qquad (13)$$
$$(n = 6, r = 0.964,\ r^2 = 0.929,\ s = 2.053,\ F = 51.92,\ r^2_{cv} = 0.670)$$

From the above equation, it seems that φ_0 fits better for these structurally related compounds. Yet, it seems that equation (11) describes these results better than those due to equations (12) and (13).

CONCLUSIONS

Equations 12 and 13 cannot be used as a model for obtaining partition coefficients. Both equations (12 and 13) were produced by chance correlation with a poor cross-validation, which is not the case described by equation 11.

Nevertheless, it is evident from the above that there is an important paradigm to be established. Log k' has led to log k_w, but k_w does not take into account all the shortcomings of chromatographic measurement. This is mainly due to the fact that problems with column manufacture, for instance, affect k_w. Moreover, it involves a free range concentration of the organic modifier, since it is dependent on whether one chooses the linear or parabolic portion of the mutual partitioning behaviour between the mobile and stationary phases. The "dragging' of solvent molecules also plays an important role. This happens mainly when the modifier is methanol.

However, it is clear that k', due to experimental conditions, has a bearing on the way k_w is determined. This means that k' is necessary to describe k_w but not sufficient to describe log P. In spite of this, it seems that until a better method is suggested, k'_w shall remain the best approximation of log P.[16]

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REFERENCES

1. R. Kaliszan, J. Chromatography A, **656**, 417 (1993).

2. K. Valks, P. Sligel, J. Chromatography A, **631**, 49 (1993).

3. L. C. Tan, P. W. Carr, J. Chromatography A, **656**, 521 (1993).

4. D.P. Nowotnik, T. Feld, A. D. Nunn, J. Chromatography A, **630**, 105 (1993).

5. H. Mei-Ming, J. G. Dorsey, J. Chromatography A, **631**, 63 (1993).

6. A. Leo, C. Hansch, D. Elkins, Chemical Reviews, **71**, 525 (1971).

7. A. J. Leo, Chemical Reviews, **93**, 1281 (1993).

8. D. J. Minick, J. H. Frenz, M. A. Patrick, D. A. Brent, J. Med. Chem., **31**, 1923 (1988).

9. E. Tomlinson, J. Chromatography A, **113**, 1 (1975).

10. J. Dearden, Environ. Health Perspect., **61**, 203, (1985).

11. T. Braumann, J. Chromatography, **373**, 191 (1986).

12. J. G. Dorsey, M. G. Khaledi, J. Chromatography A, **656**, 485 (1993).

13. R. Collander, Acta Chem. Scand., **5**, 774 (1951),

14. W. Melander, J. Stoveken, C. Horvath, J. Chromatography A, **19**, 35 (1980).

15. R. P. W. Scott, C. F. Simpson, J. Chromatography A, **197**, 11 (1980).

16. R. Kaliszan, in **Quantiative Structure-Chromatographic Retention Relationships**, Chemical Analysis, Vol. 93, John Wiley & Sons, New York, 1987.

17. R. S. Tsai, W. Fan, N. El Tayar, P. A. Carrupt, B. Testa, L. B. Kier, J. Am. Chem. Soc., **115**, 9632 (1993).

18. R. P. W. Scott, **Silica Gel and Bonded Phases, Their Production, Properties and Use in LC,** R. P. W. Scott, C. F. Simpson eds., John Wiley & Sons, 1993.

19. A. E. Beezer, Chemical Laboratory, University of Kent at Canterbury, personnal communication.

[†] Work presented at the International Symposium on Lipophilicity and Toxicology, Lausanne, Switzerland, 21-24 March, 1995.

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