SEPARATION OF CHLORAMPHENICOL INTERMEDIATES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON MICROPAK-NH$_2$ COLUMNS

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SUMMARY

A column containing a chemically bonded phase, MicroPak-NH$_2$, was used to separate six components of a chloramphenicol-intermediate mixture. Two-component eluents comprising a non-hydrogen-bonding apolar solvent (cyclohexane) and a polar hydrogen-bonding solvent (dioxan, tetrahydrofuran or isopropanol) were tested. A correlation was found to exist between the values of the capacity factor (k') and the Hildebrand parameter (δ). Three-component eluents comprising cyclohexane and one of the binary mixtures dioxan–isopropanol or tetrahydrofuran–isopropanol were also studied. Optimum separation conditions with respect to analysis time, degree of resolution and detection limit were established. The system was successfully used to analyse production samples.

INTRODUCTION

Chloramphenicol is a widely used antibiotic. At a certain stage in its synthesis, the several contaminants shown in Fig. 1 can occur. Compound 1 is the main product, and compounds 2–6 are the contaminants expected, but slight changes in the syn-
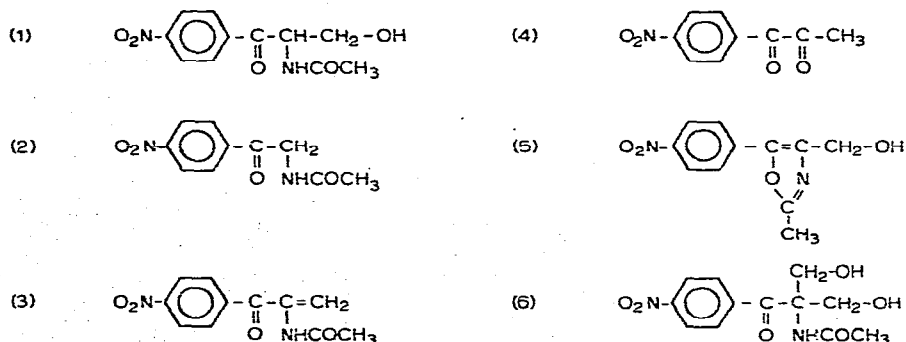

Fig. 1. Compounds of the chloramphenicol intermediate mixture to be separated; compound 1 is the main product; compounds 2–6 are the contaminants.

thesis parameters can significantly alter the amounts of these contaminants. This paper presents the final part of a research project undertaken to develop a simple, rapid and sensitive method for separation and trace-level determination in industrial practice.

A semi-quantitative thin-layer chromatographic method[1] has been used hitherto for controlling the amount of compounds 2–6 in crude 1. High-performance liquid chromatography (HPLC) on an ion-exchange column[2] was successfully employed to separate three of the compounds involved, and five of the compounds were satisfactorily separated on a column (0.3 m × 1.8 mm I.D.) of Merck HR 60 high-performance silica of nominal particle-diameter range 10–15 μm with an eluent consisting of 20% of dioxan and 80% of cyclohexane[3]. The compounds could also be separated on a column of Varian MicroPak-CN[4], although no real trace-level detection could be achieved and compound 4 was hardly retained at all on this column.

As, for certain reasons, chemically bonded phases were to be preferred[3], MicroPak-NH$_2$ seemed to be the next logical choice, and we carried out an extensive study on three 2-component and two 3-component eluents in attempts to establish the shortest analysis time consistent with adequate resolution and trace-level determination.

EXPERIMENTAL

Equipment

A liquid chromatograph consisting of a Model 6000 pulseless high-pressure pump (Waters Associates, Milford, Mass., U.S.A.), a 0.125-in. low-pressure injector head (Varian Aerograph, Walnut Creek, Calif., U.S.A., custom-modified) a water-jacketed[5] column (0.25 m × 0.125 in. O.D.) of MicroPak-NH$_2$ (Varian) and a 254-nm UV detector (Varian) with a 1-mV dual-channel recorder (Varian, type A-25) were used. The column temperature was maintained at 50.0° with the help of a type U-10 controlled-temperature water bath (MLW, Medingen, G.D.R.).

Materials

Compounds 1–6 were obtained from the EGYT Pharmaceutical Factory (Budapest, Hungary). The eluents were prepared mostly from Uvasol-grade solvents (E. Merck, Darmstadt, G.F.R.) or analytical grade solvents (Reanal, Budapest, Hungary), the latter being further purified[6].

RESULTS AND DISCUSSION

The main type of interaction expected between compounds 1–6 and the -NH$_2$ group of the stationary phase was hydrogen bonding. Accordingly, two solvents similar in selectivity but different in polarity (tetrahydrofuran and dioxan), and a third, different in both polarity and selectivity (isopropanol), were chosen to modify the cyclohexane eluent.

Table I shows the k' and $\alpha_{i,j}$ values obtained by varying the dioxan content from 2.5 to 100% (v/v). With dioxan concentrations less than 40%, excessively high k' and α values were encountered, whereas with a dioxan content of more than 80%, k' and α were much too low for useful separation. Fig. 2 shows the separation of the

TABLE I

VALUES OF CAPACITY AND SELECTIVITY FACTORS FOR COMPOUNDS 1–6 WITH DIOXAN–CYCLOHEXANE AS ELUENT

The values listed were obtained on a 0.25-m × 0.125-in. O.D. MicroPak-NH$_2$ column operated at an eluent flow-rate of 2.0 ml/min.

Compound or compound pair	Dioxan content of eluent (%, v/v)									
	2.5	5	10	15	20	30	40	60	80	100
Value of capacity factor (k')										
1	—	—	—	—	257	71.6	32.8	7.94	3.18	1.15
2	—	—	—	121	58.9	21.6	10.6	3.29	1.42	0.65
3	169	74.2	31.5	18.3	7.45	3.09	1.68	0.67	0.33	0.01
4	7.5	4.24	3.3	2.29	1.02	0.60	0.43	0.18	0.03	0.00
5	—	—	79.0	44.5	17.8	7.20	3.78	1.37	0.63	0.31
6	—	—	—	—	—	—	170	19.9	6.70	1.80
Value of selectivity factor (α_{ij})										
3/4	22.6	17.5	9.55	8.00	7.30	5.17	3.90	3.72	—	—
5/3	—	—	2.51	2.43	2.38	2.33	2.25	2.20	1.91	—
2/5	—	—	—	4.93	3.30	3.00	2.80	2.40	2.25	2.10
1/2	—	—	—	—	4.38	3.31	3.10	2.41	2.24	1.77
6/1	—	—	—	—	—	—	5.18	2.52	2.11	1.57

six compounds with an eluent comprising 60% of dioxan and 40% of cyclohexane. The separation is very good, but UV self-absorption by the eluent is high, the transmittance hardly attaining 4%, so that low-level detection by UV measurement is not practicable.

Tetrahydrofuran has a lower UV cut-off (215 nm) than dioxan, and its elution properties are generally believed to be similar; therefore, it was our next choice. Only those eluent compositions yielding reasonable k' values were tested, and the
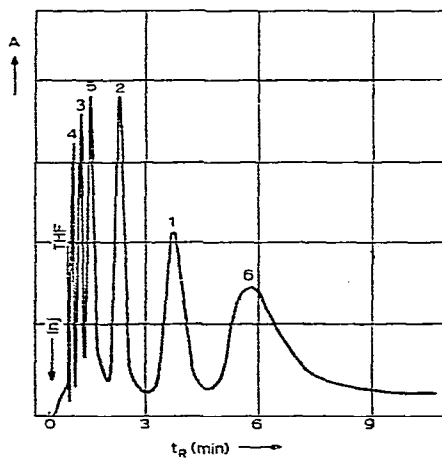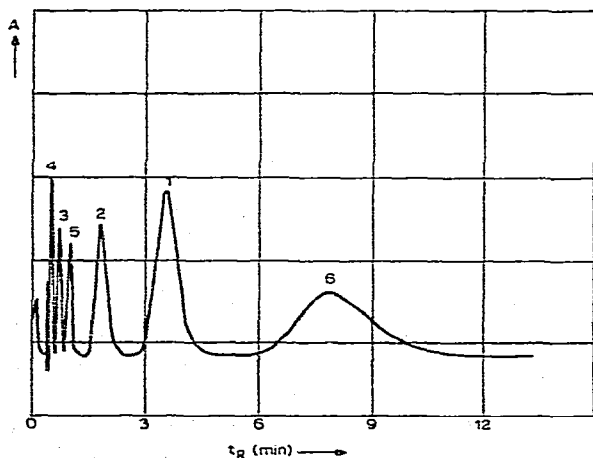

Fig. 2. Separation of compounds 1–6 on a MicroPak-NH$_2$ column (0.25 m × 0.125 in. O.D.): flow-rate, 2.0 ml/min; pressure, 1800 p.s.i.; temperature, 50°; eluent, 60% dioxan–40% cyclohexane.

Fig. 3. Separation of compounds 1–6. Conditions as in Fig. 2, but with 60% tetrahydrofuran–40% cyclohexane as eluent.

TABLE II

VALUES OF CAPACITY AND SELECTIVITY FACTORS FOR COMPOUNDS 1–6 WITH TETRAHYDROFURAN–CYCLOHEXANE AS ELUENT

The values listed were obtained under the conditions cited in Table I.

Compound or compound pair	Tetrahydrofuran content of eluent, % (v/v)		
	40	50	60
Value of capacity factor (k′)			
1	24.5	11.0	6.24
2	10.21	4.85	2.97
3	2.07	1.04	0.63
4	0.30	0.16	0.14
5	3.47	1.82	1.17
6	73.2	21.9	10.9
Value of selectivity factor ($\alpha_{i,j}$)			
3/4	6.90	6.50	4.50
5/3	1.68	1.77	1.86
2/5	2.95	2.66	2.53
1/2	2.39	2.27	2.11
6/1	2.99	1.92	1.75

results are shown in Table II; it can be seen that $k′$ and α decrease with increasing tetrahydrofuran content, which is similar to the behaviour observed with dioxan as modifier. The value of $\alpha_{5/3}$, however, behaves differently, but, considering the practical separation problem, it is advantageous that $\alpha_{5/3}$ has the lowest absolute value in this phase system. Fig. 3 shows the separation of compounds 1–6 with an eluent comprising 60% of tetrahydrofuran and 40% of cyclohexane; the separation is adequate

TABLE III

VALUES OF CAPACITY AND SELECTIVITY FACTORS FOR COMPOUNDS 1–6 WITH ISOPROPANOL–CYCLOHEXANE AS ELUENT

The values listed were obtained under the conditions cited in Table I.

| Compound or compound pair	Isopropanol content of eluent, % (v/v)						
	10	15	20	25	35	50	100
Value of capacity factor (k′)							
1	18.7	10.0	6.25	3.6	1.82	1.38	0.29
2	13.2	7.25	5.2	3.03	1.6	1.3	0.28
3	3.83	2.7	1.95	1.14	0.74	0.67	0.17
4	0.38	0.32	0.33	0.19	0.14	0.12	0.09
5	3.82	2.54	1.86	1.05	0.71	0.65	0.16
6	52.6	19.7	10.9	4.95	2.04	1.43	0.29
Value of selectivity factor ($\alpha_{i,j}$)							
5/4	10.1	7.95	5.63	5.52	5.31	5.58	17.8
3/5	1.01	1.06	1.05	1.09	1.04	1.03	1.06
2/3	3.45	2.68	2.68	2.67	2.16	1.94	1.65
1/2	1.42	1.38	1.21	1.18	1.14	1.06	1.03
6/1	2.81	1.97	1.74	1.38	1.12	1.04	1.00
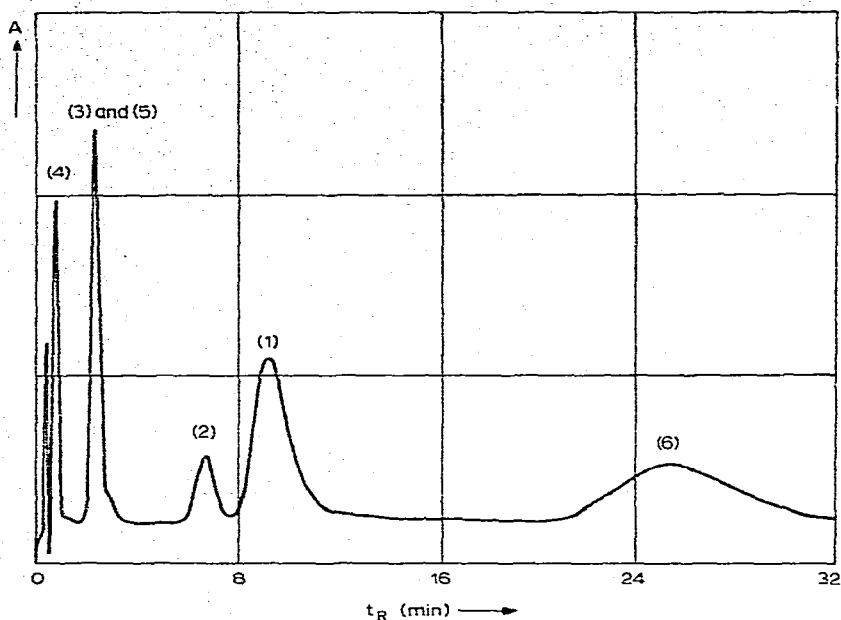
Fig. 4. Separation of compounds 1–6. Conditions as in Fig. 2, but with 10% isopropanol–90% cyclohexane as eluent.

for most practical purposes, but UV self-absorption of the eluent is still high (the transmittance is about 42%), so that trace-level detection can not be achieved.

The next polar modifier that we tested was isopropanol; at 254 nm this is practically transparent, so that UV self-absorption causes no problems. Table III shows the values of k' and α obtained; it can be seen that much lower polar-modifier concentrations than in the previous systems result in reasonable k' values. However, in this system, the elution order is reversed for compounds 3 and 5, and the value of $\alpha_{3/5}$ is practically unity, so that this pair of compounds cannot be separated; the values of $\alpha_{1/2}$ and $\alpha_{6/1}$ are also smaller than before. Fig. 4 shows the separation of compounds 1–6 with an eluent comprising 10% of isopropanol and 90% of cyclohexane; in this system, compound 4 is eluted close to the unretained peak, while compounds 3 and 5 are not resolved. This system is therefore useless for practical separations.

Attempts were made to establish a meaningful correlation between the k' values and the eluent compositions with respect to the structure of the compounds studied. In Fig. 5, the k' values from Tables I, II and III are plotted against the content

TABLE IV

HILDEBRAND PARAMETERS OF THE SOLVENTS STUDIED*

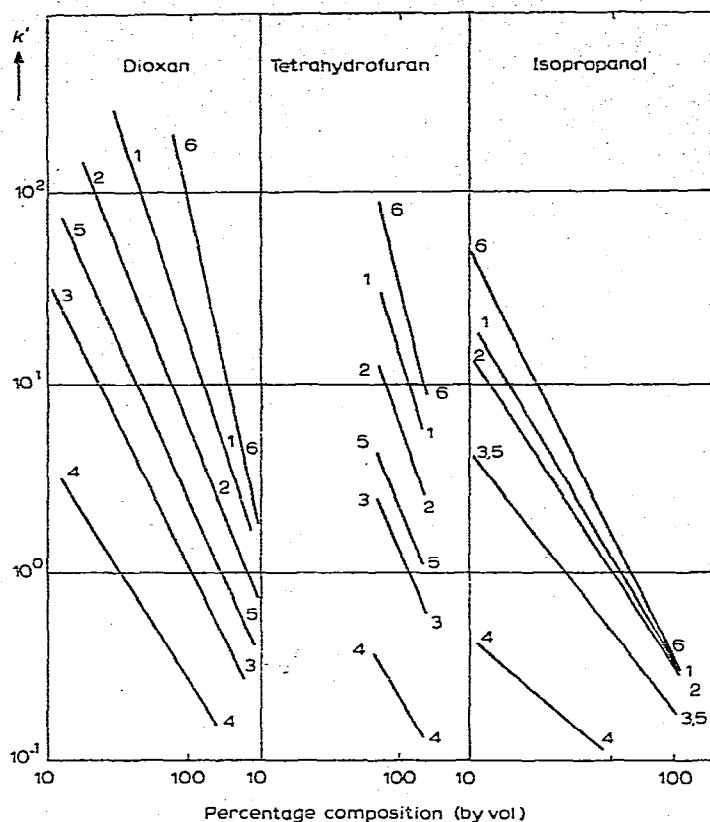| Solvent	δ	δ_d	δ_o	δ_b	δ_a
Cyclohexane	8.2	8.2	0.0	0.0	0.0
Tetrahydrofuran	9.1	7.6	4	3.0	0.0
Dioxan	9.8	7.8	4	3.0	0.0
Isopropanol	10.2	7.2	2.5	4.0	4.0

* See ref. 9.

Fig. 5. Values of k' as a function of solvent composition. Polar modifiers are dioxan, tetrahydrofuran and isopropanol; concentration range is 10–100% by vol. (based on Tables I, II and III).

of polar modifier in the eluent (10–100%). Rectilinear relationships are obtained in this range if logarithmic co-ordinates are employed. The highest values of k' and slope are obtained with compound 6 (which has two hydroxymethyl groups) and the lowest with compound 4 (which has none), indicating that competition between the stationary phase ($-CH_2-NH_2$) and the mobile phase (e.g., dioxan or tetrahydrofuran) for the solute is more pronounced in the first case. Eluents comprising both a polar and a non-polar solvent are generally believed to obey the Hildebrand rule[7], namely:

$$\delta = \Sigma \, \delta_i x_i$$

where δ_i represents the Hildebrand parameters of the pure solvents and x_i the composition (% by volume) of the eluent.

In Table IV are presented the Hildebrand parameters of the solvents concerned[8]. From the composition values shown in Tables I, II and III and the δ values shown in Table IV, the actual values of δ, δ_d, δ_o, δ_a and δ_b were calculated, and the values of log k' were plotted against the logarithms of these parameters. The graph of log k' against log δ is similar to Fig. 5 and does not allow us to draw further conclusions. However, when the relationship between log k' and log δ_b is considered, the
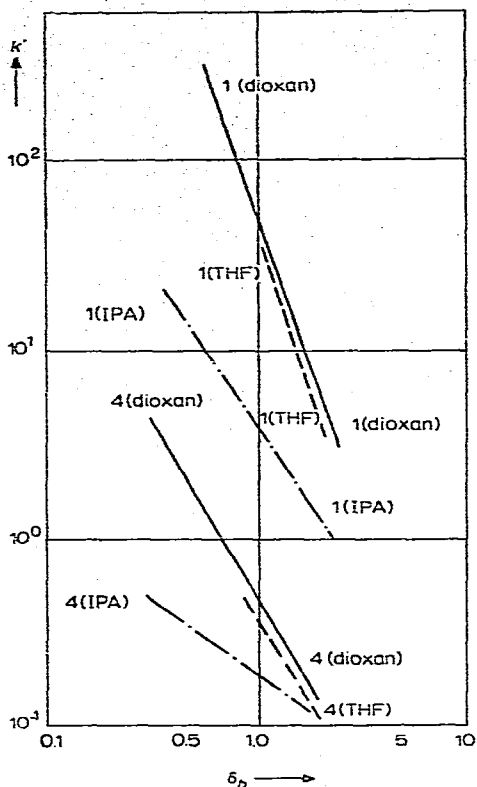
Fig. 6. Values of k' as a function of the actual δ_b values of the eluents. THF = tetrahydrofuran; IPA = isopropanol.

situation is better. For the sake of clarity, Fig. 6 presents only the graphs obtained for the extreme compounds 1 and 4; the rest are similar. It can be seen that the graphs are close to and parallel with each other when dioxan or tetrahydrofuran is used as modifier, but significantly differ from the line obtained with isopropanol as modifier. The slight difference between the dioxan and the tetrahydrofuran graphs could not be accounted for from the Hildebrand-parameter values.

Since isopropanol produces significant change in both the α and the k' values, attempts were made to prepare three-component eluent systems that might yield better separations than any of the two-component systems already tested; Table V shows the k' and α values found. It can be seen that the best results were obtained with an eluent comprising 5% of isopropanol, 30% of dioxan and 65% of cyclohexane. The separation of compounds 1–6 in this system is shown in Fig. 7. The separation obtained with an eluent comprising 5% of isopropanol, 30% of tetrahydrofuran and 65% of cyclohexane was similarly good, except for compounds 3 and 5.

The elution conditions shown in Fig. 7 were accepted as standard for the separation of a large number of samples of crude 1. Based on the peak-height values obtained from three repetitive injections from five different standard solutions, the calibration factors found with a flow-rate of 1.5 ml/min were as follows: compound 1,

TABLE V

VALUES OF CAPACITY AND SELECTIVITY FACTORS FOR COMPOUNDS 1–6 WITH ELUENTS CONTAINING ISOPROPANOL, DIOXAN (OR TETRAHYDROFURAN) AND CYCLOHEXANE

The values listed were obtained under the conditions cited in Table I. The eluent compositions are in % (v/v).

Compound or compound pair	Isopropanol content of eluent containing 20% of dioxan*		Isopropanol content of eluent containing 30% of dioxan*				Isopropanol content of eluent containing 30% of tetrahydrofuran*	
	5	10	3	5	7	10	2.5	5
Value of capacity factor (k')								
1	20.0	7.93	19.2	9.33	8.15	5.37	20.1	11.25
2	9.12	4.67	8.0	4.52	4.11	3.12	9.18	6.03
3	2.26	1.47	1.82	1.21	1.18	1.01	2.07	1.38
4	0.46	0.38	0.44	0.29	0.21	0.20	0.28	0.24
5	3.24	1.87	2.93	1.90	1.75	1.37	3.06	2.00
6	54.7	14.2	65	19.7	15.23	8.58	35.9	14.3
Value of selectivity factor (α_{ij})								
3/4	4.92	3.87	4.15	3.06	5.65	5.00	7.40	5.75
5/3	1.43	1.27	1.61	1.57	1.47	1.37	1.48	1.45
2/5	2.83	2.50	2.73	2.38	2.34	2.28	3.03	3.01
1/2	2.19	1.70	2.40	2.06	1.98	1.72	2.17	1.87
6/1	2.73	1.79	3.50	2.11	1.87	1.60	1.79	1.27

* The balance of the eluent is cyclohexane.
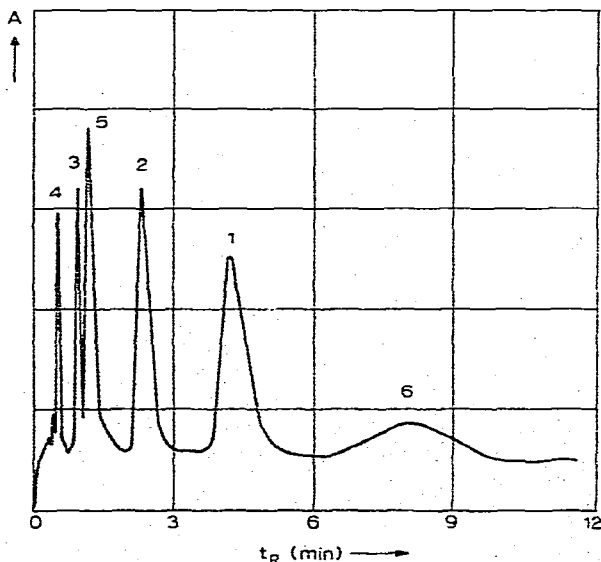

Fig. 7. Separation of compounds 1–6. Conditions as in Fig. 2, but with 5% isopropanol–30% dioxan–65% cyclohexane as eluent.

0.1107 mm/μg $\pm$ 1.36%; compound 2, 0.477 mm/μg $\pm$ 1.89%; compound 3, 2.075 mm/μg $\pm$ 7.76%.

The system outlined above has been in use for over a year without appreciable change in its chromatographic characteristics.

SYMBOLS

The symbols listed below are used in this paper.

k' = capacity factor
a_{ij} = selectivity factor (= k'_i/k'_j)
x_i = concentration of the components of the eluents (%, v/v)
δ = Hildebrand parameter
δ_d = dispersion solubility parameter
δ_o = orientation solubility parameter
δ_b = proton acceptor solubility parameter
δ_a = proton donor solubility parameter

ACKNOWLEDGEMENTS

REFERENCES

1 Quality Control Dept., EGYT Pharmaceutical Factory, Budapest, 1973.
2 Gy. Vigh and J. Inczédy, *J. Chromatogr.*, 102 (1974) 381.
3 Gy. Vigh, *Thesis*, Veszprém (Hungary), 1975.
4 Gy. Vigh and J. Inczédy, *J. Chromatogr.*, 116 (1976) 472.
5 Gy. Vigh, *J. Chromatogr.*, 117 (1976) 424.
6 Special Publ. No. AN-71-105, Waters Associates, Framingham, Mass., 1972.
7 R. A. Keller, B. L. Karger and L. R. Snyder, in R. Stock and S. G. Perry (Editors), *Gas Chromatography, 1970*, Institute of Petroleum, London, 1971.
8 R. L. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 1974.