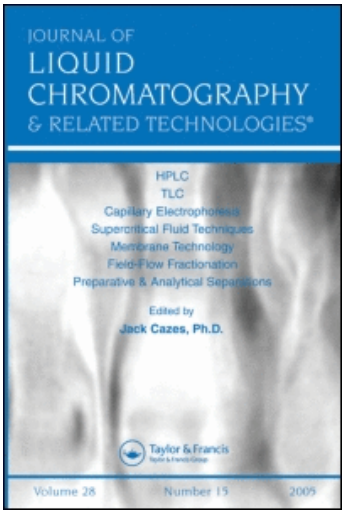

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EVALUATION OF A SIMPLE HPLC CORRELATION METHOD FOR THE ESTIMATION OF THE OCTANOL-WATER PARTITION COEFFICIENTS OF ORGANIC COMPOUNDS

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ABSTRACT

A simple reverse-phase high performance liquid chromatographic method is evaluated for the estimation of octanol-water partition coefficients (log P) of organic compounds by correlation with their chromatographic capacity factors (k'). Using an unmodified commercial octadecylsilane column and a mobile phase consisting of methanol and an aqueous buffer, a linear relationship is established between the literature log P values of 68 compounds and the logarithms of their k' values. For the determination of the partition coefficients of unknowns, one of two sets of standards is used to calibrate the system, the choice being dependent on the hydrogen-bonding character of the compounds being evaluated. The overall method is shown to be rapid and widely adaptable and to give log P data which are comparable to results obtained by classical or other correlation methods.

INTRODUCTION

The octanol-water partition coefficient (commonly expressed as log P) is an important physical parameter which has been directly correlated with the biological activities of a wide variety of organic compounds (1). While there has recently been much effort to calculate this parameter on the basis of chemical

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structure alone (1-3), imperfections in this method and the need for reference values often still requires the experimental measurement of log P values. Experimental methods for this determination include the direct chromatographic (4) or spectroscopic (2) assay of compounds in an equilibrated octanol-water system, potentiometric titrations of compounds in a biphasic octanol-water mixture (5) and determinations based upon established correlations of log P values of compounds with their thin-layer (6) or column liquid chromatographic (7,8) behavior. Of these latter chromatographic methods, there has been considerable interest in the development and utilization of relationships between octanol-water partition coefficient values and reverse-phase high performance liquid chromatography (HPLC) capacity factors (k'). Such relationships are based upon the observed similarities in the hydrophobic partitioning processes occurring in an octanol-water mixture and in a reverse-phase HPLC system with an aqueous mobile phase.

Recently, there has been much work on the attempted improvement of correlations between log P and k' values by increasing the similarities between the octanol-water and reverse-phase HPLC partitioning systems. Such attempts have included the reduction of free silanol sites in the column by exhaustive silylation (9,10), the presaturation of the column with octanol (11,12) and the use of totally aqueous mobile phases (12). While many of these modifications have been somewhat successful at improving the

correlation between log P and k', they have achieved only limited applicability due to increases in the complexity of equipment and experimental time required for their implementation.

Recent advances in methods of stationary phase preparation have resulted in the commercial production of reverse-phase HPLC columns with high homogeneity and high levels of surface alkyl bonding. Based on our premise that the utilization of such a modern column should give a higher degree of correlation between k' and log P than that previously obtained, we have developed and evaluated a simple, rapid HPLC method for the determination of partition coefficients of organic compounds from their k' values, using an unmodified commercial reverse-phase column and a standard aqueous mobile phase.

EXPERIMENTAL

Materials: All solvents were glass distilled (Burdick and Jackson). The chemicals used were obtained from commercial sources (mostly from Aldrich Chemical Company) and were used without further purification.

Apparatus: The HPLC system consisted of an Altex high pressure pump, a Waters U6K injector and an octadecylsilane column (Alltech RP-18, 10 μm particle size, 250 mm x 4.6 mm i.d.). The system was fitted with a Waters Model 440 absorbance detector with an extended wavelength module operated at a fixed wavelength of 214 nm. Chromatographic data were recorded and processed on a Perkin-Elmer Sigma 10 data system.

Throughout this study, the mobile phase consisted of 55% methanol and 45% aqueous ammonium phosphate buffer (0.05M). The pH of this mobile phase (seven unless otherwise specified) was adjusted by the addition of phosphoric acid and/or ammonium hydroxide. The flow rate of the mobile phase was set at a constant 2 ml/minute.

Procedure: Generally, 10 µl of each sample as a solution in methanol or water (1 mg/ml) were injected, although larger amounts were occasionally injected for compounds with low detector responses. The chromatographic capacity factor, k', of each compound was calculated by the formula:

$$k' = \frac{t - t_o}{t_o}$$

where t is the compound's retention time and t_o is the retention time of an unretained substance, determined by injection of an aqueous solution of sodium nitrite. Logarithms are all expressed in base ten.

RESULTS AND DISCUSSION

In accord with the goal of developing a simple, easily adaptable method, the system used in the study consisted entirely of commercial equipment and a standard aqueous mobile phase. While the choice of the specific column was arbitrary, the column type, octadecylsilane (C-18), was chosen on the basis of the good correlations between log P and log k' which have been obtained with related columns in previous studies (8,13-15).

While the use of a totally aqueous mobile phase would maximize partitioning between the column and mobile phase on the basis of hydrophobicity, such an approach also results in unacceptably long retention times for compounds with high log P values. To reduce this problem, an organic modifier was added to the mobile phase. Methanol was chosen since it has been shown to interfere the least with hydrophobic partition mechanisms in reverse-phase HPLC among common organic solvents (15-17). Under the conditions of this study, a mobile phase consisting of 55% methanol and 45% aqueous phosphate buffer allowed compounds with log P values as high as 3.5 to be eluted in 30 minutes or less.

The relationship between octanol-water partition coefficients and HPLC retention behavior in this system was established by the determination of k' values of 68 compounds of widely varying functionality and structure type (See Table 1). Figure 1 shows a plot of the log P values of these compounds (obtained from the literature) versus the logarithm of their k' values obtained under the conditions of this experiment. Considering the wide range of hydrophobicities and functional groups in these compounds, the degree of correlation between log P and log k' ($r = .966$) clearly indicates a linear relationship between these two parameters, which allows a simple estimation of log P values of compounds from their k' values.

The use of any HPLC system for the evaluation of octanol-water partition coefficients by correlation requires calibration

TABLE 1

Experimental Capacity Factors and
Literature Octanol-Water Partition Coefficients†

Compound	Log k'	Log P	Compound	Log k'	Log P
Benzyl alcohol	.151	1.16	Ethyl propionate	.421	1.50
Cinnamic alcohol	.529	1.95	Ethyl acetate	.092	0.34
p-Nitrobenzyl alcohol	.138	1.26	Phenyl acetate	.480	1.49
Allyl Alcohol	-.353	0.17	Methyl benzoate	.790	2.18
Benzonitrile	.361	1.56	Ethyl benzoate	1.07	2.64
Phenylacetonitrile	.323	1.56	Methyl salicylate	.980	2.46
p-Tolunitrile	.643	1.95*	Benzyl acetate	.750	1.96
Cinnamonitrile	.620	1.96	Acetanilide	.104	1.16
2,4-Dimethylphenol	.742	2.30	Pthalimide	.007	1.15
2,6-Dimethylphenol	.703	2.36	Formanilide	.060	1.12
1-Naphthol	.826	2.71	Benzamide	-.261	0.65
p-Cresol	.429	1.94	Thiobenzamide	.073	1.49
p-Cyanophenol	.040	1.63	N-Methylaniline	.477	1.66
Catechol	-.216	0.86	N-Propylaniline	1.06	2.45
p-Methoxyphenol	.036	1.37	p-Toluidine	.314	1.39
Thymol	1.28	3.30	Quinoline	.588	2.03
Benzene	.827	2.01	Indole	.554	2.25
n-Propylbenzene	1.78	3.62	2,6-Lutidine	.435	1.68
Toluene	1.16	2.74	2-Acetylpyridine	.066	0.85
Naphthalene	1.43	3.37	Aniline	.022	0.90
m-Dibromobenzene	1.70	3.75	o-Ansidine	.204	1.23
o-Dibromobenzene	1.54	3.64	2-Picoline	.266	1.20
Biphenyl	1.77	4.06	Acridine	1.19	3.39
Phenanthrene	2.02	4.46	Skatole	.865	2.60
Bromobenzene	1.22	2.99	Acetophenone	.446	1.66
Chlorobenzene	1.14	2.49	Benzophenone	1.22	3.18
p-Xylene	1.48	3.15	Propiophenone	.751	2.20
o-Xylene	1.42	2.77	2-Hexanone	.291	1.38
m-Xylene	1.48	3.20	p-Quinone	-.235	0.20
Anisole	.803	2.08	Anthraquinone	1.40	3.48*
Phenyl n-propyl ether	1.44	3.18	2-Bromoaceto-phenone	.632	2.43
Diphenyl ether	1.73	4.21	Chloroform	.563	1.94
Phenetole	1.06	2.51	Dichloromethane	.270	1.25
Nitropropane	.089	0.69	Trichloroethylene	1.05	2.29

†Values from Reference 1, unless otherwise specified.
*Calculated value, based on the method in Reference 22.

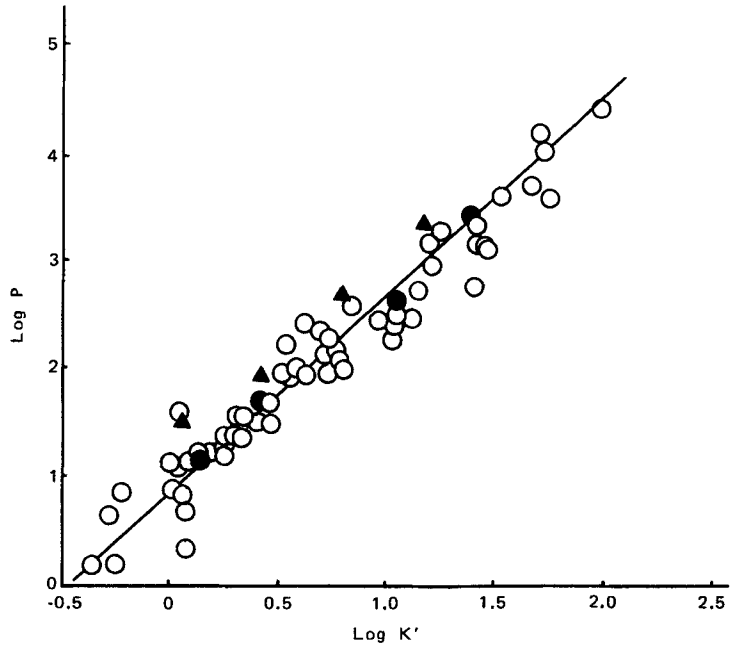

Figure 1. Log P vs Log k' for the 68 Compound Data Set.

● Non-phenolic Calibration Standards
▲ Phenolic Calibration Standards

of the system against standards with known log P values. While highest accuracy is ensured by the utilization of a large number of standards such as the 68 compounds described above, this is clearly impractical. For this reason, four compounds, benzyl alcohol, acetophenone, toluene, and naphthalene, were chosen as a "standard" calibration mixture for the evaluation of the log P's of unknowns using this method. The specific choice of these four compounds was based on four considerations:

1. They all have high UV detector responses.

2. The log P's span a range of 1.16 - 3.37 units, allowing for the calibration of the system over a wide range.
3. The compounds do not ionize over the usable pH range of the HPLC system (ca 2-8), and thus maintain their partitioning properties without regard to the pH of the mobile phase.
4. The partition coefficients of these compounds and their capacity factors are reasonably consistent with the correlation line established by the larger 68 compound data set (Figure 1).

The octanol-water partition coefficients of 25 compounds calculated from their k' values and the calibration curve established by the four "standard" compounds are listed in Table 2. With the exception of values determined for the phenols, all other log P values are in accord (± 0.2 log P units) with previously determined literature values.

Inaccuracies in log P values obtained by chromatographic correlation methods for phenolic compounds have been observed previously and have been attributed to a number of causes, including hydrogen bonding of such compounds to residual silanol sites on the reverse-phase column (9,12,18). While the extent of residual silanol sites in the column used in this study is unknown, the fact that such inaccuracies in the HPLC correlation method have previously occurred for phenolic compounds even with exhaustively silylated columns (9) is indicative of causes other

TABLE 2

Experimental and Literature Partition Coefficients

Compound	Log P_{exp}	Log P_{lit}	Difference
Cinnamic alcohol	1.78	1.95	-.17
p-Nitrobenzyl alcohol	1.13	1.26	-.13
Benzonitrile	1.50	1.56	-.06
Phenylacetonitrile	1.44	1.56	+.12
Cinnamonitrile	1.94	1.96	-.02
m-Xylene	3.37	3.20	+.17
m-Dibromobenzene	3.74	3.75	-.01
Phenanthrene	4.28	4.46	-.18
Anisole	2.24	2.08	+.16
Phenetole	2.68	2.51	+.17
Ethyl propionate	1.60	1.50	+.10
Methyl benzoate	2.22	2.18	+.04
Methyl salicylate	2.54	2.46	+.08
Acetanilide	1.16	1.07	+.09
Formanilide	1.00	1.12	-.12
N-methylaniline	1.70	1.66	+.04
p-Toluidine	1.42	1.39	+.03
Quinoline	1.88	2.03	-.15
2,6 Lutidine	1.63	1.68	-.05
Propiophenone	2.16	2.20	-.04
2-Hexanone	1.39	1.38	+.01
1-Naphthol	2.28	2.71	-.43
2,6-dimethylphenol	2.07	2.36	-.29
p-Cresol	1.62	1.94	-.32
Catechol	.53	.86	-.33

Log P_{exp} = Partition coefficient calculated from k' values and calibration curve established by benzyl alcohol, acetophenone, toluene, and naphthalene. Calibration equation: Log P = 1.67 Log k' + 0.90 (r = 0.997).

Log P_{lit} = Log P values from Reference 1.

than bonding to residual silanol sites. It is known that partitioning of a compound between water and octanol is governed not only by its hydrophobicity, but also by the degree to which the compound can hydrogen-bond to octanol itself (19). In their early studies, both Leo and Hansch (20,21) and Seiler (22) found that correlations between octanol-water and other solvent-water partition coefficients could be improved through separate consideration of these hydrogen-bonding effects. To a first approximation, an analogous treatment of the log P - log k' correlations in this study can be achieved by splitting the data into at least two subsets on the basis of the compounds' hydrogen-bonding character.

Table 3 lists the log P vs log k' linear regression parameters obtained for this HPLC system when the 68 tested compounds are considered altogether as well as split into one set containing phenolic compounds (strong hydrogen-bond donors) and one containing the rest. While the correlation coefficients of the split data sets are only slightly better than for the overall data set, the other regression parameters of the correlation lines for phenolic and non-phenolic compounds are significantly different from each other, suggesting different types of partitioning mechanisms for the two sets of compounds. Of particular significance is the large difference in the intercepts of the correlation lines (>0.4 log P units), which was also observed by Seiler (22) in correlations between the octanol-water

TABLE 3

Log P vs Log k' Linear Regression Parameters for the 68 Compound Data Set

Parameter	All Data	Phenols Only	All Except Phenols
Slope	1.65	1.53	1.69
Intercept	0.949	1.32	0.876
Correlation Coefficient	.966	.985	.973

and other solvent-water partition coefficients of strongly and weakly hydrogen-bonding compounds. Analogous to the results of those studies, the magnitude of the intercept of the log P - log k' correlation line appears to be directly related to the extent to which hydrogen bonding is involved in the partitioning of the compounds between octanol and water.

Demonstrated differences in partitioning mechanisms of strongly hydrogen-bonding and other compounds requires consideration of at least two sets of calibration standards in the use of the HPLC correlation system for the evaluation of log P values. The calibration standards used for low and non-hydrogen-bonding compounds were described earlier, and result in acceptably accurate values for these types of compounds. In accord with the requirements established for those standards, four phenols, p-methoxyphenol, p-cresol, 1-naphthol, and thymol, were chosen as standards for the evaluation of the log P's of strongly hydrogen-bonding compounds. Table 4 lists the

TABLE 4

Partition Coefficients Based on Nonhydrogen Bonding and Phenolic Calibration Curves

Compound	Log P_n	Log P_p	Log P_{lit}
2,4-Dimethylphenol	2.14	2.49	2.30
2,6-Dimethylphenol	2.67	2.43	2.36
p-Cyanophenol	.96	1.37	1.63
Catechol	.54	.97	.86
Salicylic acid	1.13	1.92	2.23 (2.00)*
p-Toluic acid	1.97	2.39	2.27 (2.26)*
Phenoxyacetic acid	.91	1.53	1.42
Benzoic acid	1.44	1.87	1.87 (1.78)*

Log P_n = Partition Coefficient based on calibration curve consisting of benzyl alcohol, acetophenone, toluene, and naphthalene.

Equation: Log P = 1.67 Log k' + 0.90 (r = 0.997).

Log P_p = Partition Coefficient based on calibration curve consisting of p-methoxyphenol, p-cresol, 1-napthol, and thymol.

Equation: Log P = 1.59 Log k' + 1.31 (r = 0.997).

Log P_{lit} = Partition Coefficient from Reference 1.

*Data from Reference 12.

calculated log P's of some strongly hydrogen-bonding compounds (phenols and carboxylic acids) based on each of the two sets of calibration standards. Ionization of the acids in the operating pH range of the HPLC system (ca pH 2-8) required the evaluation of the apparent partition coefficients of these

compounds (D) at three or four pH's, and then extrapolation of these values to the value (P) at zero ionization, in accord with the equation described by Unger et al (12):

$$D = P + K_a (-D/H)$$

Log P values obtained for both the phenols and the carboxylic acids based on the phenolic standards are clearly more in accord with the literature values than those based on the non-hydrogen-bonding standards, and are comparable to those obtained with a much more complicated correlation system utilizing an octanol-saturated column (12). The choice of the proper calibration system based upon the structure of the compound whose partition coefficient is to be evaluated is essential for highest accuracy of values obtained by this method.

CONCLUSIONS

In this study, a simple, rapidly adaptable HPLC method for the evaluation of octanol-water partition coefficients is described, and demonstrated to give values which are in accord with literature values for a wide variety of compounds. Indeed, the overall accuracy of the method may in fact be better than the data indicates, since the degree of accuracy of a number of literature values is unknown.

Since the method requires consideration of the degree of hydrogen-bonding character in the compound being evaluated, some prior knowledge of the structure of the compound is required for

highest accuracy. Another limitation of the method, of course, is the possibility of inaccuracy of a value obtained for a particular compound due to wide deviations from the overall correlations established for the general case. While splitting of compounds into further subsets and utilizing different mobile phases could increase the accuracy of the method, the data set is not large enough to draw any conclusions concerning this, and more importantly, further modification of the method may increase its complexity to an unreasonable level. In any event, the method described here is sufficiently accurate for the evaluations of the partition coefficients of compounds for correlations with their biological activity, and for evaluation of the relative solubilities of compounds in aqueous and organic media.

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