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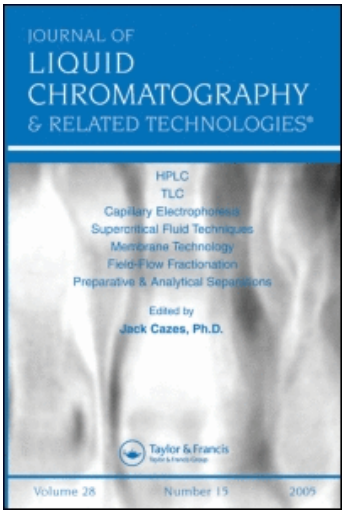

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General Approach for the Estimation of Octanol/Water Partition Coefficient by Reversed-Phase High-Performance Liquid Chromatography

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GENERAL APPROACH FOR THE ESTIMATION OF OCTANOL/WATER PARTITION COEFFICIENT BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

General approach for the relation of reversed-phase high-performance liquid chromatographic retention behaviour to the hydrophobic properties of drugs was developed. Hydrophobicity of compounds was characterized by the logarithm of 1-octanol/water partition coefficient (log $P_{o/w}$). Reversed-phase retention times of 26 model compounds (log $P_{o/w}$ values of them ranged from -1.22 to 3.84) were measured using 3 to 5 different mixture of acetonitrile and water as eluent and the logarithm of the capacity ratios (log k') was calculated.

The linear relationship between the log k' values and the percentage of the acetonitrile in the eluent was tested for each molecule. The slope and the intercept (log k'_o) of these straight lines were

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calculated and introduced to the following regression equation:

$$\log P_{o/w} = a \text{ 'slope'} + b \log k'_o + c$$

a, b and c were constants obtained by least squares method. Correlation coefficient of the equation was 0.95. The quotient of the values of a and b gave the percentage of the acetonitrile in the eluent (48%) by which the chromatographic partition system became the best model of the 1-octanol/water partition system. The proposed method provides the possibility of estimating $\log P_{o/w}$ values for compounds which can not be eluted by one isocratic eluent composition.

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INTRODUCTION

Hydrophobic properties of new biologically active agents characterized by the logarithm of 1-octanol/water partition coefficients ($\log P_{o/w}$) are widely used in quantitative structure-activity relationship (QSAR) investigations [1,2]. The traditional shake-flask method for determining $\log P_{o/w}$ has many disadvantages, such as the relatively large amount of material required, changes in partition process due to impurities of solute, the tedious and time consuming measurements of the concentration of compounds in both phases. Therefore, this method is often replaced by different chromatographic procedures, for example by thin-

layer chromatography (TLC) [3,4], reversed-phase high-performance liquid chromatography (RP-HPLC) [5,6,7,8,9,10], and by gas-liquid chromatography (GLC) [11,12].

All attempts to use RP-HPLC for determining $\log P_{o/w}$ are based on eq. 1.

$$\log k' = \log P + \log V_s/V_m \qquad (1)$$

where k' is the capacity ratio calculated from the retention time (t_R) and dead time (t_o) according to $(t_R - t_o)/t_o$; P is the partition coefficient of the compound in the given chromatographic partition system; V_s/V_m is the ratio of the mobile and the stationary phase volumes.

If a Collander [13] type relationship (eq. 2) holds between the log P values measured in two different partition systems, eq. 3 can be set up:

$$\log P_{2/w} = a_1 \log P_{3/w} + b_1 \qquad (2)$$

$$\log P_{o/w} = a_2 \log k' + b_2 \qquad (3)$$

Subscript w indicates water and subscript 2 and 3 refer to two different non-aqueous solvents in the partition system. a_1, b_1, a_2 and b_2 are constants obtained by the least squares method. Leo [14] showed the limitations of the validity of eq. 2, namely, that the plot $\log P_{2/w}$ vs. $\log P_{3/w}$ is linear only if solvents 2 and 3 similar in character, or if the compounds examined are structurally related.

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The validity of eq. 3 was proved only when one of the above requirements is met. In this point of view papers dealing with this subject can be devided into two groups. In the first approach structurally closely related compounds were investigated in a reversed-phase chromatographic system using methanol, acetone and acetonitrile as organic modifier in the eluent. For example McCall [5], Carlson et al. [7], Guerra et al. [8] have already published good correlations between log $P_{o/w}$ and log k' for phenols, anilines, benzene and nitroimidazole derivatives. In these cases the similarity of the chromatographic partition system to the 1-octanol/water partition system is not necessary for obtaining good correlation.

In the second approach the chromatographic partition system is the same as 1-octanol/water partition system, namely, 1-octanol coated column and 1-octanol saturated water as eluent were used for log k' determination. In such cases good correlation between log $P_{o/w}$ and log k' values for structurally different compounds was published by Unger et al. [8] Mirrlees et al. [9]. This latter approach seems to be more general from the theoretical point of view, although it has practical disadvantages. For example, chromatographic conditions cannot be optimized for a

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wide series of compounds and the reproduction of the same 1-octanol-coated column seems to be difficult.

In this work the chance of the good correlation between RP-HPLC retention data and the log $P_{o/w}$ values of structurally heterogeneous compound set using conventional RP-18 column and acetonitrile - water mixture as eluent was investigated. The hydrophobicity range of the model compounds was wide (log $P_{o/w}$ values ranged from -1.22 to 3.87). As they cannot be eluted using the same eluent composition microcomputer analysis of the HPLC retention data of drugs as a function of the eluent composition has been also carried out.

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THEORY

Horváth et al. [15] described the retention processes of an unionized solute on an apolar stationary phase adapting the solvophobic theory. According to their model the interaction between the solute and the stationary phase can be regarded as a reversible association of the solute molecules (S) with the hydrophobic ligands (L) at the surface. The strength of the interaction between S and L is also influenced by the property of the eluent which forces the solute to associate.

The experimentally found linear relationship between the log k' and the percentage of the organic phase in the eluent (OP%) can be described by eq. 4.

$$\log k' = a_3 \, OP\% + b_3 \qquad (4)$$

where b_3 can be considered as $\log k'_0$, the logarithm of the capacity ratio at zero percentage of the organic phase in the eluent. The physical meaning of a_3 (the 'slope' of the straight line) is the change of log k' caused by 1% addition of the organic phase to the eluent. The plot of eq. 4 is linear only in a given range of OP% values [16], therefore this relationship should be experimentally tested.

Eq. 4 provides the possibility of calculating the log k' values belonging to optional percentage of the organic phase in the eluent. In this way by the help of eq. 4 the applicability of eq. 5 can be extended to those cases when log k' values of the compounds cannot be measured at the same chromatographic circumstances. Combining eq. 3 and eq. 4 together one obtains eq. 5.

$$\log P_{o/w} = a_2 \text{ 'slope' } OP\% + a_2 \log k'_0 + b_2 \qquad (5)$$

Testing the validity of eq. 5 OP% values should be considered constant for all of the investigated compounds. One cannot know in advance that value of OP%. In order to estimate the best eluent composition

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by which 1-octanol/water partition system can be chromatographically modelled eq. 5 was set up as a regression equation with two independent variable (eq. 6).

$$\log P_{o/w} = a_4 \text{ 'slope'} + b_4 \log k'_o + c_4 \quad (6)$$

The regression coefficients a_4, b_4 and c_4 can be calculated using the least squares method. As $a_4=a_2$OP% and $b_4=a_2$, the quotient of the a_4 and b_4 will show the optimum eluent composition by which the best correlation can be found between log $P_{o/w}$ and RP-HPLC retention data.

In the case of closely related compounds the 'slope' values can be considered constant [17], which means that the straight lines described by eq. 4 are parallel, thus log k' values measured at any concentration of the organic phase will be directly proportional to log $P_{o/w}$ values of compounds. We should be careful, however, when using log k' values measured at a certain OP% value without knowing the 'slope' values of the compounds, because if the 'slope' values are not the same for all compounds tested or does not show high correlation to log k'_o values different ranks of log k' values can be obtained at different OP% values. In this case one can not know the chromatographic conditions at which the rank of log k'

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values is the same as the rank of log $P_{o/w}$ values of the compounds. Therefore eq. 6 can be suggested to examine relationship of the log $P_{o/w}$ and retention data for different types of compounds.

Correlation coefficient and the standard error of the estimate of eq. 6 will show the theoretical limit of the generalization of log $P_{o/w}$ estimation using HPLC retention data measured on RP-18 column and acetonitrile-water mixture as eluent. One can obtain the optimum composition of methanol-water, acetone-water and any other mixture as well using the measured 'slope' and log k'_o data obtained by the respective mixture and calculating a_4 and b_4 coefficients in eq. 6.

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MATERIALS AND METHODS

26 model compounds were tested. The compounds were selected according to the following criteria:

1. Log $P_{o/w}$ values of the compound is available in the literature.
2. The compound is in a neutral (unionized) form between pH 2 and pH 7. This is necessary in order to avoid ion pairs during chromatographic measurements because ion pair formation can influence the partition processes of the compounds. On

the other hand, log $P_{o/w}$ values in general refer to the neutral molecules.

3. The compounds possess a wide range of log $P_{o/w}$ values.

The selected compounds and their log $P_{o/w}$ values are shown in Table 1. Compounds were obtained from the Semmelweis University Medical School. Most of the compounds are listed in the 6th Edition of Hungarian Pharmacopea and they comply with its requirements regarding purity.

Log $P_{o/w}$ values were available from Hansch and Leo's compilation [18]. If one compound had more than one different log $P_{o/w}$ value, that value measured by Hansch or Fujita and their co-workers was used in the correlation analysis. In certain cases great discrepancies could be observed among the log $P_{o/w}$ values determined in different laboratories, as be seen in Fig. 1. This circumstance may increase the error of our results.

Log k' values of the model compounds were measured by using 3 to 5 different percentages of acetonitrile in the eluent. The experimental conditions of the measurements are summarized in Table 2.

The percentage of the acetonitrile in the eluent (OP%) was increased by 5% steps from 5% to

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TABLE 1.

Compounds Investigated and their log $P_{o/w}$ Values [18].

Compound	log $P_{o/w}$
Resorcin	0.80
Sulphadimidine	0.32
Sulphamethoxypyridazine	0.40
Barbital	0.65
Phenobarbital	1.42
Chloramphenicol	1.14
Salicylamide	1.28
Phenacetin	1.58
Vanillin	1.37
Benzaldehyde	1.45
Acetanilide	1.16
Nicotinamide	-0.57
Benzoic acid	1.87
Salicylic acid	2.25
Acetylsalicylic acid	1.23
Coffein	-0.07
Hydrochlorothiazide	-0.07
Cortexolone	2.46
Dexamethasone	1.99
Desoxycortone	2.88
Sulphaguanidine	-1.22
Isoniazide	-1.14
Methylsalicylate	2.46
Hydrocortisone	1.61
Progesterone	3.87
Testosterone	3.31

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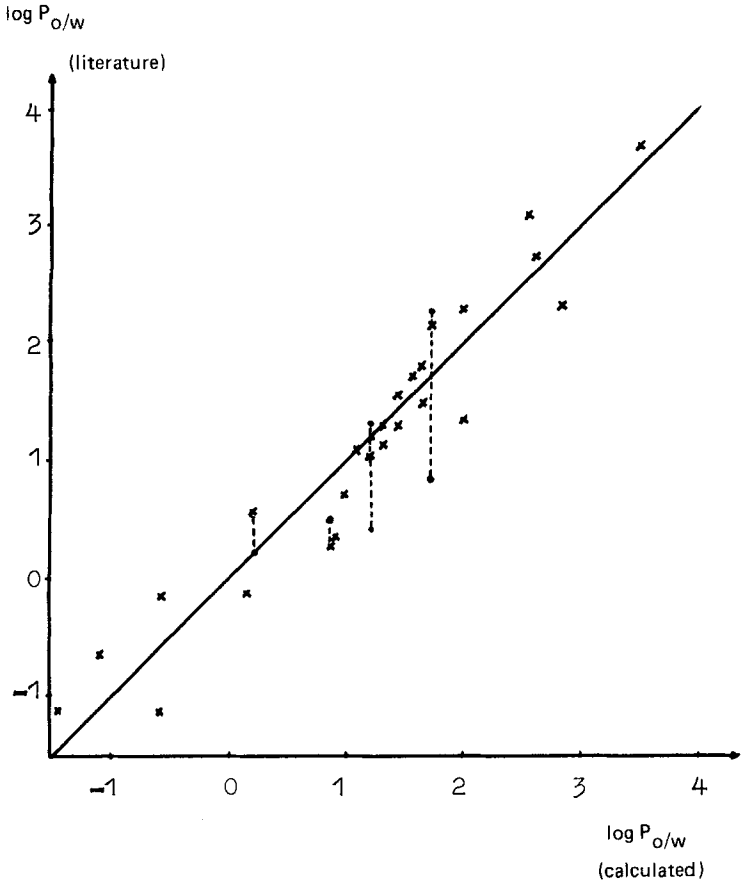

FIGURE 1.

Plot of the Calculated and the Literature log $P_{o/w}$ Values According to eq. 7.

The sign x means the values used in the calculation and the sign o----o shows the value of log $P_{o/w}$ from different laboratories.

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TABLE 2.

Experimental Conditions of the HPLC Measurements

Column:* RP-18 LiChrosorb, 250 mm, ID 4.6, dp 10 μ

Injector:* Rheodyne Model 7010 Sample Injection Valve

Detector:* ISCO Model 226 Absorbance Monitor

Detection: 254 nm

Pump: Labormim Liquopump Model 312

Integrator: Chinoin Digint Model 24

Recorder: Endim Model 621.01

Temperature: 22 °C ± 2 °C

Pressure: 30-50 bar

Eluent: 5 to 90 % acetonitrile (Reanal, purified for HPLC) and 0.05 M KH_2PO_4 buffer (pH=4.6)**

Dead time determination: $NaNO_3$ (Reanal)

Calculations:*** Apple II+ microcomputer

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* Instruments were kindly provided by Chromatronix Inc.

** For the measurements of acidic compounds 1-2 drops of 85% H_3PO_4 were added to the eluent (pH=2) in order to get symmetrical peaks.

*** The computer was kindly provided by Dime's Group Inc.

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90%. The pH was 4.6 inorder to avoid the ionization of solutes. This pH value was selected on the basis of pK_a values for weak basic, neutral and weak acidic model compounds. The pH change of the eluent from 4.6 to 2 in case of acidic compounds (salicylic acid, acetyl salicylic acid, benzoic acid) was necessary considering their pK_a values. The peak symmetry and the shortest retention time indicated that the compounds were in the neutral form during the chromatographic procedure and the speed of the partition process was much higher than the rate of elution. Dead time determination using $NaNO_3$ (Reanal) was carried out at each measurement of the t_R value. An appropriate amount of $NaNO_3$ was added to each injected solution and log k' values were calculated from the respective dead time and the retention time values. The linearity of OP% vs. log k' plot was tested by the least squares method for each compound.

All calculations including the processing of the measured retention data and least squares analysis were carried out with an Apple II+ microcomputer using a program written by us in BASIC language. Log k' values of all compounds measured at different percentages of acetonitrile are stored in data files, which can be continuously enlarged and easily used in correlation analysis.

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RESULTS AND DISCUSSION

Table 3. shows the most important parameters of the log k' - OP% relationships: the 'slope' values, the log k'_o values and the correlation coefficients for each of the model compounds. Log k' values of each compound were measured by using that percentage of acetonitrile by which t_R value fell between $2t_o$ and $3t_o$ covering at least 15% range of the organic phase concentration.

Data points belonging to very low retention times (log k'<-0.60) were omitted from the calculations. These data points usually decreased significantly the value of the correlation coefficients.

Substituting the 'slope' and the log k'_o values thus obtained into eq. 6 the following parameters were calculated:

$$\log P_{o/w} = 90.23 \text{ 'slope'} + 1.854 \log k'_o + 1.911 \quad (7)$$

$$n=26 \quad R=0.949 \quad s=0.413 \quad F=104.7 \quad F_{(2,24\ p\ 0.95)}=3.4$$

where n is the number of compounds, R is the multiple correlation coefficient, s is the residual error, and F is the Fischer-test value. The relationship described by eq. 7 is significant according to the R and F values. Comparing the calculated and the known log $P_{o/w}$ values of each compound none of them can be considered a significant outlier. Although the resi-

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TABLE 3.

The Measured Coefficients of eq. 4

Compound	slope	log k'_o	cor.coef.
Resorcin	-0.0150	0.259	0.990
Sulphadimidine	-0.0280	0.854	0.997
Sulphamethoxypyridazine	-0.0285	0.892	0.990
Barbital	-0.0402	1.063	0.981
Phenobarbital	-0.0319	1.341	0.999
Chloramphenicol	-0.0414	1.625	0.997
Salicylamide	-0.0255	0.871	0.984
Phenacetin	-0.0226	1.002	0.981
Vanillin	-0.0244	0.866	0.999
Benzaldehyde	-0.0303	1.575	0.999
Acetanilide	-0.0270	1.021	0.991
Nicotinamide	-0.0382	0.251	0.941
Benzoic acid	-0.0284	1.252	0.987
Salicylic acid	-0.0301	1.425	0.988
Acetylsalicylic acid	-0.0272	1.077	0.974
Coffein	-0.0299	0.552	0.979
Hydrochlorothiazide	-0.0456	0.887	0.912
Cortexolone	-0.0138	0.757	0.993
Dexamethasone	-0.0139	0.568	0.997
Desoxycortone	-0.0147	1.120	0.990
Sulphaguanidine	-0.0272	0.011	0.932
Isoniazide	-0.0382	0.060	0.947
Methylsalicylate	-0.0244	1.727	0.997
Hydrocortisone	-0.0129	0.436	0.991
Progesterone	-0.0192	1.831	0.995
Testosterone	-0.0143	1.085	0.998

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dual error is ten times as high as the usual error of log $P_{o/w}$ determination by the traditional shake-flask method, it is not too high in view of the deviation of the log $P_{o/w}$ values obtained from different laboratories (Fig. 1).

The intercorrelation of the two independent variables in eq. 7 is very low, therefore both variables are significant in the equation. The correlation matrix of variables is given in Table 4.

The quotient of the regression coefficients of the 'slope' and the log k'_o values gives 48.6 . It means, that the same relationship between log $P_{o/w}$ and log k' values according to eq. 3 could have been obtained if log k' values belonging to 48% acetonitrile-water mixture as eluent were used. It also means that the chromatographic partition system containing 48% acetonitrile and 52% water mixture and RP-18 column shows the greatest similarity to the 1-octanol-water partition system.

In those cases when the hydrophobicity range of the investigated compounds is wide and one cannot measure log k' values for each compound using the proposed mixture as eluent eq. 6 is suggested for estimating log $P_{o/w}$ values. When the 'slope' values are nearly the same for all of the investigated

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TABLE 4.

Correlation Matrix of Variables Used in eq. 7.

	log $P_{o/w}$	log k'_o	'slope'
log $P_{o/w}$	1.000	0.683	0.616
log k'_o	0.683	1.000	-0.062
'slope'	0.616	-0.062	1.000

compounds, or the 'slope' and the log k'_o shows intercorrelation, then the log $P_{o/w}$ and log k' values measured at any eluent mixture will show significant relationship.

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