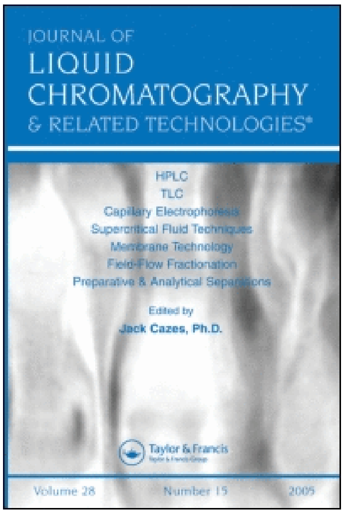

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# Liquid Chromatographic Estimation of Octanol/Water Partition Coefficients with a High Efficiency, Nonporous, Ultrasmall Particle Size Reverse Phase Stationary Phase

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# LIQUID CHROMATOGRAPHIC ESTIMATION OF OCTANOL/WATER PARTITION COEFFICIENTS WITH A HIGH EFFICIENCY, NONPOROUS, ULTRASMALL PARTICLE SIZE REVERSE PHASE STATIONARY PHASE

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## ABSTRACT

HPLC retention data, obtained over a range of organic modifier fractions (ϕ) in binary mobile phases, can be used to estimate a compounds octanol/water partition coefficient (log $P_{o/w}$). Such a determination is facilitated by a high efficiency, low capacity column. The utility of a nonporous, ultrasmall particles size (1.5 µm), $C_{18}$ stationary phase capable of such performance was assessed using aqueous mobile phases containing either acetonitrile or methanol. In general, compounds were effectively eluted from this stationary phase at lower values of ϕ than with more typical stationary phases, thus increasing the accuracy and speed with which log $K_w$, the capacity factor in a 100% aqueous mobile phase, could be determined. Values of log $K_w$ obtained for seventeen model compounds were highly correlated with the compound's log $P_{o/w}$. Comparison of retention trends in the methanol and acetonitrile mobile phases suggests differences in the retention mechanisms for both organic modifiers.

## INTRODUCTION

The dependance of analyte retention on the organic modifier level in binary mobile phases in reverse phase HPLC is of interest for several reasons.[1] While such information has chromatographic significance (e.g., by providing insight into retention mechanisms and allowing for the optimization of chromatographic separations), an important use of this information is in the estimation of octanol-water partition coefficients (log $P_{o/w}$). The use of log $P_{o/w}$ as an indicator of a compounds lipophilic nature is well established and has become a standard method for modeling biological, environmental and physicochemical processes.[1-8] While log $P_{o/w}$ may be determined by calculation with traditional shake flask experiments and via chromatographic retention data, the use of HPLC retention data offers the benefits of accuracy, consistency, specificity, relative ease of determination and a broader dynamic range.

In the HPLC approach, a compound's log $P_{o/w}$ is related to its capacity factor obtained with a 100% aqueous mobile phase ($K_w$) via a Collander-type expression:

$$\log P_{o/w} = a(\log K_w) + b \qquad (1)$$

While direct measurement of $K_w$ is desirable, it is not practically possible for many, even modestly, lipophilic compounds using conventional $C_{18}$ or $C_8$ stationary phases and column configurations due to strong retention. In such instances, log $K_w$ is obtained by extrapolation of plots of capacity factor (k') versus volume fraction ($\phi$) of organic solvent in a binary water-organic mobile to $\phi = 0$:

$$\log k' = \log K_w + S\phi \qquad (2)$$

While equation 2 usually provides a reasonable fit to experimental retention data over a limited range in $\phi$, both concave and convex deviations in plots of log k' versus $\phi$ have been observed, especially for lipophilic compounds which require high $\phi$ mobile phases for their effective elution.[1,5,9] Curvature of the log k' versus $\phi$ plots results in an inaccurate determination of log $K_w$ which in turn is manifested in poor log $P_{o/w}$ versus log $K_w$ correlations.

It is anticipated that the accuracy of such correlations would, thus, be enhanced by using a reverse phase column which offers high efficiency coupled with reduced chromatographic capacity. In such a situation, high efficiency separations, effected with lower $\phi$ mobile phases, could be used to facilitate the

## Table 1

### Marker Compounds Used and their Partition Coefficients

| Marker Compound | Abbreviation | log $P_{0/w}$ |
|---|---|---|
| Acetanilide | AD | 1.46 |
| Dimethyl phthalate | DMP | 1.95 |
| Acetophenone | AP | 1.63 |
| 2-phenyl-2-propanol | PP | 1.90 |
| Methyl paraben (*) | MTPB | 1.96 |
| p-toluic acid | MBH | 2.34 |
| Diethyl phthalate | DEP | 2.95 |
| Ethyl paraben (*) | ETPB | 2.47 |
| 4-ethyl benzoic acid | EBH | 2.97 |
| Propyl paraben (*) | PRPB | 3.04 |
| Butyl paraben (*) | BUPB | 3.57 |
| 4-tert-butyl benzoic acid | BBH | 3.78 |
| Dipropyl phthalate | DPP | 3.84 |
| Antracene | ANTH | 4.50 |
| Dibutyl phthalate | DBP | 4.72 |
| Hexachlorobenzene | PCB | 5.44 |
| Dioctyl phthalate | DOP | 7.88 |

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(*) p-hydroxy benzoic acid, n-ester. In general the partition coefficients were obtained from the LOGKOW© data (Sangster Laboratories, first edition).

direct measurement of log $K_w$ or, at least, allow for more accurate log $K_w$ extrapolations. High efficiencies are necessary to ensure that an adequate interaction is achieved between the analyte and the stationary phase. In addition to the potentially increased log $P_{o/w}$ model accuracy, the retention data may be obtained more quickly with the high efficiency, low capacity LC column configuration.

Recently, a high efficiency, low capacity "fast" LC column based on nonporous, 1.5 μm $C_{18}$ bonded silica microspheres has become commercially available. Such a stationary phase, available in a 33 by 4.6 mm column geometry, has been documented to produce high efficiency separations of low molecular weight analytes at much lower values of $\phi$ and in much shorter total analysis times than can be obtained with more conventional $C_{18}$ stationary phases and column configurations.[10] In the study reported herein, the retention properties of seventeen marker compounds were determined over a wide range of $\phi$ values in aqueous mobile phases containing either acetonitrile or methanol. The nature of the log k' versus $\phi$ relationship for this column type was established and the applicability of the data for the determination of log $K_w$ and log $P_{o/w}$ was assessed.

## EXPERIMENTAL

### Materials

The marker compounds used and their log $P_{o/w}$ values, obtained from a computerized database,[11] are identified in Table 1. These compounds were chosen based on their high detectability (thereby allowing for good peak shapes due to small injection masses), their wide range in partition coefficients, and, for several analytes, their acid functionality. Individual stock solutions of each analyte were prepared by dissolving reagent grade reference materials in an appropriate strength binary solvent. Single analyte working standards were prepared at a concentration of ≈ 3 mg/L by diluting the stocks in 0.03 M phosphoric acid. Reagents used to prepare the mobile phases and other analytical solutions were reagent, or analytical grade, as appropriate. Water was obtained from a Barnstead NANOpureII water polishing system.

### Analytical System

The column used was obtained from Micra Scientific (Northbrook, IL) and consisted of NPS RP-18, 1.5 μm stationary phase in a 33 by 4.6 mm stainless steel column. Binary methanol/0.03 M phosphoric acid or acetonitrile/0.03 M phosphoric acid mobile phases were used. The addition of acid was necessary to ensure that the acidic analytes were eluted in their protonated, uncharged form.

The chromatographic system consisted of a Kratos Spectroflow 400 pump, a Micrometics 728 autosampler (coupled with an electronically actuated Rheodyne 7010 valve), a Kratos Spectroflow 757 UV detector, a strip chart recorder and a Hewlett Packard HP 3357 LAS computer data collection system. A minimum length of 0.007" i.d. PEEK tubing was used to connect the injector and column, while the column and detector was connected directly using an Alltech Direct-Connect™ low dead volume connector. Minimizing extra-column system void volume is essential to maintaining the columns efficiency.

## Table 2A

## Capacity Factor Data for the Various Model Analytes

| Analyte | Elution Behavior |  |  |  |
|---|---|---|---|---|
|  | In Methanol |  | In Acetonitrile |  |
|  | % Organic | log (k′) | % Organic | log (k′) |
| AD | 0 | 0.757 | 0 | 0.707 |
|  | 5 | 0.403 | 5 | 0.061 |
|  | 10 | 0.167 | 10 | -0.234 |
|  | 15 | -0.019 | ---- | ---- |
| DMP | 0 | 1.828 | 0 | 1.801 |
|  | 5 | 1.296 | 5 | 0.907 |
|  | 10 | 0.969 | 10 | 0.489 |
|  | 15 | 0.658 | 15 | -0.35 |
|  | 20 | 0.451 | ---- | ---- |
| AP | 0 | 1.191 | 0 | 1.160 |
|  | 5 | 0.801 | 5 | 0.456 |
|  | 10 | 0.557 | 10 | 0.152 |
|  | 15 | 0356 | 15 | -0.025 |
| MTPB | 0 | 1.060 | 0 | 1.041 |
|  | 5 | 0.711 | 5 | 0.398 |
|  | 10 | 0.464 | 10 | 0.061 |
|  | 15 | 0.246 | 15 | -0.160 |
| PP | 0 | 1.142 | 0 | 1.096 |
|  | 5 | 0.821 | 5 | 0.533 |
|  | 10 | 0.613 | 10 | 0.212 |
|  | 15 | 0.411 | 15 | -0.006 |
|  | 20 | 0.279 | 20 | -0.122 |
| MBH | 0 | 1.297 | 0 | 1.258 |
|  | 5 | 1.105 | 5 | 0.759 |
|  | 10 | 0.819 | 10 | 0.428 |
|  | 15 | 0.620 | 15 | 0.165 |
|  | 20 | 0.483 | 20 | 0.017 |
| DEP | 5 | 2.167 | 5 | 1.720 |
|  | 10 | 1.785 | 10 | 1.230 |
|  | 15 | 1.423 | 15 | 0.831 |
|  | 20 | 1.153 | 20 | 0.529 |
|  | 25 | 0.867 | 25 | 0.188 |
|  | 30 | 0.607 | ---- | ---- |
|  | 35 | 0.330 | ---- | ---- |

(continued)

## Table 2A (continued)

| Analyte | Elution Behavior | | | |
|---|---|---|---|---|
| | In Methanol | | In Acetonitrile | |
| | % Organic | log (k′) | % Organic | log (k′) |
| ETPB | 0 | 1.574 | 0 | 1.761 |
| | *5* | *1.170* | *5* | *0.880* |
| | *10* | *0.919* | *10* | *0.493* |
| | *15* | *0.668* | *15* | *0.196* |
| | *20* | *0.499* | 20 | -0.007 |

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Note: Data in *italics* were used to generate the log $K_w$ calculation plots.

## Table 2B

### Capacity Factor Data for the Various Model Analytes

| Analyte | Elution Behavior | | | |
|---|---|---|---|---|
| | In Methanol | | In Acetonitrile | |
| | % Organic | log (k′) | % Organic | log (k′) |
| EBH | 0 | 1.807 | 1.760 | |
| | *5* | *1.508* | *5* | *1.248* |
| | *10* | *1.284* | *10* | *0.898* |
| | *15* | *1.056* | *15* | *0.524* |
| | *20* | *0.896* | *20* | *0.288* |
| | *25* | *0.701* | ---- | ---- |
| | *30* | *0.496* | ---- | ---- |
| PRPB | ---- | ---- | 0 | 2.081 |
| | *5* | *1.721* | *5* | *1.408* |
| | *10* | *1.424* | *10* | *0.993* |
| | *15* | *1.156* | *15* | *0.588* |
| | *20* | *01946* | *20* | *0.288* |
| | *25* | *0.716* | ---- | ---- |
| | *30* | *0.495* | ---- | ---- |
| BUPB | *5* | *2.225* | *5* | *1.921* |
| | *10* | *1.937* | *10* | *1.479* |
| | *15* | *1.641* | *15* | *1.033* |
| | *20* | *1.419* | *20* | *0.734* |

(continued)

**Table 2B (continued)**

| **Analyte** | **Elution Behavior** | | | |
|---|---|---|---|---|
| | **In Methanol** | | **In Acetonitrile** | |
| | **% Organic** | **log (k′)** | **% Organic** | **log (k′)** |
| BUPB | *25* | *1.150* | *25* | *0.294* |
| | *30* | *0.893* | ---- | ---- |
| | *35* | *0.635* | ---- | ---- |
| BBH | ---- | ---- | *5* | *2.025* |
| | *10* | *2.045* | *10* | *1.619* |
| | *15* | *1.775* | *15* | *1.188* |
| | *20* | *1.573* | *20* | *0.880* |
| | *25* | *1.325* | *25* | *0.410* |
| | *30* | *0.904* | *30* | *0.139* |
| | *35* | *0.806* | ---- | ---- |
| | *40* | *0.635* | ---- | ---- |
| DPP | ---- | ---- | *10* | *2.104* |
| | ---- | ---- | *15* | *1.613* |
| | *20* | *1.970* | *20* | *1.283* |
| | *25* | *1.628* | *25* | *0.844* |
| | *30* | *1.321* | *30* | *0.536* |
| | *35* | *0.994* | *35* | *0.260* |
| | *40* | *0.707* | ---- | ---- |

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Note: Data in ***italics*** were used to generate the log $K_w$ calculation plots.

**Table 2C**

**Capacity Factor Data for the Various Model Analytes**

| **Analyte** | **Elution Behavior** | | | |
|---|---|---|---|---|
| | **In Methanol** | | **In Acetonitrile** | |
| | **% Organic** | **log (k′)** | **% Organic** | **log (k′)** |
| ANTH | ---- | ---- | *15* | *2.238* |
| | ---- | ---- | *20* | *1.928* |
| | *25* | *2.310* | *25* | *1.140* |
| | *30* | *2.043* | *30* | *0.814* |
| | *35* | *1.764* | *35* | *0.446* |
| | *40* | *1.509* | *40* | *0.284* |

(continued)

**Table 2C (continued)**

| Analyte | Elution Behavior | | | |
|---|---|---|---|---|
| | In Methanol | | In Acetonitrile | |
| | % Organic | log (k′) | % Organic | log (k′) |
| | *45* | *1.218* | ---- | ---- |
| | *50* | *0.944* | ---- | ---- |
| | *55* | *0.727* | ---- | ---- |
| DBP | ---- | ---- | *20* | *2.029* |
| | ---- | ---- | *25* | *1.528* |
| | *30* | *2.104* | *30* | *1.140* |
| | *35* | *1.703* | *35* | *0.796* |
| | *40* | *1.369* | *40* | *0.513* |
| | *45* | *1.024* | *45* | *0.220* |
| | *50* | *0.702* | ---- | ---- |
| | *55* | *0.453* | ---- | ---- |
| PCB | ---- | ---- | *25* | *2.061* |
| | ---- | ---- | *30* | *1.685* |
| | ---- | ---- | *35* | *1.344* |
| | *40* | *2.124* | *40* | *1.049* |
| | *45* | *1.838* | *45* | *0.705* |
| | *50* | *1.552* | *50* | *0.472* |
| | *55* | *1.337* | ---- | ---- |
| | *60* | *1.066* | ---- | ---- |
| | 65 | 0.739 | ---- | ---- |
| DOP | ---- | ---- | *40* | *2.234* |
| | ---- | ---- | *45* | *1.763* |
| | *55* | *2.349* | ---- | ---- |
| | *60* | *1.887* | ---- | ---- |
| | *65* | *1.337* | ---- | ---- |

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Note: Data in ***italics*** were used to generate the log $K_w$ calculation plots.

## Procedure

Each model solute was injected in replicate in each appropriate mobile phase. The mobile phases were used and the model compounds injected in random order. While specific model compounds were injected into all mobile phases in which they possessed reasonable retention, the mobile phases used for each compound were limited at low $\phi$ by reasonable elution times (k' values less

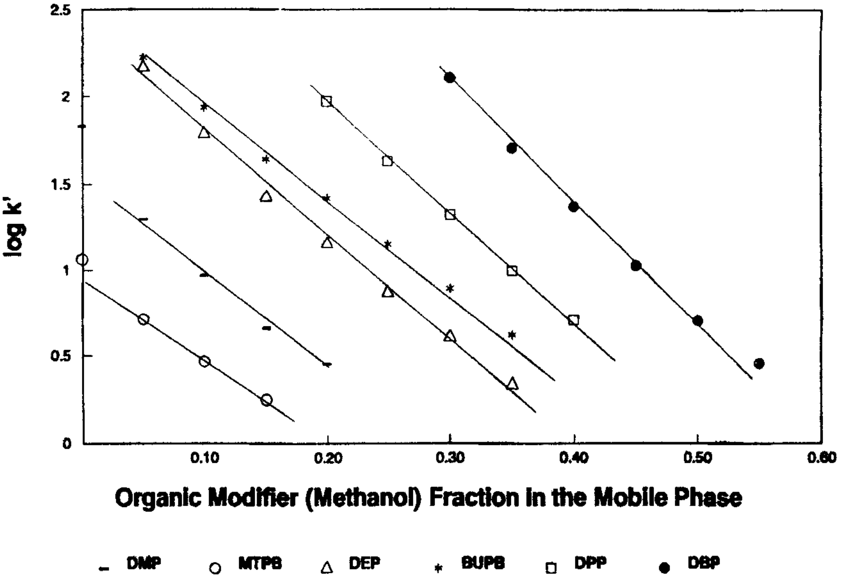

**Figure 1.** Plots of log k' versus organic modifier fraction (ϕ) for several model compounds using methanol as the organic modifier. In general the plots are quite linear and are roughly co-linear.

than 200) and at high ϕ by the need to obtain an effective column/compound interaction (k' values greater than 1). Mobile phase flow rate was 1 mL/min, sample injection size was 10 μL and the detection wavelength was 215 nm.

## RESULTS AND DISCUSSION

The capacity factor versus mobile phase composition data obtained is summarized in Tables 2A through 2C. For nine of the models compounds, log $K_w$ could be measured directly. For the other model compounds, the lowest mobile phase ϕ which could be used to obtain log $K_w$ via extrapolation was typically 0.2 or less. Only in the case of the most strongly retained analyte (DOP) was the extrapolation performed with a minimum measured ϕ greater than 0.5.

Representative plots of log k' versus ϕ are shown in Figures 1 and 2. In most cases, the linear correlation between these two variables was excellent, with the best fit linear models exhibiting correlation coefficients ($r^2$) of 0.99 or

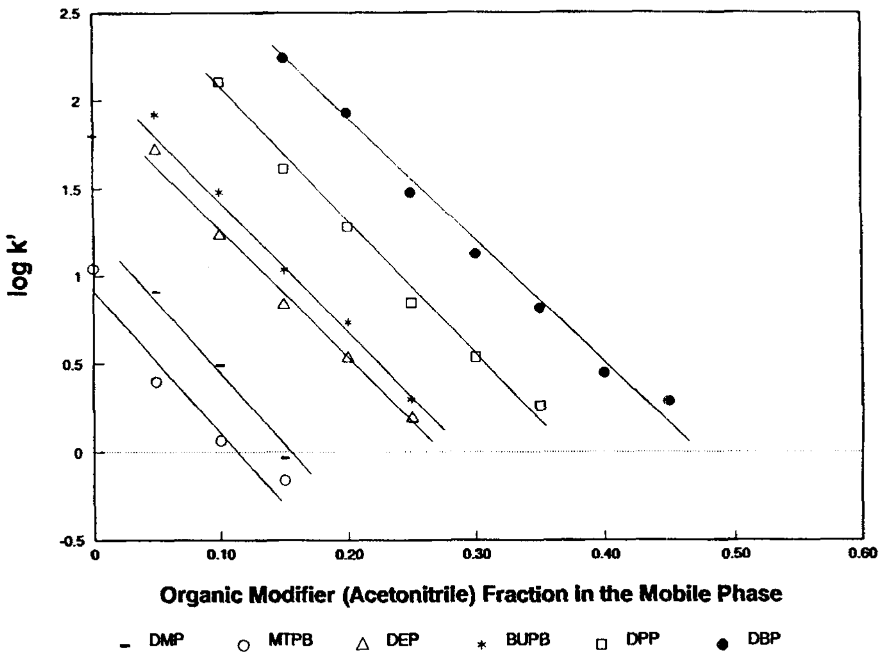

**Figure 2.** Plots of log k' versus organic modifier content (ϕ) for several model compounds using acetonitrile as the organic modifier. In general the plots are quite linear and roughly co-linear. The log k' obtained at equivalent values of ϕ is smaller in acetonitrile than in methanol, establishing acetonitrile as the stronger mobile phase modifier.

greater. For most analytes, however, some concavity in the plots were observed between ϕ values of 0.05 and 0.00 for both the acetonitrile and methanol mobile phases. For this reason, measured values of log k' at ϕ = 0.00 were not used in the linear regression analysis.

The plots of log k' versus ϕ for a given organic modifier were strikingly co-linear. For methanol, the mean and standard deviation of the slopes from these plots for all 17 model compounds were 0.051 and 0.009. For acetonitrile, the mean and standard deviation of the slopes were 0.068 and 0.011. The co-linearity of the plots reflects the structural similarities among the model compounds used and suggests that the general retention mechanism was consistent among all the model compounds. The stronger eluting power of acetonitrile versus methanol is reflected in the larger mean slope for this organic

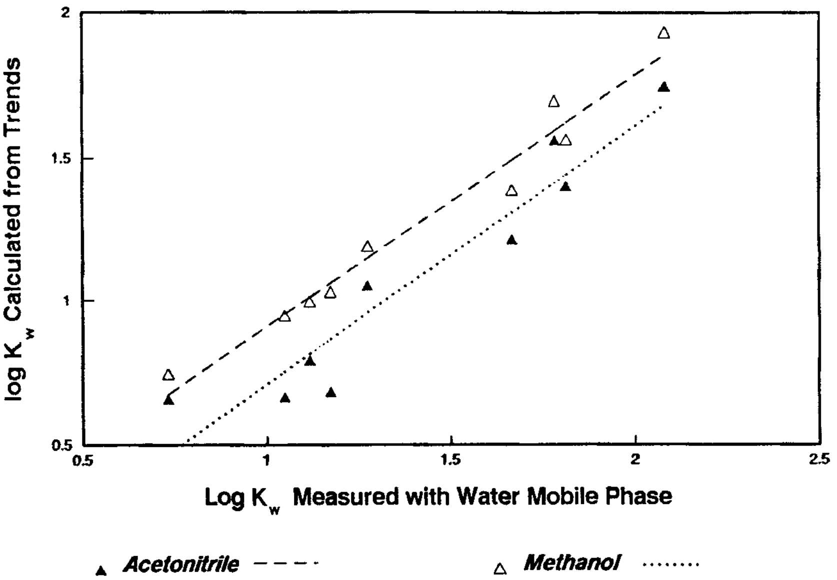

**Figure 3.** Plots of log $K_w$ actually measured versus log $K_w$ calculated using either methanol or acetonitrile as the mobile phase organic modifier. In general the calculated and measured values are linearly related; however the measured values are usually larger than the calculated ones due to concave curvature in the log k' versus $\phi$ plots.

modifier and is re-enforced by an examination of retention data in Table 2A through 2C. For every model compound studied, the $\phi$ required to produce a given k' is lower for acetonitrile than for methanol.

The effectiveness of the log $K_w$ extrapolations can be addressed in two manners. Figure 3 represents the comparison between measured and calculated values of log $K_w$. The linear model used for the comparison was

$$\log K_w\,(\text{calculated}) = a\,[\log K_w\,(\text{measured})] + b \qquad (3)$$

which, ideally, should exhibit a slope of 1.0, an intercept of 0.0 and a correlation coefficient of 1.0. For acetonitrile, pertinent curve fit data included the following: slope, 0.90 (0.11); intercept, -0.19 (0.13); $r^2$, 0.91; where the number in () is the standard error. For methanol, the pertinent curve fit data included slope, 0.87 (0.06); intercept, 0.03 (0.07); $r^2$, 0.97. While, in general, the correlations are good, the less than unit slopes reflect the generally concave nature of the log k' versus $\phi$ plots at low values of $\phi$. That is, extrapolation of

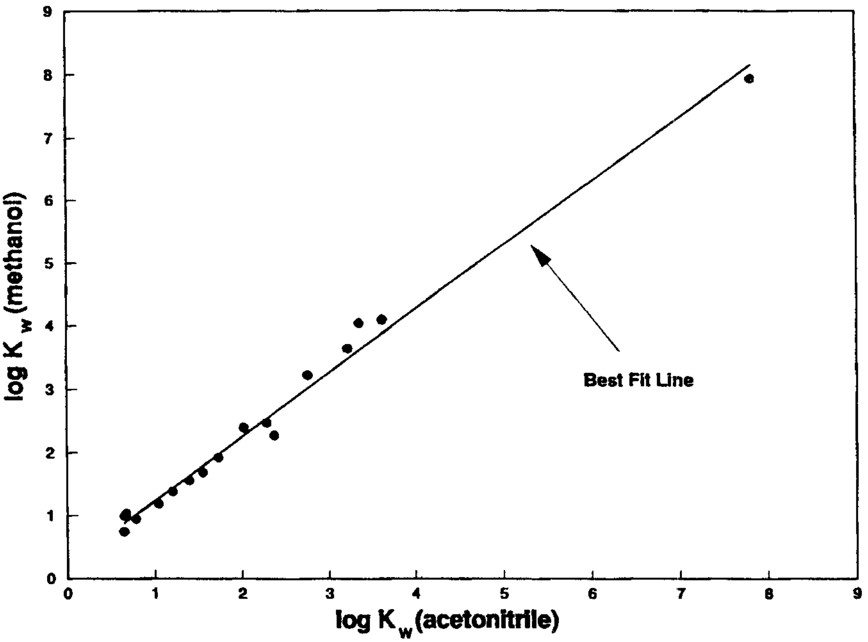

**Figure 4.** Plot of log $K_w$ calculated, methanol versus acetonitrile as mobile phase additives. Within the regression and experimental errors, the log $K_w$ values obtained with both modifiers are equivalent.

the log k' data to $\phi = 0$ generally produced a log $K_w$ estimate which was lower than the corresponding measured value.

In a recent analysis of retention trends in HPLC, Valko, Snyder and Glajch observe that the reliability of extrapolated log $K_w$ values can be assessed by comparing such values obtained from two different organic modifiers.[1] The point here is that log $K_w$ is an analyte/column property which is organic modifier independent. The relationship between log $K_w$ extrapolated from the data for all the model solutes using both organic modifiers is shown in Figure 4. In general, an excellent linear correlation is observed. Linear regression curve fit parameters for the model

$$\log K_w \text{ (methanol)} = a[\log K_w \text{ (acetonitrile)}] + b \qquad (4)$$

include slope, 1.02 (0.03); intercept, 0.22 (0.20); correlation coefficient ($r^2$), 0.99. Thus within the precision of the experimental measurements, the expected

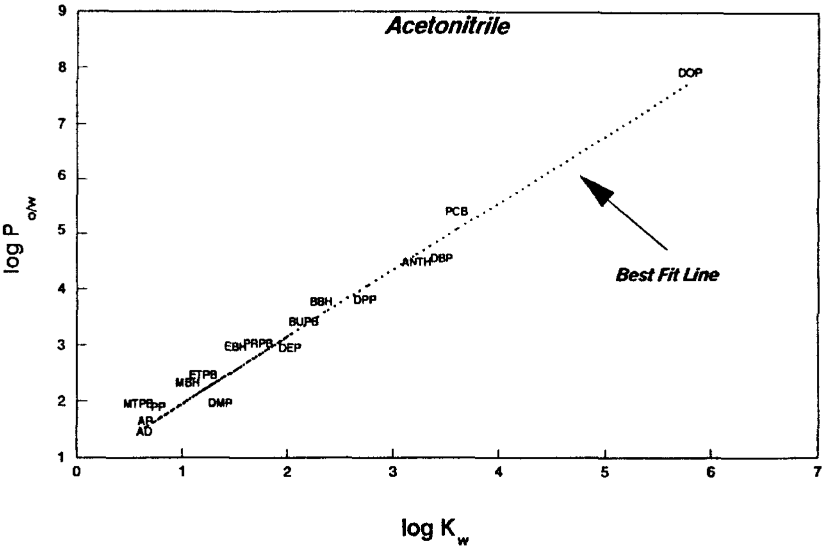

**Figure 5**. Plot of log $P_{o/w}$ versus log $K_w$ for acetonitrile as the organic modifier. The two variables are linearly correlated, with a correlation coefficient ($r^2$) of 0.977. The random distribution of the data around the best fit line suggests that the model contains no significant compound class bias.

1 to 1 relationship between log $K_w$ extrapolated from both organic modifiers is observed. The ultimate goal of this research is to examine the relationship between the extrapolated log $K_w$ data and available log $P_{o/w}$ data specifically per equation 1. Plots of log $P_{o/w}$ versus log $K_w$ are shown in Figures 5 and 6; the corresponding linear regression equations are as follows:

For acetonitrile, $\log P_{o/w} = 1.18 (\log K_w) + 0.87, r^2 = 0.977$

For methanol, $\log P_{o/w} = 0.90 (\log K_w) + 1.11, r^2 = 0.961$

Standard errors for the curve fit parameters included slope, 0.05 and intercept, 0.25 for acetonitrile and slope, 0.05 and intercept, 0.34 for methanol. As illustrated by the Figures and confirmed by the correlation coefficients, the partition coefficient models are quite accurate for the model compounds used. Examination of these figures indicates that the distribution of the data around the best fit line is essentially random and thus that the models contain no significant compound class bias.

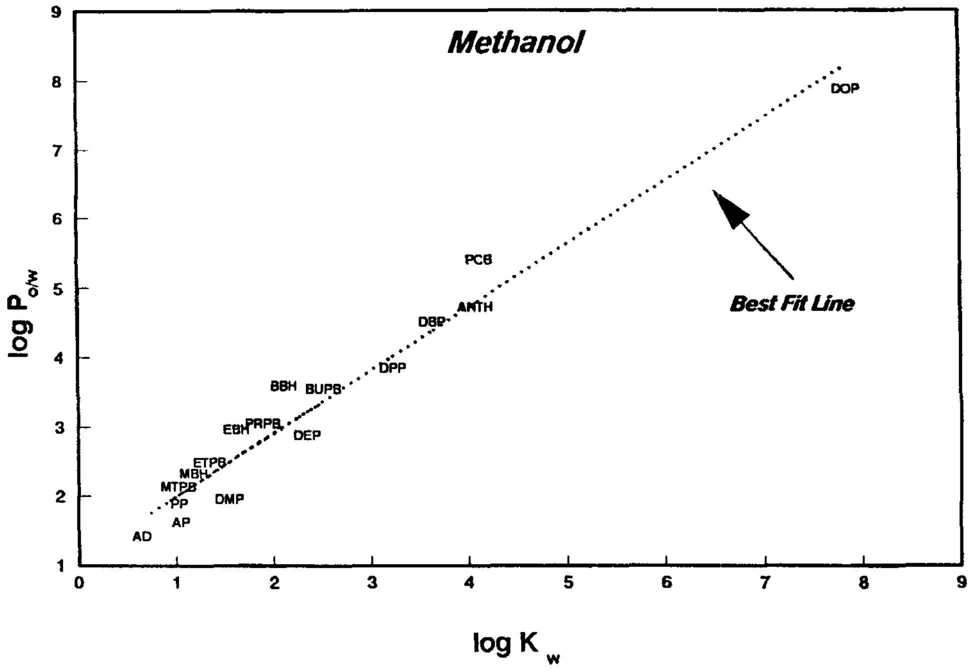

**Figure 6.** Plot of log $P_{o/w}$ versus log $K_w$ for methanol as the organic modifier. The two variables are linearly correlated, with a correlation coefficient ($r^2$) of 0.961. The random distribution of the data around the best fit line suggests that the model contains no significant compound class bias.

As observed by Braumann, Weber and Grimme,[2] the magnitude of the slope and intercept of the Collander-like expressions are significant. The model's intercept provides a measure of the hydrophobicity of the stationary phase used versus octanol; the similar positive values obtained indicate that the stationary phase used was significantly more hydrophobic than octanol. These authors attributed this behavior to the solvation of the $C_{18}$ stationary phase ligands by the organic modifier and free energy effects associated with water-organic mixtures relative to pure water.

The slopes of the regression equations are a measure of the solvent system's sensitivity to changes in the hydrophobicity of the model compounds. While both organic modifiers exhibit sensitivities to compound hydrophobicity which are similar to octanol (slopes near 1.0), it is clear that acetonitrile is more sensitive to solute nature than is methanol. Thus acetonitrile is the more

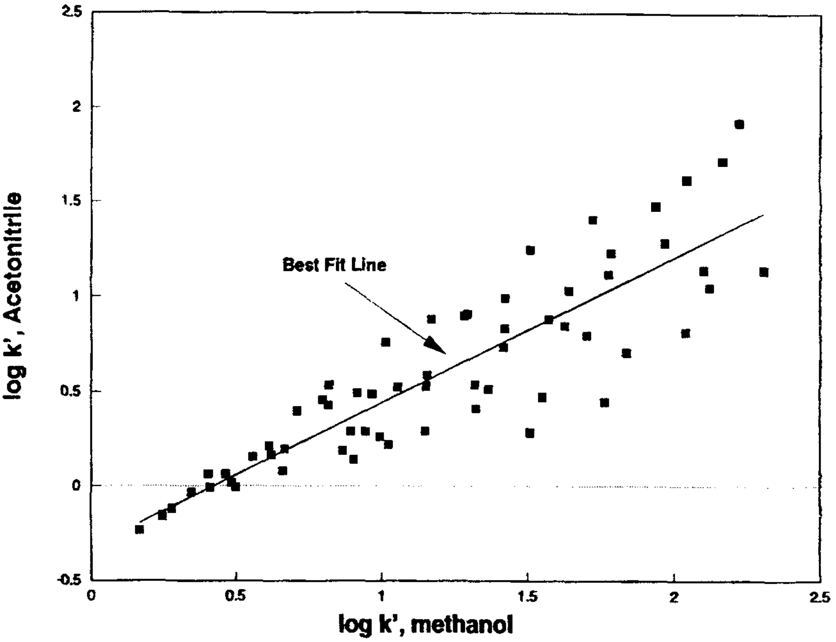

Figure 7. Plot of log k' measured for various compounds at similar values of $\phi$ in either methanol or acetonitrile containing mobile phases. While the log k' values for the two organic modifiers are directly related, the scatter in the data suggests that the retention mechanism in both organic modifiers is markedly different.

discriminating organic modifier and its use may facilitate the effective separation of mixtures of compounds. The two organic modifiers used are expected to have significantly different analyte retention profiles due to their marked chemical differences.[2,4,5,6,10]

Methanol, with both hydrogen donor and acceptor character, changes the ordering of water molecules in the mobile phase to only a limited extent and, thus, will not effect the mobile phases interaction potential with polar solutes. Acetonitrile, a weak hydrogen bond acceptor, will exert a more marked effect on the structure of the mobile phase and, hence, have a greater impact on the energetics of the solvophobic effect. Acetonitrile, with its high dipole moment,[5] may also participate in selective dipole-dipole interactions with certain solutes. Additionally, the participation of residual silanols in the retention process is more pronounced in organic modifiers other than methanol.[5,12]

### Table 3

### Comparison of Capacity Factors Obtained with Acetonitrile vs Methanol; Data Sorted by Analyte

| | | Regression Model Parameters | | |
|---|---|---|---|---|
| **Analyte** | **$\phi$ Range** | **Slope** | **Intercept** | **$r^2$** |
| DMP | 0.05 - 0.15 | 1.47 | -0.98 | 0.994 |
| AP | 0.05 - 0.15 | 1.08 | -0.42 | 0.991 |
| PP | 0.05 - 0.20 | 1.21 | -0.49 | 0.986 |
| MTPB | 0.05 - 0.15 | 1.20 | -0.47 | 0.993 |
| MBH | 0.05 - 0.20 | 1.39 | -0.68 | 0.992 |
| DEP | 0.05 - 0.25 | 1.17 | -0.68 | 0.992 |
| ETPB | 0.05 - 0.20 | 1.31 | -0.68 | 0.996 |
| EBH | 0.05 - 0.20 | 1.58 | -1.31 | 1.000 |
| PRPB | 0.05 - 0.20 | 1.45 | -1.08 | 1.000 |
| BUPB | 0.05 - 0.25 | 1.50 | -1.42 | 0.999 |
| BBH | 0.10 - 0.30 | 1.31 | -1.17 | 0.961 |
| DPP | 0.20 - 0.35 | 1.05 | -0.81 | 0.991 |
| ANTH | 0.25 - 0.40 | 1.10 | -1.42 | 0.982 |
| DBP | 0.30 - 0.45 | -0.85 | -0.65 | 1.000 |
| PCB | 0.40 - 0.50 | 1.01 | -1.11 | 0.988 |
| Overall | 0.05 - 0.50 | 0.76 (0.05) | -0.32 (0.24) | 0.772 |

Note: The above data represents all values for $\phi$ for which there were log k' data for both methanol and acetonitrile. Analytes which had only two such data points and, thus, were not subjected to regression analysis included AN and DOP. Data in () are the standard error of regression. The regression model used was:

log k' (acetonitrile) = a[log k' (methanol)] + b.

A comparison of all log k' data for analytes whose retention was measured in both acetonitrile and methanol containing mobile phases with the same $\phi$ value is shown in Figure 7 (representing 61 data pairs). While in general, the log k' values for both organic modifiers are directly related, linear regression analysis of the entire dataset produces a poor correlation ($r^2 = 0.77$). The poor correlation does not result from the differing behavior of individual compounds as mobile phase $\phi$ changes; as shown in Table 3, the linear correlation betweenlog k' in acetonitrile versus that in methanol at constant $\phi$ is excellent for each individual compound studied.

### Table 4

### Comparison of Capacity Factors Obtained with Acetonitrile Versus Methanol; Data Sorted by Mobile Phase Composition (ϕ)

| Mobile Phase Composition (ϕ) | Number of Compounds | Regression Model Parameters | | |
|---|---|---|---|---|
| | | Slope | Intercept | $r^2$ |
| 0.05 | 11 | 0.97 | -0.29 | 0.991 |
| 0.10 | 12 | 0.96 | -0.38 | 0.995 |
| 0.15 | 11 | 0.85 | -0.39 | 0.977 |
| 0.20 | 10 | 0.83 | -0.42 | 0.990 |
| 0.25 | 5 | 0.71 | -0.46 | 0.948 |
| 0.30 | 4 | 0.70 | -0.45 | 0.914 |
| 0.35 | 3 | 0.46 | -0.18 | 0.516 |
| 0.40 | 3 | 0.87 | -0.84 | 0.794 |
| 0.05 - 0.20 | 46 | 0.95 | -0.40 | 0.953 |
| 0.25 - 0.50 | 15 | 0.68 | -0.47 | 0.84 |

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Note: The above data represents all values for ϕ for which there were log k' data for both methanol and acetonitrile. Mobile phases with values of ϕ greater than 0.50 had only two such datapoints and thus were not subjected to regression analysis. The regression model used was:

log k' (acetonitrile) = a [log k' (methanol)] + b

Considering data for each of the studied compounds separately, log k' in the acetonitrile and methanol containing mobile phases are linearly correlated and, the retention characteristics in a mobile phase containing one of the modifiers, can be used to accurately estimate the retention characteristics in a mobile phase containing the other modifier at the same volume fraction ϕ.

Differences in the slope and intercepts of the log k' comparisons in Table 3 suggest part of the non-linearity observed in Figure 7 results from different solute/solvent interactions in the mobile phases containing the two organic modifiers. Table 4, which sorts the log k' acetonitrile versus methanol dataset as a function of ϕ, provides an insight into the dominant driving force for the non-linearity of the relationship shown in Figure 7. As shown in Table 4 and Figure 8, the relationship between log k' in acetonitrile versus log k' in methanol is highly linear over two distinct ranges of ϕ; ϕ values between 0.05 and 0.20 and ϕ values greater than 0.25. While this discontinuity in relative retention behavior at a ϕ value of approximately 0.25 is a clear indication of a changing

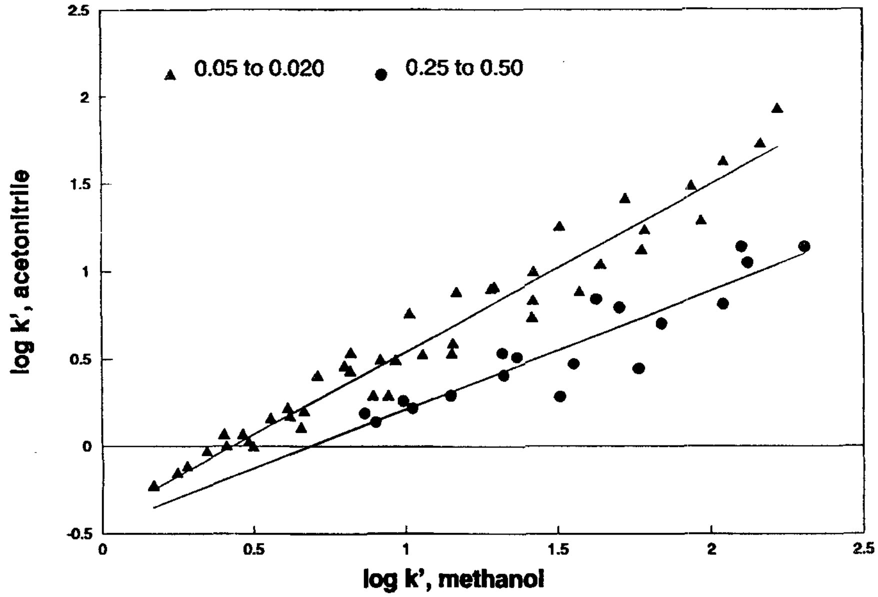

Figure 8. Data from Figure 7 sorted into two subsets; those for $\phi$ less than 0.25 and those for $\phi$ of 0.25 or greater. The clear breakdown of the data into two subsets as a function of $\phi$ suggests that the markedly different retention mechanisms for methanol versus acetonitrile mobile phases occurs above a $\phi$ value of 0.25. The somewhat poorer correlation of the data above $\phi$ of 0.25 suggests that compound/solvent interactions play a more significant role in defining the relative retention characteristics of methanol versus acetonitrile at the higher organic modifier volume fractions.

retention mechanism in one of the mobile phase types, the nature of this change in mechanism was not examined in detail in this study. It is suggested, however, that the influence of compound identity on the relative retention behavior of all compounds studied in the methanol or acetonitrile mobile phases is small at $\phi$, less than 0.25 as evidenced by the excellent linear correlation for this data subset. However, the poorer correlation above $\phi$ = 0.25 suggests that compound/solvent specific interactions play a more important role in defining the relative retention characteristics of the compounds in mobile phases prepared with organic modifiers present at high volume fractions.

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