Octadecyl, Phenyl and Cyano Phases Comparison for the RP-HPLC Prediction of Octanol-Water Partition Coefficient

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ABSTRACT

On phenyl and cyano phases log k´ values were determined, for a series of benzodiazepines. The log k´ values linearly extrapolated at 100 % water were highly significantly correlated to log P values.

A comparison with the most commonly used octadecyl phase demonstrates that phenyl is the best phase for log P prediction, cyano the most sensitive to the presence of particular moieties in the solute molecule, whereas octadecyl allows to obtain the most sensible measure of solute lipophylicity.

INTRODUCTION

Solute octanol-water partition coefficients (log P) play an important role in quantitative structure-activity relationships of pharmacologically active substances (1,2). Reversed phase HPLC determination of the capacity factor

provides a simple, accurate, reproducible method to determine lipophylic character. The octadecyl bonded phase (C_{18}) has been extensively used to predict log P values since, for a wide variety of compounds, the capacity factors on a C_{18} column appear correlated to the log P values (3,4,5,6). However the log P - chromatographic data correlation coefficients have often been significantly lower than unity, thus different reversed phase columns have been suggested as a better prediction tool (7,8,9).

In this study the phenyl and cyanopropyl stationary phases were investigated to predict benzodiazepine log P values and the behaviour of these columns was compared to that of C_{18}, previously determined (10). An attempt was made to explain how the chromatographic retention is affected by the presence of some particular substituents in the solute molecule.
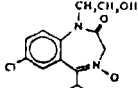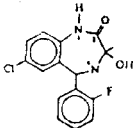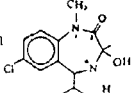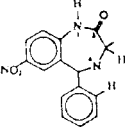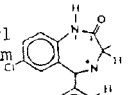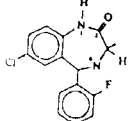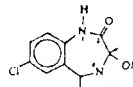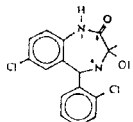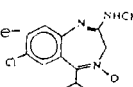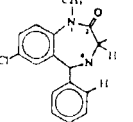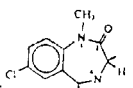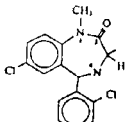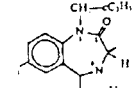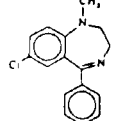
MATERIALS AND METHODS

The HPLC determinations were performed with the same experimental details previously reported for C_{18} column (10). Commercial columns (each 30 cm x 3.9 mm I.D.) μ Bondapak Phenyl and μ Bondapak CN (Waters Assoc.) were used. Table 1 reports molecular structure of benzodiazepines, previously measured log P values (11) and log k´ values extrapolated from the measurements on C_{18}.

RESULTS

The relationship between log k´ and the mobile phase composition (expressed as volume fraction φ of methanol) is generally represented by a quadratic expression (12,13,14); that part of the parabola corresponding to water rich eluents can be regarded as linear. For phenyl and CN columns it was possible to select a methanol concentration range in which the linear relationship holds. As expected (12), on both columns, the linearity breaks down for the more lipophylic compounds at a higher methanol concentration. Tables 2 and 3 report the high significancy of the calculated straight lines. By means of these linear relationships the log k´ values were calculated at 100% water in the mobile

TABLE 1

Molecular structure, log P and log k´ values on C_{18} phase of benzodiazepines.

Compound			log P	log k´
1			.44	2.18
2			1.48	2.90
3 Temazepam			1.99	3.17
4 Nitrazepam			2.12	3.20
5 N-demethyl diazepam			2.14	3.25
6			2.15	3.26
7 Oxazepam			2.17	3.16
8 Lorazepam			2.38	3.26
9 Chlordiaze- poxide			2.50	3.29
10 Diazepam			2.66	3.59
11			2.67	3.68
12			3.25	4.17
13 Prazepam			3.72	4.77
14 Medazepam			4.05	5.04

phase. These values were considered as a measure of hydrophobic stationary phase-water solute partitioning.

Let us first consider the chromatographic behaviour of the studied systems. The intercept values in Tables 2 and 3 were compared to corresponding C_{18} values (Table 1) showing that, for each compound, log k´ values extrapolated on phenyl and CN phases are about 4/5 and 1/2 respectively of the corresponding C_{18} values. This trend is not unexpected (15, 16) but simply reflects the different retentivity of the studied columns. In fact, RP retention decreases as stationary phase polarity increases and approaches that of the mobile phase (17). The polarity of the bonded phase increases when polar groups (e. g. CN) are introduced or when the alkyl chain length decreases, thus the retention strength decreases in the order: C_{18} > phenyl > CN.

The larger range of log k´values extrapolated on the C_{18} phase with respect to those extrapolated on phenyl and CN phases suggests that C_{18} can be considered a more sensitive measure of lipophylicity. As a measure of the log k´ dependence on mobile phase composition, the mean slopes for C_{18} , phenyl and CN phases were 5.07 (10), 3.98 and 4.03 (Tables 2,3) respectively. This trend agrees with previously reported results (9, 12). For each column the slope varies with the number and type of moieties in the solute molecule: a decrease in solute polarity is followed by an increase in slope value.

Any correlation between the logarithmic partition coefficient and chromatographic data can be brought to light either with the extrapolated log k´ values or with the slopes of log k´- relationships : that is according to the following relationships (17):

$$\log k´ = A + B \log P \qquad (I)$$

$$b = A´ + B´\log P \qquad (II)$$

The results are reported in Table 4. The correlation coefficients indicate that, for all the three columns studied, chromatographic data are highly correlated to log P values; as expected (9, 18) the extrapolated log k´ values show better correlation with log P than do the slope values. A comparison of statistical parameters of correlations 1-6 in Table 4 shows that the correlation

TABLE 2

Linear relationship between log k´ values and methanol concentration
(log k´ = a − b φ) on phenyl phase.

Compound	a	b	r	Methanol % range
1	1.80	2.71	0.999	4o-70
2	2.19	3.25	1	40-70
3	2.67	3.61	1	40-70
4	2.69	4.01	0.998	40-70
5	2.78	3.76	1	45-70
6	2.77	3.94	1	40-70
7	2.71	3.96	0.998	40-70
8	2.74	4.05	0.999	40-70
9	2.96	4.09	0.996	45-70
10	3.12	4.09	0.998	50-75
11	3.16	4.21	0.999	50-75
12	3.46	4.43	0.996	50-75
13	3.72	4.62	0.999	55-80
14	4.04	5.01	0.999	55-80
mean values	2.92(+.33)	3.98(+.32)		

TABLE 3

Linear relationship between log k´ values and methanol concentration
(log k´ = a − b φ) on cyano phase.

Compound	a	b	r	Methanol % range
1	1.23	3.36	0.999	20-50
2	1.49	3.65	0.998	20-50
3	1.62	3.67	0.999	20-50
4	1.55	3.43	0.999	20-50
5	1.78	4.03	0.999	20-50
6	1.83	4.22	0.999	20-50
7	1.66	4.09	0.999	20-50
8	1.68	3.97	0.997	20-50
9	1.67	3.87	0.999	20-50
10	1.97	4.20	0.998	23-50
11	1.93	4.19	0.998	23-50
12	2.12	4.49	0.999	26-50
13	2.29	4.56	0.997	30-50
14	2.44	4.66	0.996	33-50
mean values	1.80(+.19)	4.03(+.23)		

TABLE 4

Linear regression data of the correlations: $\log k' = A + B \log P$ (I)

and $b = A' + B' \log P$ (II)

Stationary phase	A	B	r	S.D..
Octadecyl				
(1) intercept (eq.I)	1.56(+.24)	0.800(+.094)	0.975	0.30
(2) slope (eq.II)	2.99(+.25)	0.861(+.096)	0.977	0.31
Phenyl				
(3) intercept (eq.I)	1.38(+.12)	0.635(+.046)	0.990	0.15
(4) slope (eq.II)	2.51(+.17)	0.612(+.065)	0.979	0.21
Cyano				
(5) intercept (eq.I)	0.97(+.14)	0.346(+.053)	0.958	0.17
(6) slope (eq.II)	3.08(+.28)	0.39(+.11)	0.880	0.35
(7) intercept (eq. I) n=5 (with OH)	1.130(+.032)	0.240(+.018)	0.997	0.28
(8) intercept (eq. II) n=7 (without OH)	1.096(+.089)	0.324(+.030)	0.994	0.053

r=regression coefficient; S. D.=Standard Deviationof extimate.

All the reported errors are calculated in a 95% confidence interval.

between octanol-water partition coefficients and chromatographic data on phenyl phase is the best. Thus et al. (9) obtained the same results for aromatic compounds and attributed this finding to a better masking effect of the residual silanol groups by the phenyl moieties and/or the possibility of $\pi-\pi$ interactions. The poorer correlation observed for the CN phase is significantly improved when calculated separately for two groups: compounds containing an OH group or not (correlations 7 and 8 in Table 4). A z test on r (19) shows that the difference between the correlation coefficients of relationships 7, 8 and that of relationship 5 (Table 4) are statistically significant (P < 0.05). These results may indicate that the retention on this polar phase is more selective towards the presence of an OH group than are the C_{18} and phenyl phases.

By inverting equation (I) a log P value can be calculated from the extrapolated log k′ value for each benzodiazepine. The difference between the true (log P) and calculated (log $P_{calc.}$) value may measure whether Reversed Phase HPLC determinations are valid or not in predicting log P values. The results for the considered columns are presented in Figure 1. One can note that, with few exceptions, for the intermediate-polarity phenyl phase the residuals are about 0.1 and randomly distributed over the entire lipophylicity scale. On the other hand, for extreme polarity C_{18} and CN phases more pronounced deviations and more complex patterns are observed. This is simply an another, more detailed way of proving that phenyl is more suited to polarity prediction studies, at least for this series of compounds. An attempt to fully explain these differences in terms of specific molecular interactions was inconclusive most likely because many different solute-stationary phase and solute-mobile phase interactions are working at the same time (19).

Nevertheless, an analysis of the residuals reported in Figure 1 may give some indication as to how solute structure affects chromatographic retention with respect to the octanol reference system. In fact, for the CN phase the most significant positive and negative residuals are correlated to some specific moieties in the benzodiazepine molecules. A positive difference (i.e., a stronger interaction with the reference octanol than with the CN phase) appears for compounds 4 and 9 carrying specific groups, such as $-NO_2$ and N-O, as well as for compounds 3, 7 and 8 which have an OH group in position 3. The only exception is
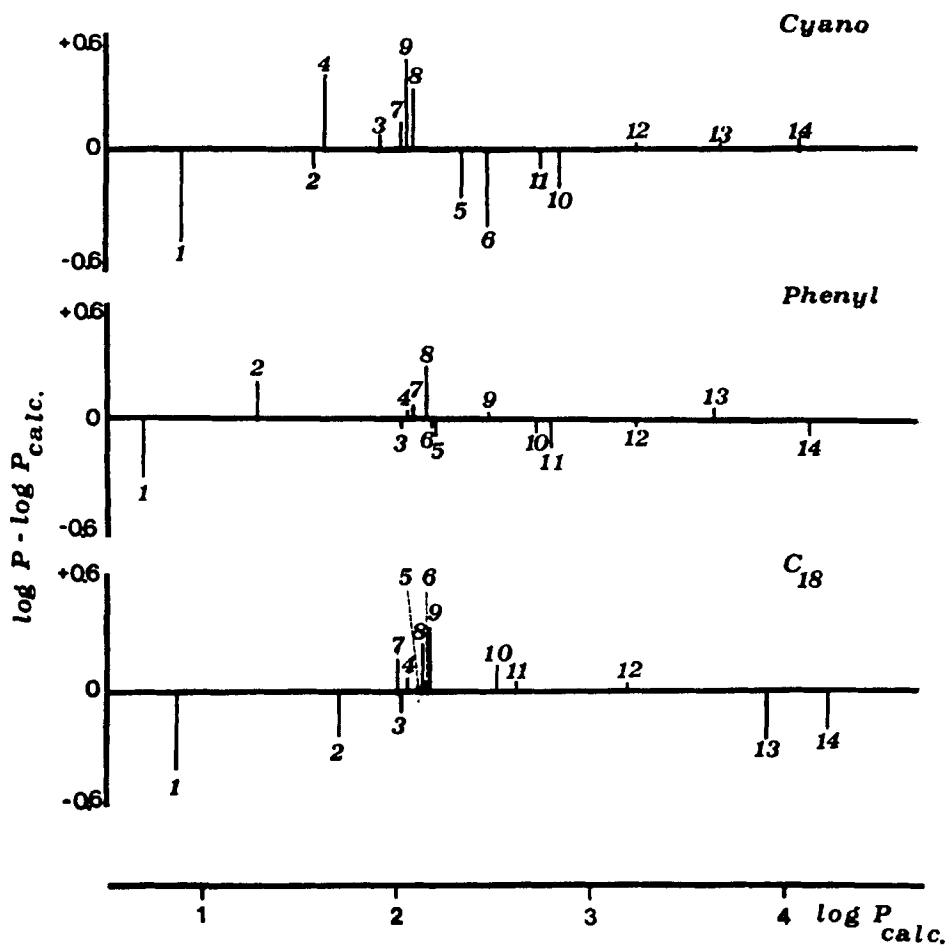
FIGURE 1

Behaviour of the difference (log P - log $P_{calc.}$) as a function of log P.

for compound 2 which also has an OH group in position 3. The correlation between the negative differences and structure features is less simple, however a persistent negative residue, (i.e., a stronger interaction with the CN phase) is observed whenever a fluorine atom is present in position 2´ (compounds 2, 6 and 11).

The analysis of the residues on C_{18} phase points out a less clear behaviour.

CONCLUSION

The present study showed that the chromatographic data measured on all three columns in question are highly correlated with log P values for a series of benzodiazepines. Further investigations extended to different mobile-stationary phases systems would be interesting to elucidate how the solute molecular structure affects retention on different bonded stationary phases. These data also appear promising in improving the study of quantitative structure-pharmacological activity relationships.

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