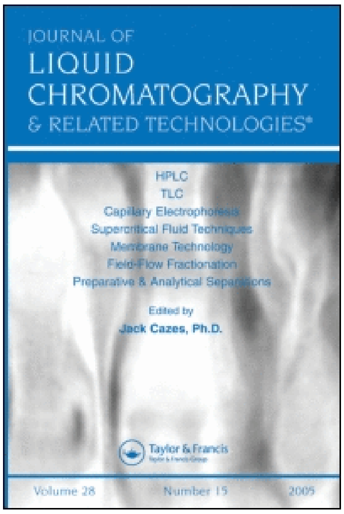

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Hydrophobicity and Retention in Reversed Phase Liquid Chromatography

Marius D'amboise[a]; Toshihiko Hanai[a]
[a] Department of Chemistry University of Montreal, Canada

To cite this Article D'amboise, Marius and Hanai, Toshihiko(1982) 'Hydrophobicity and Retention in Reversed Phase Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 5: 2, 229 — 244
To link to this Article: DOI: 10.1080/01483918208069068
URL: http://dx.doi.org/10.1080/01483918208069068

JOURNAL OF LIQUID CHROMATOGRAHPY, 5(2), 229-244 (1982)

HYDROPHOBICITY AND RETENTION IN REVERSED PHASE LIQUID CHROMATOGRAPHY

Marius D'AMBOISE, and Toshihiko HANAI
Department of Chemistry
University of Montreal
P.O. Box 6210, Station A
Montreal, Canada, H3C 3V1

ABSTRACT

Reversed phase liquid chromatography retention data for several compounds are examined in relationship to their hydrophobicities. Alcohols and various aromatics are used to compare hydrophobicities in aliphatic and aromatic compounds. Capacity factors, k', can be correctly evaluated by using the hydrophobic factors (log P) derived from the hydrophobic fragmental constants. Appropriate solvent mixtures to achieve good separations can be choosen from graphical data.

INTRODUCTION

The behaviour of a solute in a chromatographic system is a constant preoccupation for the analytical chemist. Distribution coefficients of ionizable compounds have been related to capacity ratios in a system using polystyrene gel as packing (1). Retention of dipolar acids on ion-exchange resin has been explained through ion-ion interaction. Optimization of chromatographic conditions for non-ionizable compounds has been discussed by many researchers ; solvent effects (2), molecular size effects (3), and branching effects (4) give useful information for the prediction of the retention time. Molecular size effects have also been used to study the influence of alkyl groups on capacity factors (5-8).

Optimization of procedures in reversed phase liquid chromatograhy, RPLC, requires some knowledge of the separation mechanism. Various modes of interaction are possible. Non-polar solid supports give rise to absorption on a surface which may behave both as if it were a solid and a liquid. Solute retention can be envisaged as a reversible association between the hydrocarbonaceous surface and the solute molecule. This type of interaction is called hydrophobic interaction (9). It is the result of a repulsion between the chromatographic support and water as well as between water and the solute : it may be said that water forces the association between the solute and the support. The interaction can also be viewed as a partition between one liquid, the aqueous eluent and a second pseudoliquid, the solid hydrocarbonaceous support.

Rekker's hydrophobic fragmental constants (10) have been useful in evaluating hydrophobicities of aromatic as well as aliphatic compounds. The concept of hydrophobicity is quite helpful in the development of new drugs. It may also be useful in the optimization of reversed phase liquid chromatography.

Attempts, based on the hydrophobicities of various alcohols, were made in order to predict the relative retention of theses solutes on a polystyrene gel using methanol-water or acetonitrile-water mixtures as eluent (11). In the present paper, capacity factors of alcohols, fatty acids, and various aromatic compounds such as polyaromatic hydrocarbons, alkylbenzenes and benzoates are related to the hydrophobicities of the molecules. Solvent composition permitting good separations is also discussed in terms of the observed relationship between retention and hydrophobicity.

Hydrophobicity

The hydrophobicity of a solute is conveniently estimated from its partition coefficient, P, between an organic solvent and water. The larger the value of P, the more hydrophobic the substance is. Rekker (10) has shown that

$$\log P = \sum a_i F_i \qquad [1]$$

where a_i represents the number of times a particular fragment "i" is found in a given molecule and F_i is the hydrophobic fragmental constant of fragment "i". Thus a molecule can be visualized as a series of fragments, each of which contributes to its hydrophobic character.

An extensive study of the partition coefficients of various solutes (aromatics and aliphatics) in octanol-water led to the various fragmental constants reported by Rekker (10). Arbitrarily, but based on practical considerations, the log P value of a given solute as calculated using equation [1] and the F_i values derived from the partition coefficients in octanol-water, is called the hydrophobic factor of this solute. In liquid chromatography, provided the hydrophobic effect is the sole interaction,

$$\log P = A^* + \log k'$$

In this equation, A* is a constant and k' is the capacity factor. Thus, a straight line with unit slope should be observed in a system using octanol-water as eluent. It is easily shown that for retention using another eluent,

$$\log k' = a + \beta \log P \qquad [2]$$

where "a" is a constant and the slope β , a characteristic of the eluent.

EXPERIMENTAL

A chromatographic system was assembled from various modules. Pumps were Waters, model 6000 A (Water Associates Inc., Milford, MA 01757), and Altex, model 100 (Altex Scientific Inc., Berkeley, CA 94710). Detectors were Waters differential refractive index detector, Model R401, and a Hitachi spectrophotometer, model 100-20 (Hitachi, Mountain View, CA 94043) equipped with and 8-μL flowthrough cell from Altex. Injector was Altex, model 905-19. Recorders were Brinkmann, model 2541 (Brinkmann Instruments, Inc., Westbury, NY 11590), or Linear Instruments, Model 915 (Linear Instruments Corp. Irvine, CA 92714).

Chemically bonded octadecyl packing was LC 7 from Johns-Manville (Johns-Manville, Denver, CO 80217) packed in our laboratory

TABLE 1

HYDROPHOBIC FACTORS

COMPOUNDS	log P
Polyaromatics	
1. Benzene	2.16
2. Naphtalene	3.18
3. Anthracene	4.20
4. Pyrene	4.50
Alkylbenzenes	
5. Toluene	2.59
6. Ethylbenzene	3.12
7. Isopropylbenzene	3.52
Benzoates	
8. Methylbenzoate	2.15
9. Isopropylbenzoate	3.09
10. Butylbenzoate	3.74
11. Isopentylbenzoate	4.15
Substitued Benzenes	
12. Aniline	1.03
13. Phenol	1.54
14. Benzoic Acid	1.79
15. Chlorobenzene	2.81
16. Bromobenzene	3.02

COMPOUNDS	log P
Alcohols	
17. Butyl Alcohol	0.80
18. Pentyl Alcohol	1.33
19. Hexyl Alcohol	1.86
20. Heptyl Alcohol	2.39
21. Octyl Alcohol	2.92
22. Decyl Alcohol	3.98
23. Dodecyl Alcohol	5.04
Fatty Acids	
24. Hexanoic Acid	1.87
25. Octanoic Acid	2.93
26. Decanoic Acid	3.99
27. Dodecanoic Acid	5.05
28. Tetradecanoic Acid	6.11
29. Hexadecanoic Acid	7.17
30. Octadecanoic Acid	8.23
31. Eicosanoic Acid	9.29
32. Palmitoleic Acid	6.58
33. Oleic Acid	7.64
34. Linoleic Acid	7.05
35. Linolenic Acid	6.46
36. Arachidonic Acid	6.98

by a balanced slurry method in 19.7 cm long, 3.2 mm I.D. stainless steel column fitted with zero dead volume attachments, Altex, No 250-21, or Swagelok (Crawford Fitting CO., Solon Ohio 44139). Chemicals came from various sources ; the list of the different solutes studied is given in Table 1. Solvents were HPLC grade ; water was first distilled in glass and further purified through a Milli-Q system (Millipore, Corp., Bedford, MA 01730).

RESULTS AND DISCUSSION

Capacity factors (k') were evaluated from at least two reproducible chromatograms for each compound. Various solvent mixtures were used as eluents. Uric acid, eluting faster than any other solute, served to measure the dead volume of the column. The hydrophobic factor, log P, was calculated by summing up Rekkers's hydrophobic fragmental constants (eq 1) of all fragments in the molecule ; the values obtained are given in table 1. As observed in Figures 1 to 3, a good linearity exists between log k' and the hydrophobicity of the molecule. Equation 2 is generally obeyed ; least squares analysis shows that the linear model explains 95+% of the variations observed.

Figure 1 shows the relationship between log k' and log P for alcohols, polyaromatics, benzoates, alkylbenzenes, benzene derivatives and fatty acids in 50% acetonitrile in water as eluent. All these compounds, except the fatty acids, lie on the same straight line. Capacity factors of fatty acids are always lower than those observed for the other types of compounds having similar hydrophobicities. This behaviour might be due to ionization of the acids in acetonitrile-water mixtures and/or because of the high polarity of the solutes.

In Figure 2, the variation of capacity factors (log k') as a function of hydrophobicities is shown for various acetonitrile-water mixtures. Equation 2 holds for solvent mixtures containing between 20 and 80% acetonitrile in water, and for non-ionizable

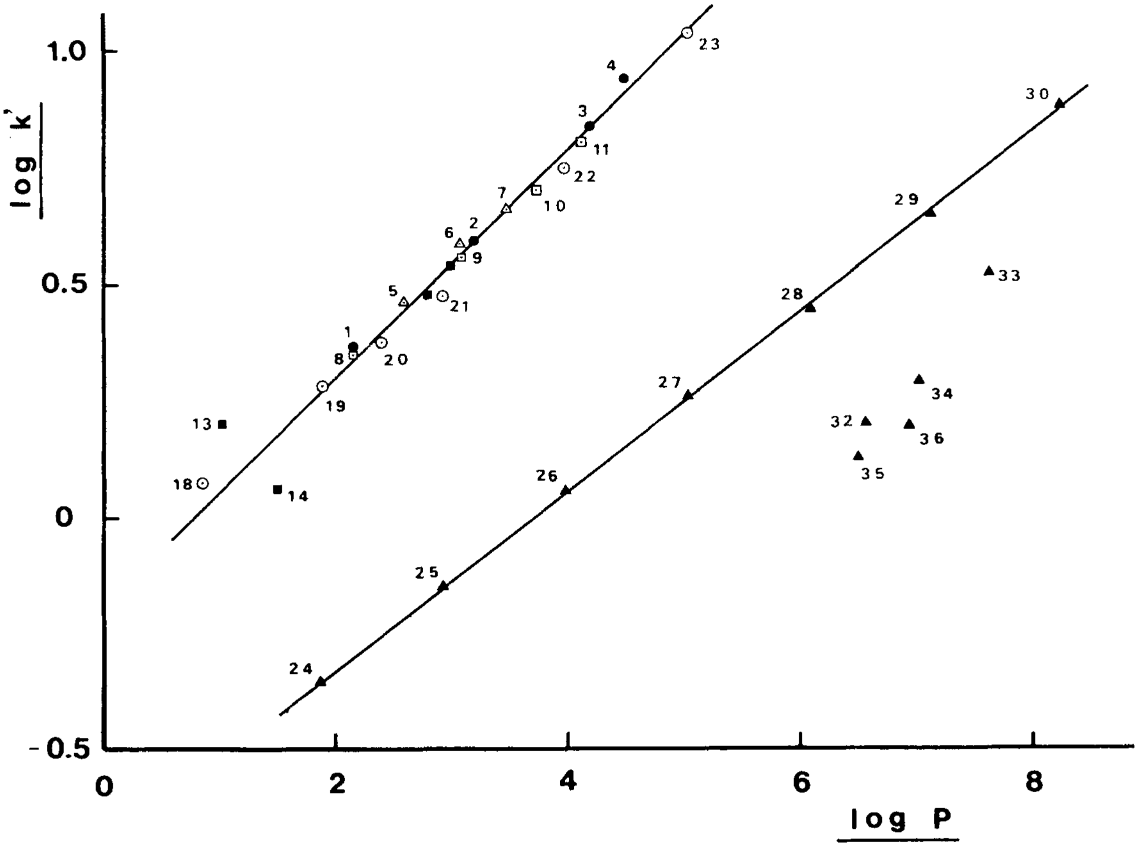

FIGURE 1. Relationship between the logarithm of capacity ratio and hydrophobic factors in 50% $^{v}/v$ aqueous acetonitrile on octadecyl packing. ⊙ alcohols , ● polyaromatics ; ⊡ benzoates ; ⚠ alkylbenzenes ; ■ benzene derivatives ; ▲ fatty acids. Numbers : see Table 1.

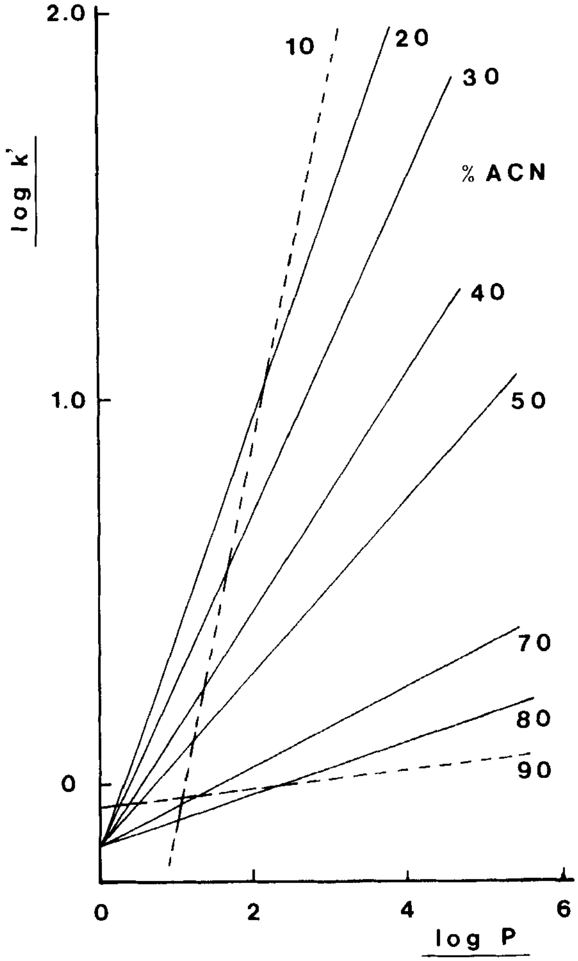

FIGURE 2.

Variation of the capacity ratio (log k') as a function of hydrophobicity (log P) in various acetonitrile (% ACN) eluents. N.B. For clarity in the graphical representation, experimental points are not indicated on the drawing.

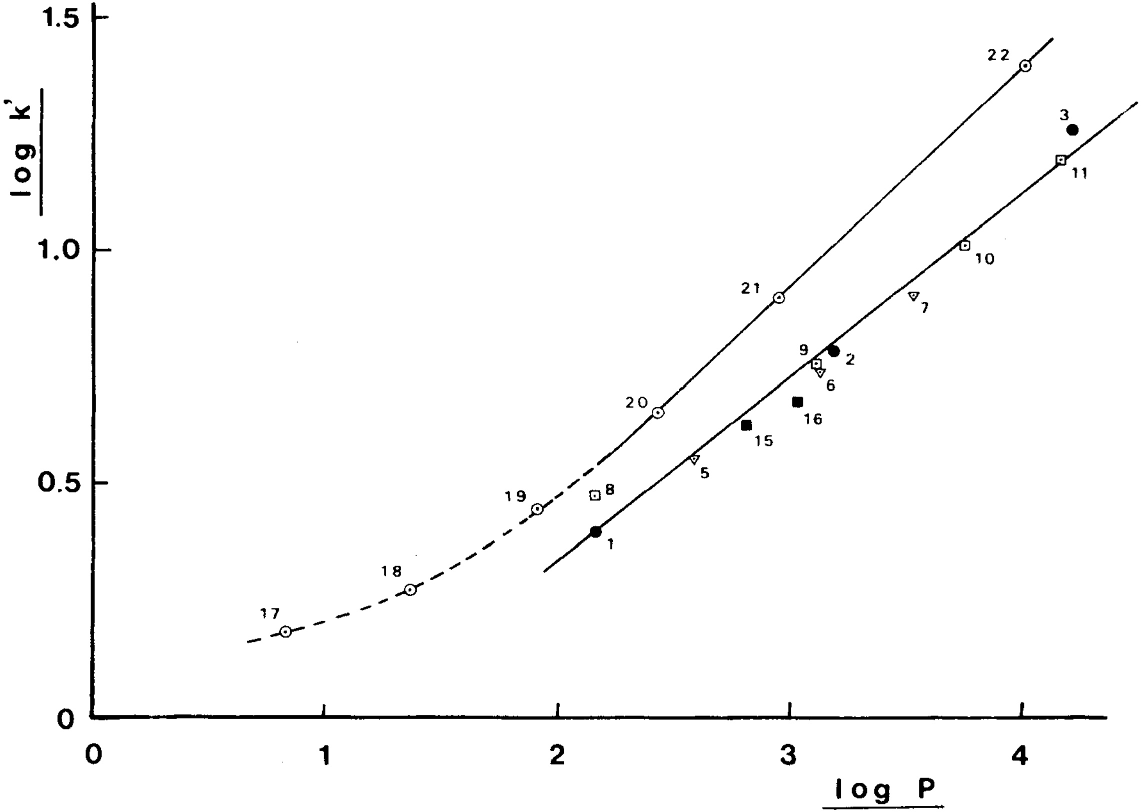

FIGURE 3. Relationship between the capacity ratios (log k') and the hydrophobicity (log P) in 50% aqueous methanol on octadecyl packing. Symbols : see Figure 1.

compounds such as alcohols, polyaromatic hydrocarbons, alkylbenzenes, benzoates and halogenated benzenes. The convergence of the family of lines to the same point suggests that constant "a" of equation [2] is a characteristic of the system itself. The variation of the slope was predictable from eq. 2. The behaviour at extreme concentrations, higher than 80% ACN and lower than 20% ACN in water indicates that the mechanism of retention is more complex than pure hydrophobic effect. We believe that hydrogen bonding competes with hydrophobic effects at high acetonitrile content whereas coating of the packing material with water molecules could be responsible for the behaviour at low acetonitrile content. A similar observation was made by Horvath (9).

The graph of Figure 3 describes the relationship between log k' and the hydrophobic factor in 50% aqueous methanol. A linear relationship is observed for all compounds except for alcohols in which a curvature is seen at low hydrophobicities. The regression line for alcohols is also above the line for other compounds. This indicates that hydrophobicity of a given fragment in aliphatic groups is higher than that in aromatics in methanol-water solvent mixtures. The study has also been performed at other MeOH-Water ratios and also in aqueous ethanol and aqueous tetrahydrofuran. Similar results were obtained with all eluents. The least squares analysis in the case of the 50% aqueous eluents are given in Table 2. It is worth mentioning the following observations from those results :

TABLE 2

Summary of Least Squares Coefficients for log k' vs log P

Solutes*	Eluents (% in water)	log k' = a + β log P		
		a, s_a	β, s_β	R^2**(%)
1 to 23	50% ACN	- 0.182 0.011	0.241 0.035	96.1
acids (24 to 30)	50% ACN	- 0.763 0.002	0.197 0.014	99.9
1 to 16	50% MeoH	- 0.625 .028	0.445 0.095	95.7
alcohols (17 to 23)	50% MeoH	- 0.268 0.025	0.403 0.062	98.5
(19 to 23)	50% MeoH	- 0.444 0.015	0.460 0.045	99.8
1 to 12	50% EtoH	- 0.227 0.015	0.229 0.048	96.0
alcohols	50% EtoH	- 0.277 0.010	0.255 0.030	99.7
1 to 4	50% THF	0.162 0.026	0.109 0.093	90.0
benzene derivatives	50% THF	0.094 0.022	0.154 0.060	90.7
benzoates	50% THF	0.064 .009	0.183 0.030	99.5
alcohols	50% THF	0.005 0.001	0.136 0.004	99.9^8
acids	50% THF	0.0431 0.0006	0.144 0.004	100%

* Numbers refer to compounds in Table 1.

** R^2 (%) : Percentage of the total variation explained by the model.

i) the negative intercepts in the cases where ACN, MeOH or EtOH are used in eluents.

ii) although a linear relationship was observed in aqueous THF, the straight lines calculted for the various categories of compounds were all different in this solvent.

iii) the high slope of log k' vs log P in methanol as compared with the values in the other solvents.

iv) the increase in slope as the mole fraction of organic modifier increases.

The slopes, β, of the straight lines can be useful in the choice of the proper solvent mixture for the achievement of a given separation. Moreover, the regression lines may serve for the identification of unknown compounds. Figure 4 shows the slope, β, as a function of the organic content of the eluent. For any pair of solutes, A and B, equation 2 can be rearranged in terms of the separation factor, α, to give :

$$\beta = \log \alpha - \log P_a/P_b \qquad [3]$$

Since hydrophobicity characterises a solute, it becomes easy to calculate the β value which will achieve a given separation (i.e. a given value of α). Thereafter, an eluent insuring a value of β equal to or larger than that calculated from equation 3 can be chosen from a graph such as that of figure 4. The solvent mixture so chosen will separate compounds A and B satisfactorily.

The discrepancies observed between the unit slope expected in octanol and the slopes observed in the various eluents can be

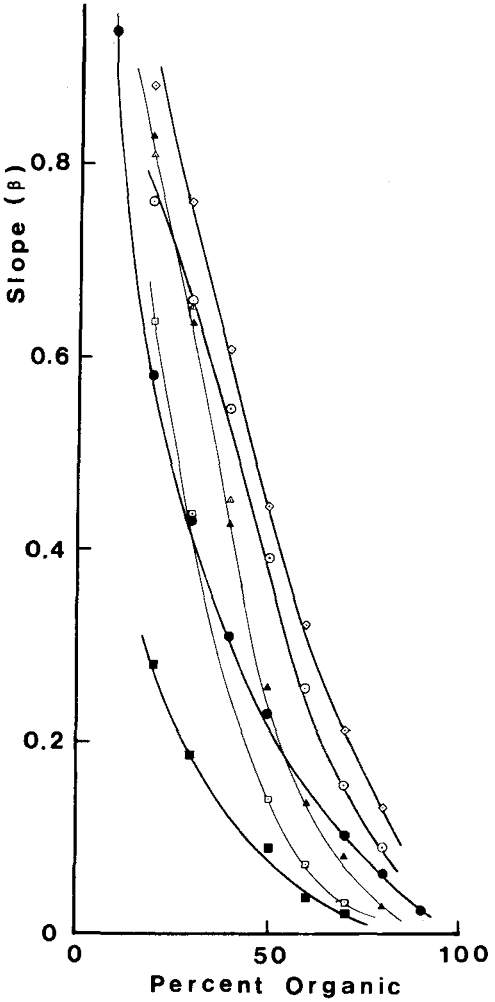

FIGURE 4.

Variation of the slope (β) for various solutes as a function of the organic content (%) in the eluent. ◇ Methanol (alcohols) ; ⊙ Methanol (polyaromatics ; ● acetonitrile (alcohols and aromatics); △ ethanol (alcohols) ; ▲ ethanol (aromatics) ; ▣ tetrahydrofuran (alcohols) ; ■ tetrahydrofuran (polyaromatics).

understood if one considers that equation [2] refers to a partition between octanol and water whereas the quantity measured, log k', is due to a partition between an aqueous organic eluent and a pseudoliquid, the hydrocarbonaceous material.

The separating ability observed in this work is similar to that reported by Hoffman and Liao (12) except for methanol which shows a larger separating ability than acetonitrile.

The use of hydrophobicities to link the retention of various compounds in liquid chromatography seems promising. Of the many ways to evaluate hydrophobicity, we have selected Rekker's (10) method. The reasons for this choice are the easiness with which the parameters may be calculated and the fact that the approach does not require additional experimental measurements as do, for instance, Sliwiok, Macioszcyk and Kowalska's relative coefficients (13). The method described in this paper to link the retention data is thus most convenient for routine laboratory work.

Finally, as shown by Murray, Hall and Kier (6), a straight linear relationship exists between log P and the connectivity index, χ. Therefore, a straight line should be observed when plotting log k' vs connectivity indices.

ACKNOWLEDGEMENTS

We are grateful to Mr. S. Dave (Johns-Manville) for a gift of a LC-7 packing. We also acknowledge the financial support of the Ministère de l'Education du Québec, FCAC grant, in conducting this research.

REFERENCES

1. Hanai, T., Walton, H.F., Navratil, J.D., and Warren, D. Liquid Chromatography of Polar Aromatic Compounds on Cation-Exchange Resins and Porous Polymer Gels. J. Chromatogr., 155, 261 (1978).

2. Bakalyar, S.R., McIlwrick, R., and Roggendorf, E. Solvent selectivity in Reversed-Phase High Pressure Liquid Chromatography. J. Chromatogr., 142, 353 (1977).

3. Karger, L.B., Gant, J.R., Hartkopf, A., and Weiner, P.H. Hydrophobic Effects in Reversed-Phase Liquid Chromatography. J. Chromatogr., 128, 65 (1976).

4. Kier, L.B., Hall, L.H., Murray, W.J., and Randic, M. Molecular Connectivity I : Relationship to Nonspecific Local Anestesia. J. Pharm. Sci. 64, 1971 (1975).

5. Hall, L.H., Kier, L.B., and Murray, W.J. Molecular Connectivity II : Relationship to Water Solubility and Boiling Point. J. Pharm. Sci. 64, 1974 (1975).

6. Murray, W.J., Hall, L.H., and Kier, L.B. Molecular Connectivity III : Relationship to Partition Coefficients. J. Pharm. Sci. 64, 1978 (1975).

7. Uchida, M., and Tanimura, T. Retention Behaviour of a Porous Styrene - Divinylbenzene Copolymer in the Separation of Fatty Acids and Related Compounds. J. Chromatogr., 138, 17 (1977).

8. Randic, M. The Structural Origin of Chromatographic Retention Data. J. Chromatogr., 161, 1 (1978).

9. Horvath, C., Melander, W., and Molnar, I. Solvophobic Interactions in Liquid Chromatograhy with Non-Polar Stationary Phases. J. Chromatogr., 125, 129 (1976).

10. Rekker, R.F. The Hydrophobic Fragmental Constant. Nauta, W.T., and Rekker, R.F., Editors. Elsevier Scientific Publishing Company, Amsterdam (1977).

11. Hanai, T. Study of Qualitative Analysis by Liquid Chromatography Using Porous Polymer Gel. Chromatographia, 12, 77 (1979).

12. Hoffman, N.E., and Liao, J.C. Separating Ability of some Polar Mobile Phases in Reverse Phase High Performance Liquid Chromatography. Anal. Lett. All, 287 (1978).

13. Sliwiok, J., Macioszczyk, A., and Kowalska, T. Chromatographic Investigation of the Hydrophobic Properties of Isomeric Naphtalene Derivatives. Chromatographia, 14, 138 (1981).