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REVERSED-PHASE LIQUID CHROMATOGRAPHY OF SOME PESTICIDES AND RELATED COMPOUNDS

SOLUBILITY–RETENTION RELATIONSHIPS

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SUMMARY

The liquid chromatographic retentions of 17 pesticides have been studied on Vydac reversed-phase in water–methanol and water–ethanol mixed solvents. The logarithm of the adjusted retention relative to methoxychlor correlated linearly with Beroza partition *p*-values recorded in heptane–90 % ethanol (correlation coefficients greater than 0.95). The resulting relationship could have value in the choice of eluting solvent ratio and prediction of relative retentions in the separation of mixtures, and in defining the polarity of unknowns in mixtures. Retention characteristics of some poly-chlorinated biphenyl mixtures are given for illustration. Relative responses of the pesticides to the 254-nm ultraviolet detector were tabulated to allow estimation of limits of detectability for individual compounds.

INTRODUCTION

Liquid chromatography (LC) has found increasing use for the separation of pesticides and their conversion products[1]. Liquid–solid, normal phase liquid–liquid, and ion-exchange systems have predominated in applications to formulation[2-4] and residue[4-7] analysis. A major application lies in separation of heat-labile or non-volatile compounds, often following conversion to UV absorbing[6,8] or fluorescing[9,10] derivatives for enhancement of detectability. A further advantage of LC may lie in the need for less extensive cleanup than required with gas–liquid (GLC) or thin-layer chromatographic (TLC) methods[5,9-11]. Ready availability of LC retention and response characteristics with several mobile–stationary phase combinations and detectors should hasten further applications in the pesticide field.

Reversed-phase (RP) chromatography has shown excellent promise for resolution of compounds of low and medium polarity[12]. Among pesticide-related compounds, RPLC retention data have been reported for a few chlorinated hydrocarbons[13], carbamate insecticides[14] and herbicides[1], and chlorinated dibenzo-*p*-dioxins[3]. Generally carried out on chemically bonded octadecylsilanes such as C_{18}/ Corasil, Permaphase ODS, and Vydac reversed-phase, RPLC separates primarily by solubility characteristics. For example, the logarithm of the capacity factor was shown

to increase linearly with the carbon number of polynuclear aromatic hydrocarbons on RPLC. reflecting their decreasing solubility in water–methanol with increasing molecular weight[15]. Pesticides represent too structurally diverse a class of chemicals for a similar simple correlation to hold. However. relative solubilities for a large number of pesticides have been tabulated by Beroza *et al.*[16] as partition *p*-values. a measure of per cent of solute distributed in the non-polar phase of an equivolume immiscible solvent pair. It was of interest to determine the relationship, if any. between *p*-values and high-pressure LC retention data developed with similar solvent systems.

EXPERIMENTAL

The liquid chromatograph was assembled from parts purchased from Laboratory Data Control (LDC) (Riviera Beach. Fla.. U.S.A.). These included a Milton-Roy mini-pump. an LDC Model 709 pulse dampener. a 1 m $\times$ 2.1 mm I.D. precision-bore stainless-steel column. and an LDC Model 1205 254-nm UV detector. The column was dry-packed in a vertical position with Vydac reversed-phase (The Separations Group, Hesperia. Calif.. U.S.A.) with gentle vibration. The packed column was jacketed for elevating its temperature with water circulated from a thermostatted bath.

On-column injection was followed by a stop flow technique. Stirred solvent mixtures were degassed prior to use by simultaneous warming and moderate evacuation for several minutes. Pesticide standards. all analytical grade or equivalent, were made to known concentrations in methanol. Collected Arochlor 1254 fractions were extracted with hexane and the hexane extract analyzed by coupled GLC–mass spectrometry using a 1.2 m $\times$ 2 mm I.D. glass column containing 2% OV-1 on Chromosorb G and a Finnigan Model 3000 peak identifier.

RESULTS AND DISCUSSION

To compare retentions. R'_M values (adjusted retention relative to methoxychlor) were used. such that

$$ R'_M = \frac{(R_e - R_0)_{compound}}{(R_e - R_0)_{methoxychlor}} \times 100 $$

where R_e and R_0 are the retentions (measured in volume or time units) of the compound concerned and that of an unretained component. respectively. Methoxychlor was selected as a reference. having retention. *p*-value. and response intermediate among the pesticides studied.

Of the six solvent systems for which *p*-values have been tabulated. heptane–90% ethanol (1:1) appeared closest to the octadecylsilane–aqueous alcohol partition system present in RPLC. R'_M values were obtained under two conditions: In one. A. the solvent was aqueous methanol at 50°: conditions recommended for RPLC[12]. In the second. B. the solvent was aqueous ethanol at 25°. this system was chosen to more closely approximate the conditions under which the *p*-values were experimentally determined[16], although it leads to less efficient chromatography than the first. For each solvent mixture. two proportions of water and the alcohol were used. to elute all 17 compounds in a convenient time. The literature *p*-values and experimental R'_M values are listed in Table I. A least squares plot of log R'_M *vs.* *p*-values (Fig. 1) shows

TABLE I

RELATIVE RETENTION AND UV$_{254}$ RESPONSE OF SOME PESTICIDES ON VYDAC REVERSED-PHASE LIQUID CHROMATOGRAPHY

Compound	Relative response[*]	p-Value[**]	$R_M^{\cdot}$	
			A[***]	B[§]
Carbaryl	83	0.06	0.94	1.6
Methyl parathion	150	0.11	3.17	7.0
Azinphosmethyl	38	0.14	3.10	4.6
Malathion	3	0.14	6.18	6.2
Dyrene	300	0.17	4.00	6.0
Dicapthon	75	0.25	10.3	21.6
Ethyl parathion	105	0.30	16.0	28.0
Diazinon	78	0.39	30.5	27.0
Methoxychlor	100	0.44	100	100
p,p'-DDD	26	0.46	196	249
Dieldrin	1	0.54	113	149
Heptachlor epoxide	1	0.57	97	137
Azobenzene	59	0.59	64	98
p,p'-DDT	26	0.64	595	655
o,p'-DDT	17	0.68	639	565
Trifluralin	140	0.72	190	249
p,p'-DDE	260	0.76	829	860

[*] Based on area, relative to methoxychlor (100).
[**] In heptane–90% ethanol[10].
[***] 29% and 50% aqueous methanol at 50° (system A).
[§] 29% and 50% aqueous ethanol at 25° (system B).

$$\text{Log } R_M^{\cdot} = 0.06 + 3.73\,(p)$$

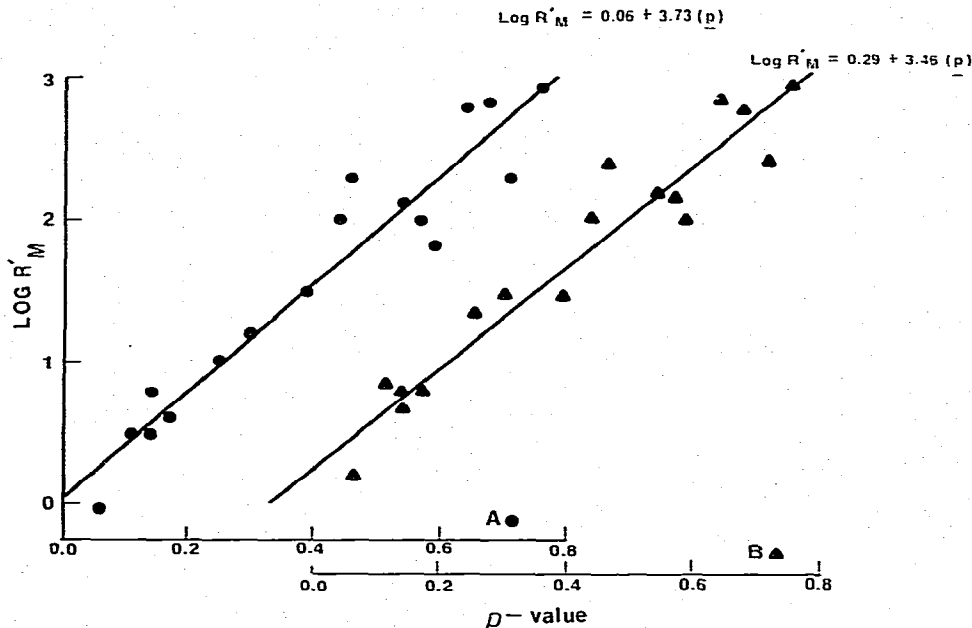$$\text{Log } R_M^{\cdot} = 0.29 + 3.46\,(p)$$

Fig. 1. Plots of the logarithm of the adjusted retention relative to methoxychlor ($R_M^{\cdot}$) and p-value (heptane–90% ethanol) in (A) 29% and 50% aqueous methanol at 50°, and (B) 29% and 50% aqueous ethanol at 25°. Lines drawn by method of least squares.

the linear trend followed between the two. The correlation coefficients were 0.957 and 0.959 for systems A and B, respectively (standard errors of estimate 0.256 and 0.232, respectively: probability <0.001 for both)[17].

The presence of individual deviations of the linear relationship is not surprising, given the structural diversity among the compounds studied and the fact that the heptane–90 % ethanol p-value solvent pair only approximates in polarity the RPLC systems. Some deviation may also arise from departure from pure partition in the column: while the dominant retention mechanism involving bonded phases is undoubtedly partition in nature, the possibility of adsorption, at the polymer surface or at unreacted sites on the solid support, cannot be excluded[18]. A certain amount of imprecision, of course, exists in both the R'_M and p-values. Errors in both should increase at high and low values: this was anticipated and led to choosing more compounds for data points at the two extremes. With these considerations in mind, the degree of correlation was reasonably good. Furthermore, the correlations for systems A and B were approximately equal: there appears to be no advantage to using the less efficient B system when applying the relationship.

Better correlations were expected within series of closely related compounds. Indeed, for thiophosphates (methyl parathion, azinphosmethyl, dicapthon, ethyl parathion, and diazinon) in system A, a high correlation coefficient (0.993) resulted. The coefficient was, however, considerably lower (0.895) for the same series in system B: furthermore, the correlation for DDT relatives (methoxychlor, p,p'-DDD, p,p'-DDT, o,p'-DDT, and p,p'-DDE) was no better in either system (0.964 in A, 0.928 in B) than that for the total group. Visual inspection of the points for the DDT relatives suggests that a non-linear function might better describe their relation to p-values. More compounds must be examined to pursue this possibility.

The relationship described herein can have practical value in several ways. Selection of optimum solvent ratios for elution of single compounds or mixtures may be facilitated. Relative elution orders of compounds of reported or experimentally determined p-values may be predicted. An idea of the polarity, and thus the nature, of unknowns can be obtained by comparing their retentions with those of a few reference standards.

Typical chromatograms of some pesticides in system A are given in Figs. 2 and 3. Superimposed in Fig. 3 is a chromatogram of Arochlor 1254, a polychlorinated biphenyl (PCB) often encountered as a contaminant in residue analysis of chlorinated insecticides[19]. Using the solubility criterion, it was expected that retention of PCB components should increase with increasing chlorine number. Six fractions were collected at the peak areas indicated on the chromatogram and analyzed by coupled GLC–mass spectrometry. Both the GLC retention times and mass spectrometric molecular ions confirmed the expected elution order: LC peak A had a major Cl_4 component, peaks B–D were enriched in Cl_5 components, and fractions E and F contained primarily Cl_6 components. Further LC analysis under the same conditions of a series of Arochlor PCB's of increasing chlorine percentages showed major (greater than 90 %) elution of Arochlor 1221 within 5 min, Arochlor 1232 within 10 min, Arochlor 1254 from 10–50 min, and Arochlor 1260 from 20–80 min of injection. These results are in agreement with those reported for migration of various chlorinated aromatics, including PCB's, on RPTLC[20], and for elution of chlorinated dibenzo-p-dioxins[3], chlorinated benzenes[13], and constituents of toxaphene[21] on RPLC. Further
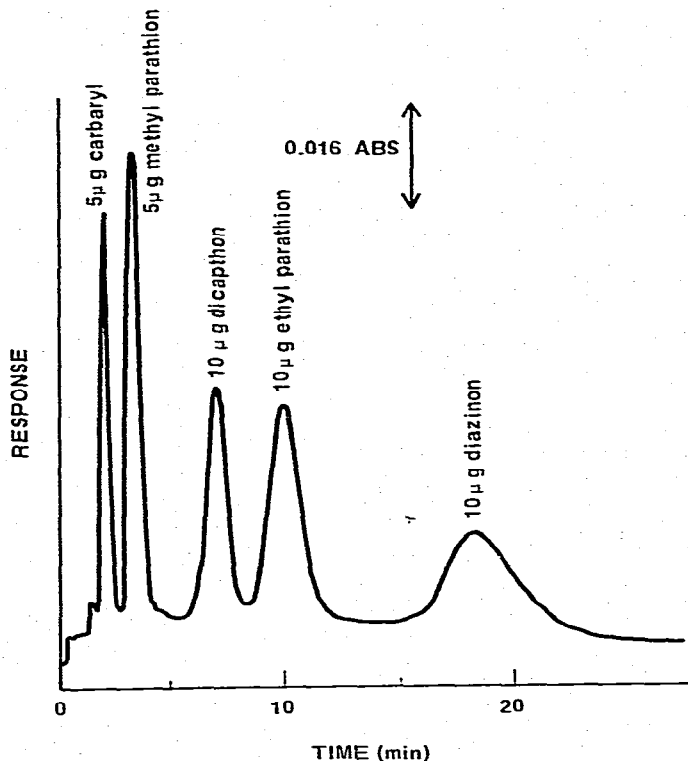
Fig. 2. Chromatogram of five pesticides on Vydac reversed-phase. Solvent. 29% aqueous methanol; temperature. 50°; flow-rate. 1 ml min; pressure. 780 p.s.i.
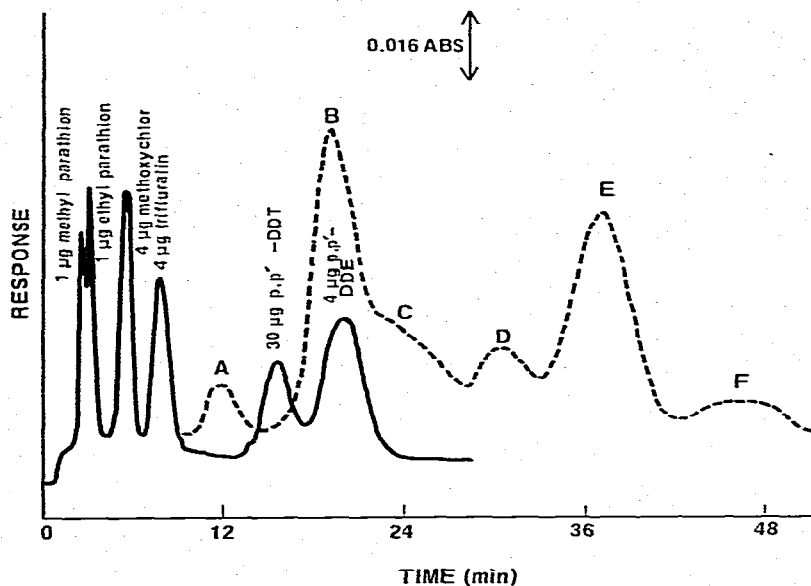
Fig. 3. Chromatogram of six pesticides on Vydac reversed-phase, with elution profile of Arochlor 1254 superimposed and marked for fraction collection. Solvent, 50% aqueous methanol; temperature, 50°; flow-rate, 1 ml/min; pressure, 850 p.s.i.

definition of the relationship between retention and chlorine number among PCB components could be of use in confirmatory residue analysis.

In the course of this study, relative responses for the 17 pesticides to the 254-nm UV detector were obtained (Table I). For compounds absorbing strongly at 254 nm, such as Dyrene and p,p'-DDE, the limit of detectability (peak height twice background) was approximately 10 ng, providing the solvent mixture was adjusted to allow complete elution within 10 min. The limits will vary with the background from one instrument to another; they may be significantly improved for many compounds by adjusting the wavelength of detection to the compound's UV absorption maximum. The limitations of the 254-nm UV detector are apparent from the wide range in the observed responses; clearly a more satisfactory detection system is desirable for broader applicability in pesticide analysis.

CONCLUSIONS

RPLC represents an excellent choice for resolving many pesticides of low and medium polarities. Choice of eluting solvent ratio and prediction of relative retentions in the separation of mixtures may be facilitated by reference to Beroza p-values for heptane–90 % ethanol. Other relationships of predictive value may be found within related groups of compounds, or in normal phase LC partition systems. Resulting compilations of retention for large numbers of chemicals should encourage further applications of LC in pesticide formulation and residue analysis.

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REFERENCES

1 D. F. Horgan, Jr., in G. Zweig (Editor), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. 7, Academic Press, New York, 1973, Ch. 2.
2 N. E. Skelly and R. F. Cornier, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 551.
3 W. B. Crummett and R. H. Stehl, *Environ. Health Perspect.*, Exp. Issue No. 5 (1973) 15.
4 R. A. Henry, J. A. Schmit, J. F. Dieckman and F. J. Murphey, *Anal. Chem.*, 43 (1971) 1053.
5 J. G. Koen and J. F. K. Huber, *Anal. Chim. Acta*, 51 (1970) 303.
6 J. J. Kirkland, R. F. Holt and H. L. Pease, *J. Agr. Food Chem.*, 21 (1973) 368.
7 F. Eisenbeiss and H. Sieper, *J. Chromatogr.*, 83 (1973) 439.
8 P. J. Porcaro and P. Shubiak, *Anal. Chem.*, 44 (1972) 1865.
9 R. W. Frei and J. F. Lawrence, *J. Chromatogr.*, 83 (1973) 321.
10 R. W. Frei, J. F. Lawrence, J. Hope and R. M. Cassidy, *J. Chromatogr. Sci.*, 12 (1974) 40.
11 D. F. Horgan, Jr., W. A. Dark and K. J. Bombaugh, *Tech. Rep.*, No. 19531, Waters Associates, Framingham, Mass.
12 J. A. Schmit, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
13 P. R. Brown, *High Pressure Liquid Chromatography*, Academic Press, New York, 1973, p. 177.
41 A. D. Thruston, Jr., *Liquid Chromatography of Carbamate Pesticides*, U.S. EPA Technical Report EPA-R2-72-079, Corvallis, Ore, October, 1972.
15 R. B. Sleight, *J. Chromatogr.*, 83 (1973) 31.

16 M. Beroza, M. N. Inscoe and M. C. Bowman, *Residue Rev.*, 30 (1969) 1.
17 *Program 1316 EB/ST3*, Rev. 1, 700 Series Program Library 9, Wang Laboratories, Tewksbury, Mass., 1971.
18 J. J. Kirkland, *J. Chromatogr. Sci.*, 9 (1971) 206.
19 L. Fishbein, *J. Chromatogr.*, 68 (1972) 345.
20 D. L. Stallings and J. N. Huckins, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 367.
21 J. E. Casida, R. L. Holmstead, S. Khalifa, J. R. Knox, T. Ohsawa, K. J. Palmer and R. Y. Wong, *Science*, 183 (1974) 520.