Journal of Chromatography, 161 (1978) 59–68

CHROM. 11,196

CHROMATOGRAPHIC ANALYSIS OF MIXTURES OF UNKNOWN COMPLEXITY

USE OF RELATIVE RETENTION AND CAPACITY FACTOR DATA

R. J. LAUB and J. H. PURNELL

Department of Chemistry, University College of Swansea, Swansea, Wales SA2 8PP (Great Britain)

(Received April 24th, 1978)

SUMMARY

The method of optimizing column composition for the chromatographic analysis of complex mixtures of unknown composition already described by us is modified to allow use of the practical parameters: relative retention, capacity factor and weight fraction composition of a binary stationary phase. The separation of a mixture of unknown chlorinated phenols and cresols is used as an illustration.

INTRODUCTION

The liquid–gas partition coefficient of any sample component eluted from a column containing two liquids (A and S) which are not mixed must be defined by the equation:

$$K_R = \Phi_A K^0_{R_{(A)}} + \Phi_S K^0_{R_{(S)}} \quad (1)$$

where $K^0_{R(A)}$ and $K^0_{R(S)}$ pertain to sample components eluted from the pure phases, A and S, respectively, and Φ represents a volume fraction. Eqn. 1 then immediately leads to:

$$\alpha_{2/1} = \frac{X_{R_2}}{X_{R_1}} = \frac{\Phi_A K^0_{R_{(A)_2}} + \Phi_S K^0_{R_{(S)_2}}}{\Phi_A K^0_{R_{(A)_1}} + \Phi_S K^0_{R_{(S)_1}}} \quad (2)$$

whence α, the relative retention of two sample components (1 and 2), is immediately calculable as a function of Φ_A and Φ_S. Plots of α against Φ_A for all solute pairs in the mixture, christened window diagrams[1], then allow identification of the optimum substrate composition by inspection. This procedure has met with complete success in application to problems in gas–liquid chromatography, gas–solid chromatography, gas–liquid–solid chromatography and high-performance liquid chromatography

insofar as predicting optimal substrate or solvent compositions for given separations[2–6] and has, further, been fully computerized[2,5]. However, the chromatographic analysis of mixtures of initially-unknown composition and complexity[7] remains its most powerful application to date.

The procedure previously described for dealing with unknown mixtures was as follows.

(i) The mixture is chromatographed with columns containing pure stationary phases A and S, respectively. The partition coefficients of all visible peaks are measured from the chromatograms.

(ii) The mixture is next eluted from several columns, each containing an A+S mechanical mixture of known Φ_A, *e.g.*, $\Phi_A = 0.333$ and $\Phi_A = 0.667$. Partition coefficients of all visible peaks are again measured.

(iii) The partition coefficient data are plotted on a common graph of K_R against Φ_A and, since K_R *versus* Φ_A must be a linear function for mechanically-mixed stationary phases, straight lines are drawn through all points that fit. Obviously, if n columns have been used, only those straight lines having n points need be considered. Several lines may be drawn with the aid of these constraints and with reference to the solute peak areas. At this stage, there may, coincidentally, be more lines than actual components. Several points, however, may be left over, that is, do not seem to correspond to any one solute.

(iv) A window diagram is constructed from the data as described above and the optimum Φ_A, evaluated from the corresponding value of α, and the length of column required to baseline separate all indicated peaks is calculated. The column is constructed and a sample run. The correct number of peaks is immediately recognised, and spurious lines on the original K_R/Φ_A plot expunged.

(v) A window diagram is constructed for the "real" components, that is, those actually present in the mixture, followed by the construction of a final column corresponding to the predicted optimum composition of A+S and calculated length. The mixture components are resolved with this column.

In our initial report of the foregoing[7], we made use of partition coefficient and stationary-phase volume fractions. Such quantities offer no difficulty of determination where stationary phases such as squalane are concerned. In contrast, problems are encountered when polymeric phases are used, in particular as regards defining the molecular weight of such materials and determining their density at elevated temperatures. To overcome these and related objections to the use of our earlier procedures, we have recently pointed out that eqn. 1 may be cast in the two alternative forms:

$$V_g^T = W_A V_{g(A)}^{T,0} + W_S V_{g(S)}^{T,0} \tag{3}$$

$$\alpha = W_S \alpha_{(S)}^0 + W_A \alpha_{(A)}^0 \beta_{(A)}^0 \tag{4}$$

Eqn. 3 expresses the linear dependence of the solute specific retention volume (at the column temperature), V_g^T, upon the weight fraction, W_A, of one component of the binary (A+S) stationary phase. While representing a useful simplification insofar as obviating the need for density data, it still demands accurate measurement of both

the column pressure drop and liquid-phase weight percent, the latter being by no means a straight-forward procedure[9].

Eqn. 4 overcomes the above difficulties by making use solely of purely practical quantities that can be read directly from the chromatogram. Thus, we may use α, the solute relative retention time with the binary phase, $\alpha^0_{(S)}$ and $\alpha^0_{(A)}$, the relative retention times with pure S and A, respectively, and $\beta^0_{(A)}$, the capacity factor ratio of the (internal or external) standard solute with pure A and with pure S:

$$\beta^0_{(A)} = \frac{k'_{(A)}}{k'_{(S)}} \tag{5}$$

One of the compelling advantages of the window diagram procedure for solute mixtures of a specified number of known components is that data only for the two end-points of the lines on K_R/Φ_A plots need be measured. Eqn. 4 correctly orders these data in terms of α and W, with respect both to position and magnitude, by inclusion of the $\beta^0_{(A)}$ factor. (When standard columns containing pure phases are used such that the weight of one of the phases per unit length, say column A, is not equal to that of the other, namely, column S, it is to be noted that $\beta^0_{(A)}$ must be multiplied by the weight ratio, W_S/W_A, to account for discrepant retention due to the differences in liquid loadings.) However, values of α must be defined at intermediate weight fraction compositions for the analysis of mixtures of unknown content; these are correctly ordered and adjusted with regard to magnitude by multiplication by β^{W_A}, that is, the quotient of the capacity factor of the standard solute with a column containing W_A amount of A and S, and that corresponding to pure S:

$$\beta^{W_A} = \frac{k'_{(W_A)}}{k'_{(S)}} \tag{6}$$

The analysis of mixtures of unknown content thus reduces to the sequence indicated above, except that α values, capacity factors, and weight fractions are employed rather than partition coefficients and volume fractions.

We illustrate below the application of eqns. 4–6 in the analysis of a mixture which was supplied independently to us and which was said to contain chlorinated phenols and cresols in an undetermined number.

EXPERIMENTAL

All separations were performed with 5-ft. packed glass columns of 6 mm O.D. × 4 mm I.D. contained in a Pye 104 FID gas chromatograph, unless otherwise noted. The stationary phases and support material (Chromosorb G AW DMCS, 120–140 mesh) were obtained from Jones Chromatography (Llanbradach, Great Britain). The solute mixture was supplied by an agricultural research station.

Liquid-phase loadings of 5.0% were used throughout this work in order to simplify the application of eqn. 4. Mechanical mixtures, that is, (support + A) + (support + S) were employed for intermediate compositions of A + S.

RESULTS

In chromatographic analysis of the type described here, the choice of a stationary-phase pair is of initial concern. Primarily, the liquid phases must, first, allow some separation of the mixture components, secondly, yield reasonable column efficiencies, thirdly, offer acceptable retention times and, fourthly, each must exhibit selectivity which is sufficiently different from the other to indicate the likely possibility of the success of an intermediate mixture of the two. Several secondary criteria (such as cost, availability, etc.) may, at a later stage, also require consideration.

As a rough rule, therefore, one of the test phases should be, in all but the most

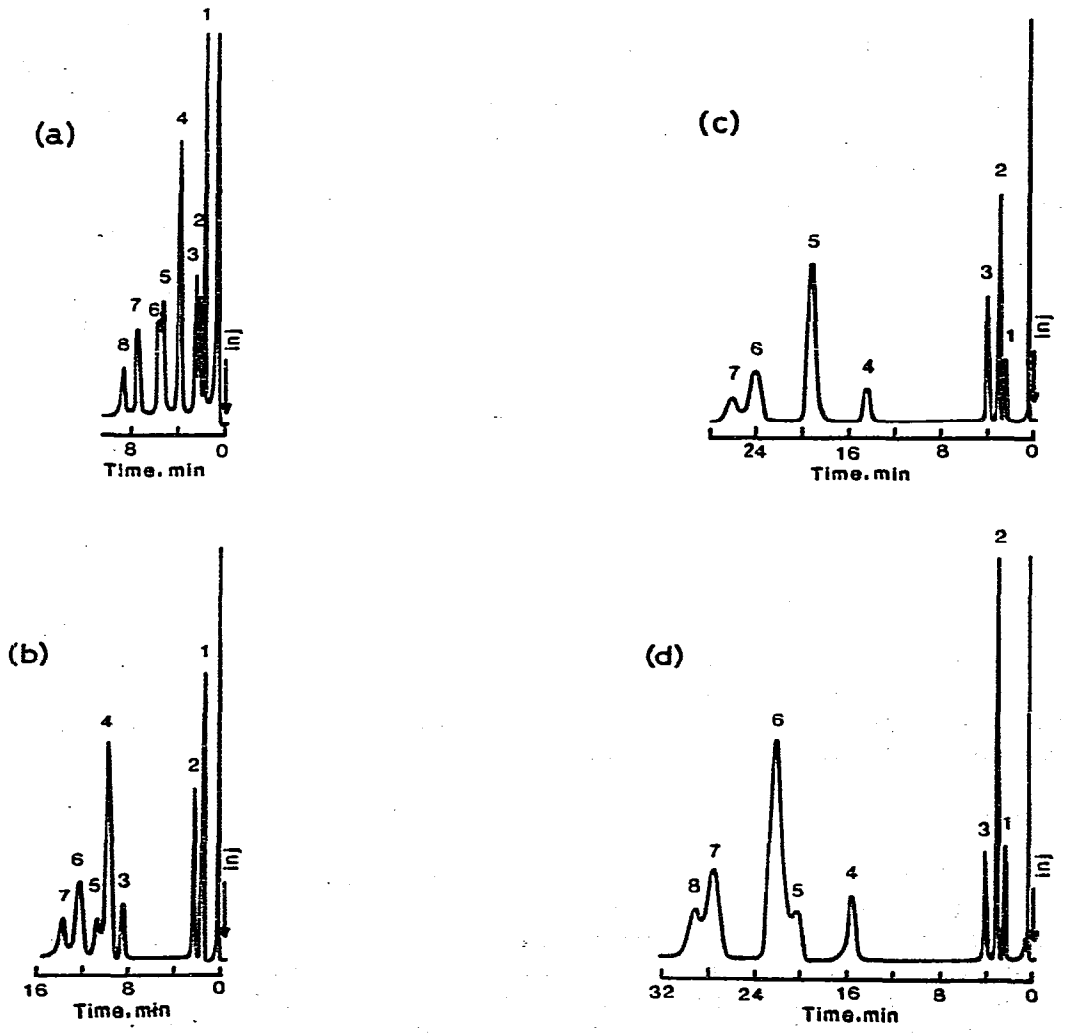

Fig. 1. Chromatograms of the mixture of unknown number of chlorinated phenols and cresols at 175° with columns (5 ft. × 6 mm O.D. × 4 mm I.D. glass) containing 5% (w/w) of (a) pure OV-17 ($w_A = 0$), (b) $w_A = 0.333$, (c) $w_A = 0.667$ and (d) pure Carbowax 20M ($w_A = 1.0$).

TABLE I

RELATIVE RETENTION DATA FOR PEAKS OF FIG. 1

Solute No.	$\alpha^{\circ}_{(OV-17)}$	$\alpha \cdot \beta^{33}$	$\alpha \cdot \beta^{67}$	$\alpha^{\circ}_{(20M)} \cdot \beta^{\circ}_{(20M)}$
1	0.369	0.966	1.337	1.685
2	0.538	1.368	1.552	2.130
3	0.606	5.344	2.153	2.874
4	1.000	6.190	8.611	11.775
5	1.425	6.842	11.330	15.276
6	1.513	7.931	14.367	16.515
7	2.150	8.817	15.545	20.710
8	2.569	—	—	21.998

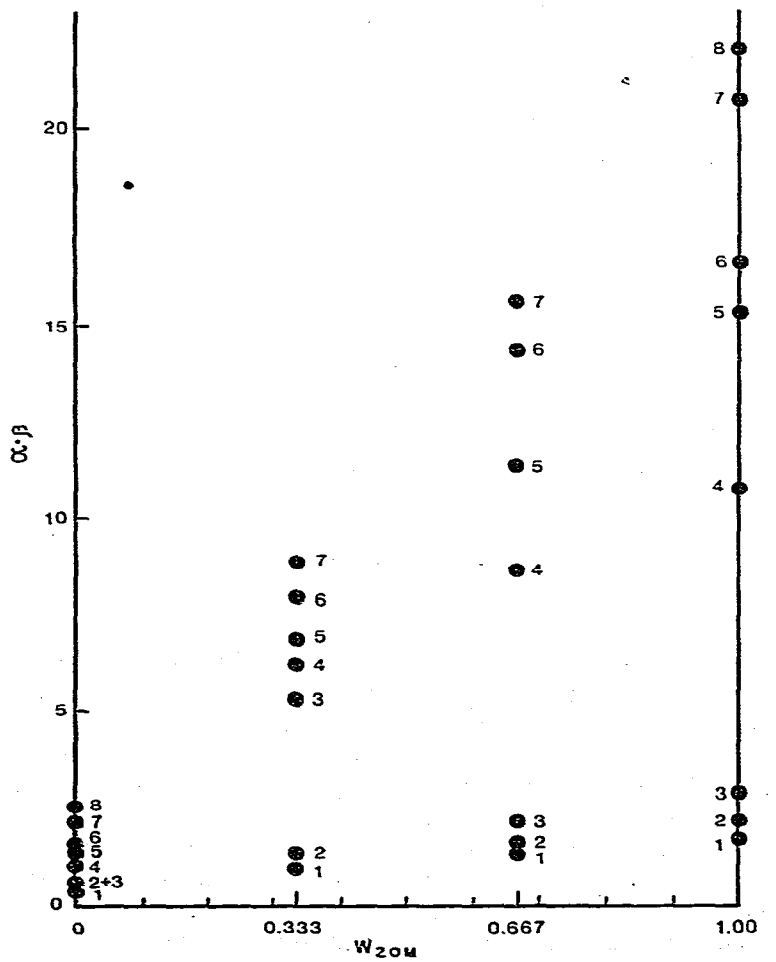

Fig. 2. Plot of retention data relative to peaks (a) 4, (b) 4, (c) 5 and (d) 6 of Fig. 1 *vs.* w_A. Points are numbered according to order of elution in Fig. 1.

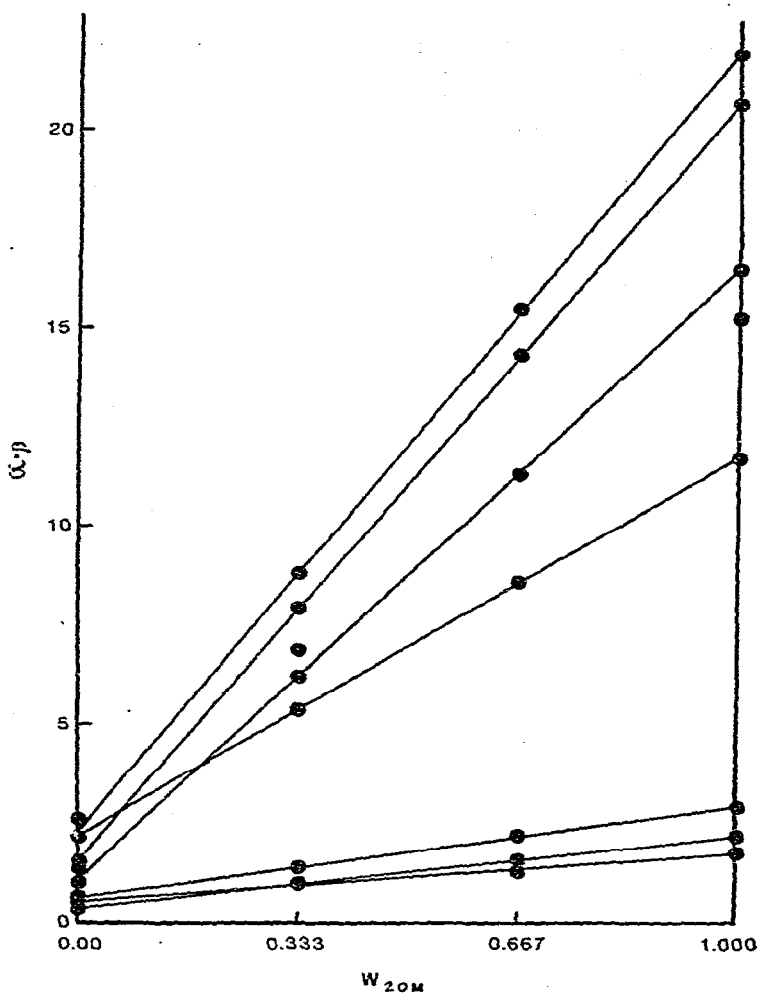

Fig. 3. Lines drawn through points of Fig. 2 which correspond to same solutes. Tentative identification based upon inspection of Fig. 1.

difficult of cases, a "boiling-point" phase, such as squalane or SE-30. However, here, they are unacceptable since they are known to give rise to marked gas–liquid interfacial adsorption with consequent peak tailing with solutes such as phenols. Thus, in this case, we chose OV-17 (50% phenylmethyl silicone) which, by virtue of its aromatic content, offered reduced peak tailing and which gives an elution order which corresponds roughly to boiling point for similar sample types.

The choice of the second member of a stationary-phase pair is by far the more crucial. The fourth criterion, that of selectivity, is the most important to be applied since no phase, regardless of its inherent efficiency, will provide enhanced separation upon being mixed with the first phase unless the solute retention characteristics found with it are sufficiently different. Thus, for example, having chosen OV-17 as one phase, it will do little good to use OV-22 (65% phenylmethyl silicone) as the second

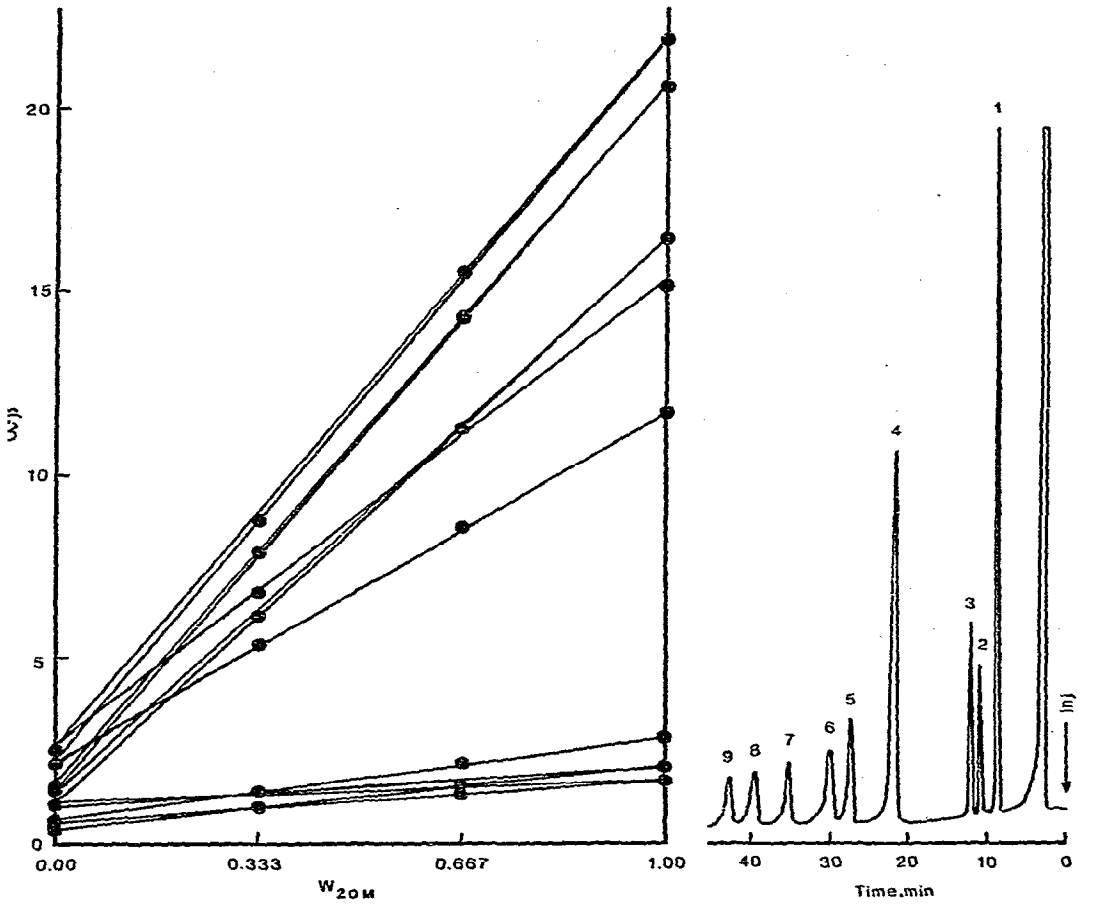

Fig. 4. All possible lines drawn through all possible combinations of sets of points of Fig. 2.

Fig. 5. Chromatogram of the mixture at 175° with a mechanical mixture of pure-component packings such that $w_{20M} = 0.02$. Column: 20 ft. × 6 mm O.D. × 4 mm I.D. glass; inlet pressure: 50 p.s.i.g.

since both phases offer very similar retention characteristics. A cursory perusal of any one of the many compendia of gas chromatographic retention data will, however, indicate one or more possibilities likely to prove satisfactory. In the present instance, Carbowax 20M was chosen on the basis that it has proved to be very selective, on other than a boiling-point basis, for a wide range of solute chemical types. Further, it offers, in our experience, high column efficiency and retention times, even for compounds such as alcohols, aldehydes and ketones, that are reasonably short.

Operating conditions, such as temperature, must now be chosen. A few test runs soon establish the conditions corresponding to reasonable elution times and column efficiencies. We found that the mixture at hand eluted with moderate efficiency from 5-ft. columns of 5% liquid loadings at 175°, and 15 to 30 p.s.i.g. inlet pressure. These conditions were, therefore, used throughout.

Fig. 1 shows the chromatograms obtained for the mixture with the stationary

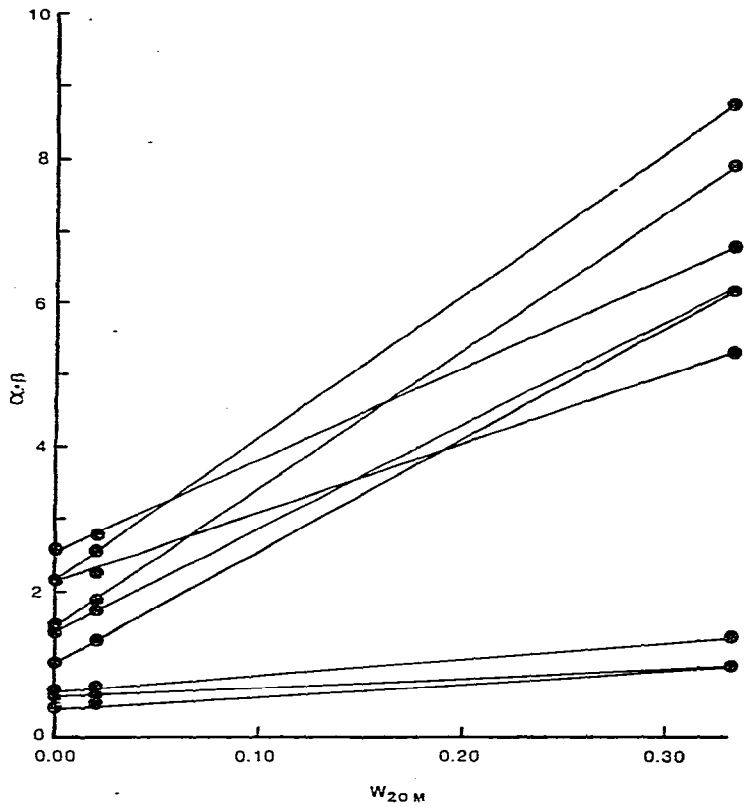

Fig. 6. Expanded-scale plot of relative retention data of Fig. 4 *vs.* w_{20M}, where fictitious lines have been removed.

phases. Table I lists the $\beta^0_{(S)}$ and $\alpha \cdot \beta$ for all visible peaks relative to the largest component in the middle of each run and the points are shown plotted in Fig. 2. Several lines may be drawn through sets of points; inspection of the peak areas of Fig. 1 assists in choice and in eliminating obviously spurious possibilities. These lines are shown in Fig. 3. Several other points, however, have not been used; these solutes must, at some or another weight fraction, have overlapped fully with other components. Fig. 4 shows all possible lines drawn through all points and we see for the first time that there may be as many as thirteen solutes present in the mixture. In order to eliminate fictitious lines, a fifth column must be used such that all peaks, if present, would be visible (although not necessarily baseline-resolved). Referring back to Fig. 4, we see that a column of 2% 20M will show all thirteen solutes. However, several pairs of these, if real, will be overlapped unless the column yields a number of plates sufficient to resolve a minimum α value of 1.05 (N_{req} = 7100 plates for 2σ separation). Since we obtained 350 plates per foot with the OV-17 packing, a column of 20 ft. is indicated. Fig. 5 shows the chromatogram obtained with such a column (50 p.s.i.g. inlet pressure) and we see that there are nine solutes in the mixture. An expanded version of Fig. 4, with the fictitious lines removed, may now be constructed and is shown in Fig. 6.

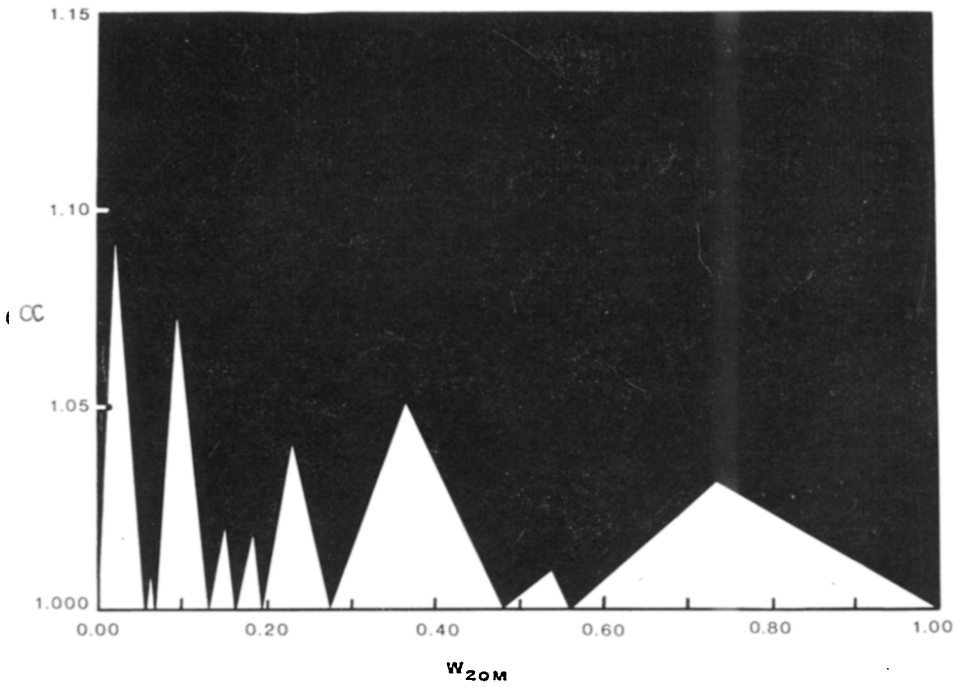

Fig. 7. Window diagram of solutes of Fig. 6. Optimum weight fraction of Carbowax 20M is predicted to be 0.02.

Finally, having identified the actual number of solutes present, a window diagram may be constructed from the data of Table I and Fig. 6, as shown in Fig. 7. This indicates that, fortuitously, 2% 20M is, in fact, the optimum column composition and that the most difficult α value is 1.095. (N_{req} = 5000 plates; L_{req} = 14.5 ft.) Thus, complete separation has, at this point, been achieved and the only parameter of interest left, namely, the time of analysis, could, according to the window diagram, be reduced significantly by shortening the column length by a factor of 3/4. Alternatively, since the 20-ft. column already at hand provides more than enough theoretical plates to effect separation, it may be used at increased flow-rate and temperature. Fig. 8 shows the chromatogram obtained with this column at 200° and 75 p.s.i.g. where the analysis time has been reduced from 45 to 16.5 min which we believe would be regarded as being of reasonably short duration from most points of view.

DISCUSSION

We regard the analysis of mixtures of unknown content as described above as a significant advance in that, throughout the procedure, use was made solely of relative retention and capacity factor data. Furthermore, two polymeric stationary phases were employed. In addition, the solute mixture consisted of underivatized chlorinated phenols and cresols which are generally considered difficult to elute from mixed phases on a quantitative basis because of gas–liquid interfacial adsorption

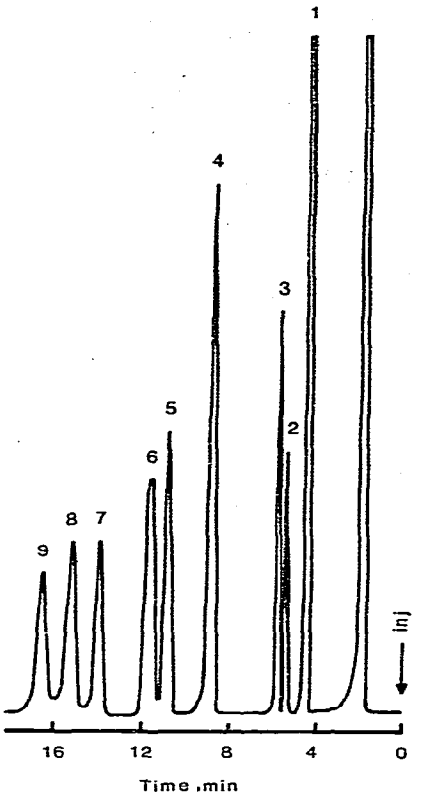

Fig. 8. Chromatogram of the mixture at 200° and at 75 p.s.i.g. inlet pressure. Column and packing identical to those of Fig. 5.

effects, yet which were baseline-resolved by the optimum column composition predicted by the window diagram.

Since we have now applied the window diagram technique to the separation of underivatized sterols, aromatic amines and, in this report, chlorinated phenols and cresols with stationary phase pairs as diverse as methylsilicone gum with 2,4,7-trinitrofluorenone and methyltrifluoropropylsilicone, and methylphenylsilicone with polyethylene glycol, we regard its use as compatible with virtually any class of solutes with all currently-available stationary phases.

REFERENCES

1 R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 112 (1975) 71.
2 R. J. Laub, J. H. Purnell and P. S. Williams, *J. Chromatogr.*, 134 (1977) 249.
3 W. K. Al-Thamir, R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 142 (1977) 3.
4 R. J. Laub and J. H. Purnell, *Anal. Chem.*, 48 (1976) 799.
5 S. N. Deming and M. L. H. Turoff, *Anal. Chem.*, in press.
6 R. J. Laub, J. H. Purnell and P. S. Williams, *Anal. Chim. Acta*, 95 (1977) 135.
7 R. J. Laub and J. H. Purnell, *Anal. Chem.*, 48 (1976) 1720.
8 R. J. Laub, J. H. Purnell, D. M. Summers and P. S. Williams, *J. Chromatogr.*, 155 (1978) 1.
9 R. J. Laub, J. H. Purnell, P. S. Williams, M. W. P. Harbison and D. E. Martire, *J. Chromatogr.*, 155 (1978) 233.