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Computer simulation as an aid in method development for gas chromatography

III. Examples of its application

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ABSTRACT

The use of computer simulation for developing optimized gas chromatographic (GC) separations is illustrated for several samples. Resolution maps (plots of R_s *vs.* heating rate r) for different starting temperatures provide a means for the rapid exploration of separation as a function of the temperature program in the case of programmed-temperature GC. Isothermal separations are easily developed by trial and error, because of the speed of computer simulation. More complex samples may require multi-ramp temperature programs; these require a greater method development effort, but partial resolution maps can help facilitate this process.

INTRODUCTION

The best approach to gas chromatographic (GC) method development depends on the sample and the goals of separation. If the quantitation of all sample components is required, the baseline resolution of every band pair ($R_s > 1.5$) will usually be the main objective. Even greater resolution may be desirable when the sample contains bands of quite different size, or when it is anticipated that future samples may contain additional components (*e.g.*, interferences) not present in the sample used during method development. Achieving an acceptable separation within a minimum run time is also a common goal. Finally, there may be other considerations; *e.g.*, a need to restrict the maximum column temperature within certain limits.

The choice of a method development procedure is also affected by the complexity of the sample. Many samples consist of literally hundreds of individual compounds, where it is usually impractical to attempt the resolution of all components. In many such cases, the separation and quantitation of a smaller number of "key" bands may be required. For samples that contain fewer components, isothermal separation will be preferred in some cases, because temperature-programmed runs (including the time for cooling the column oven after the separation) often require more time.

Method development can be somewhat tedious for the case of temperature-

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programmed separations (of primary interest in this study), because resolution and run time depend on several different variables: heating rate, starting temperature, and the shape of the temperature program (linear or multi-ramp). Thus a large number of experiments may be required to adequately explore the various separation options. Computer simulation can greatly reduce the time and effort required for GC method development, while achieving better final methods as measured by resolution, run time, etc. The preceding two papers [1,2] have described the basis of a computer-simulation approach to method development for GC. The accuracy of this software (DryLab GC) for the prediction of separation has been confirmed, and the value of optimizing the heating rate in temperature-programmed separations (or the temperature in isothermal runs) has been demonstrated.

In the present paper we will illustrate how computer simulation can be applied to GC method development for samples of different types and for different separation goals.

EXPERIMENTAL

Equipment, materials and procedures are described in Part I [1].

RESULTS AND DISCUSSION

The general similarity of temperature-programmed GC and gradient elution high-performance liquid chromatography (HPLC) has already been noted [1–3] and the development of optimized gradient elution procedures using computer simulation has been described in several previous papers [4–15]. These generalizations for gradient elution can be applied with little modification for the similar development of programmed-temperature GC separations. This will allow us to simplify and condense the following discussion. Three different samples will be used to illustrate how computer simulation can facilitate GC method development. Since it has been established in Part I [1] that DryLab GC can provide accurate predictions of separation, we will make use of both simulated and experimental runs in the following treatment.

Herbicide sample

The two initial chromatograms used for computer simulation of this 13-component sample are shown in Fig. 1A and B. Excellent resolution ($R_s > 7$) is noted for all bands in both runs, suggesting that the separation of this sample will not be difficult. Our major concern will therefore be with other aspects of separation: run time, isothermal *vs.* programmed separation, etc.

It is generally useful to examine a resolution map (Fig. 2) at the beginning of method development; *i.e.*, a plot of minimum resolution *vs.* heating rate r (for linear temperature programs). An arbitrary column plate number N can be assumed at this point, but it is recommended that bandwidths for two, well-resolved, early- and late-eluting peaks from the chromatogram of Fig. 1A (or B) be measured. This allows DryLab GC to predict plate numbers for each band in the chromatogram, as a function of experimental conditions (initial temperature, heating rate, shape of the temperature program, etc.) and the retention characteristics of the solute. All simulations described in this paper are based on the determination of column plate number in this way, as described in ref. 1.

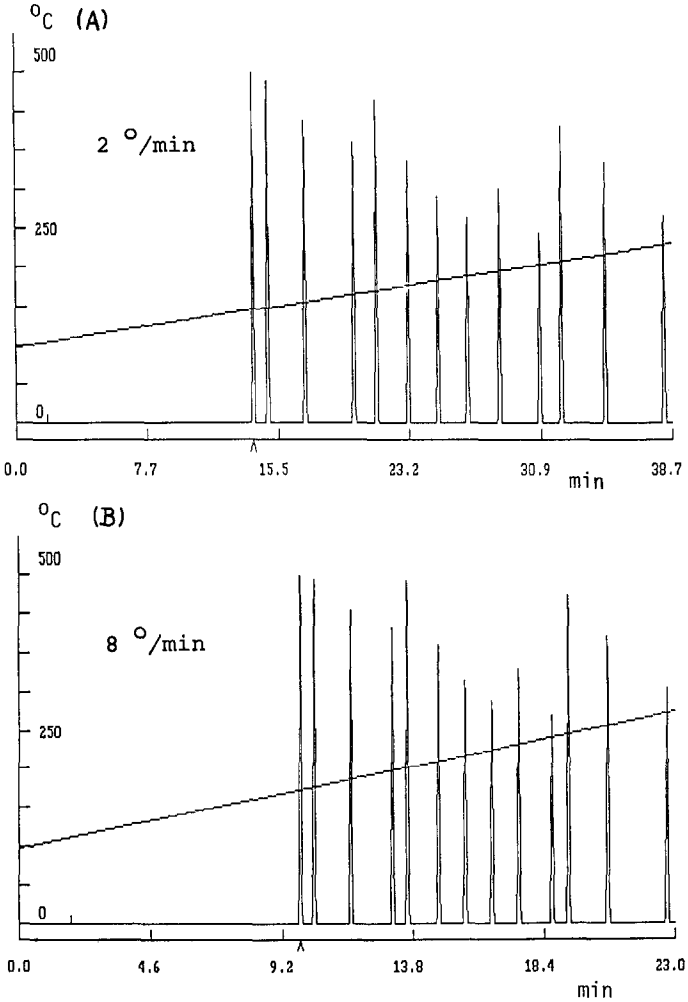

Fig. 1. Experimental chromatograms (recreated by computer simulation) for the herbicide sample; temperature programs shown as overlays. Conditions: DB-5 column, 1 ml/min, 100°C starting temperature; (A) 2°C/min; (B) 8°C/min.

Resolution maps as in Fig. 2 should be requested for different starting temperatures T_0. This allows a quick assessment of the trade-off between resolution and run time as a function of T_0. The map of Fig. 2A (T_0 = 50°C) indicates that bands 4 and 5 are the critical pair for heating rates r > 10°C/min, while bands 1 and 2 are the least resolved for lower values of r. Fig. 2A–C (for 50°C < T_0 < 150°C) show a continual increase in resolution as the heating rate is decreased (and run time increases). That is, there is no intermediate temperature which provides maximum resolution for the herbicide sample. There is also not much difference in the resolution maps of Fig. 2A–C, indicating that the starting temperature is not of critical importance, as long as T_0 < 150°C. The map for T_0 = 200°C (Fig. 2D), on the other hand, shows a marked

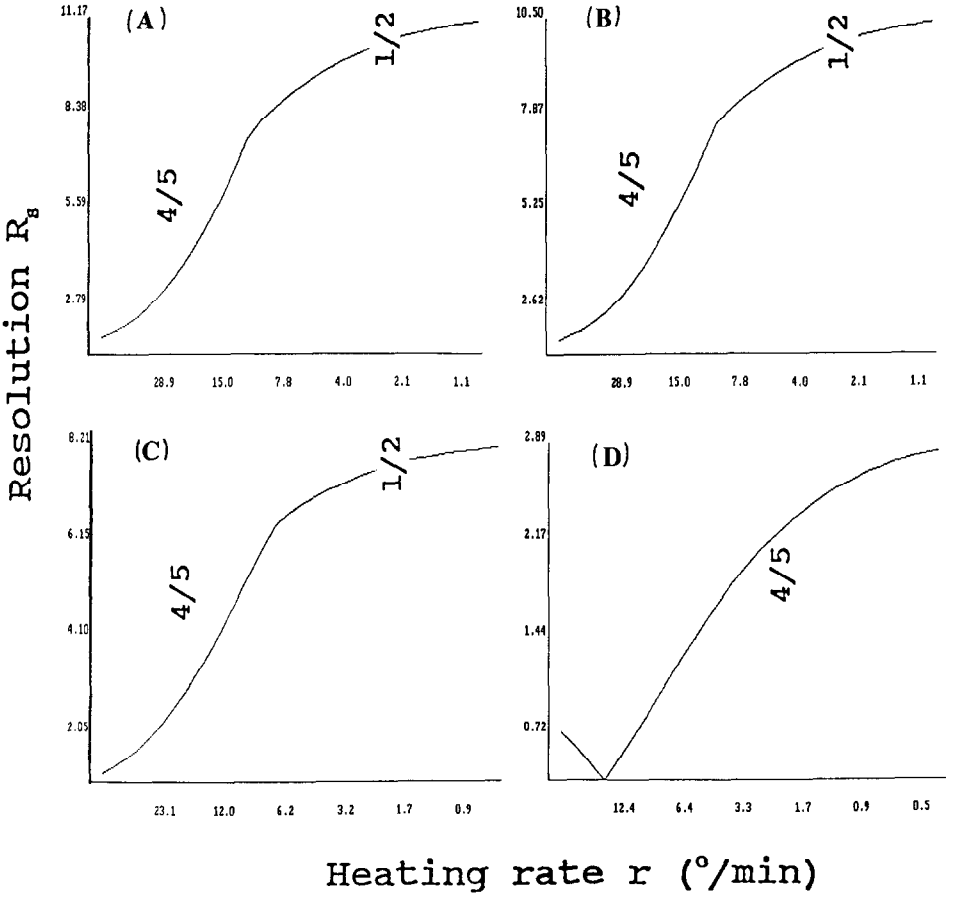

Fig. 2. Resolution maps for the herbicide sample as a function of starting temperature T_0 and heating rate r. Conditions as in Fig. 1 except where noted otherwise. (A) $T_0 = 50°C$; (B) $T_0 = 100°C$; (C) $T_0 = 150°C$; (D) $T_0 = 200°C$.

decrease in resolution for all values of r, suggesting that the optimum starting temperature is $T_0 < 200°C$.

If our goal is the separation of this sample in the shortest possible time, we can carry out computer simulations in order to predict run time and maximum column temperature (corresponding to elution of the last sample band) as a function of starting temperature, for some minimum resolution. Table I summarizes this information for the present sample, for a minimum value of $R_s = 2.0$. The shortest run time (10.0–10.2 min) is obtained with a starting temperature of 100–150°C and a heating rate of 17–23°C/min; the separation for $T_0 = 100°C$ and 23.5°C/min is shown in Fig. 3A. One consideration with these conditions, however, is the need to heat the column to 320–340°C in order to elute the last band. If the present column were not recommended for use above 250°C, the separation of Fig. 3A would then be impractical.

An alternative (assuming a maximum column temperature of 250°C) is to use a

TABLE I

CONDITIONS FOR THE SEPARATION OF THE HERBICIDE SAMPLE OF FIG. 1 IN MINIMUM TIME WITH A RESOLUTION R_s = 2.0

Linear temperature programs, computer simulations.

T_0 (°C)	r (°C/min)	Last band t_R[a] (min)	Maximum temperature[b] (°C)
50	25.5	11.6	346
100	23.5	10.2	340
150	17.0	10.0	320
200	2.5	18.9	247

[a] This is equal to the run time (excluding the time to cool down the column oven after each run).
[b] Temperature at which last band elutes.

linear program to 250°C and then maintain an isothermal hold for the balance of the separation. This option is demonstrated in Fig. 3B, for a starting temperature of 100°C and a heating rate of 23.5°C/min. Since the critical band pair is 4/5 for these

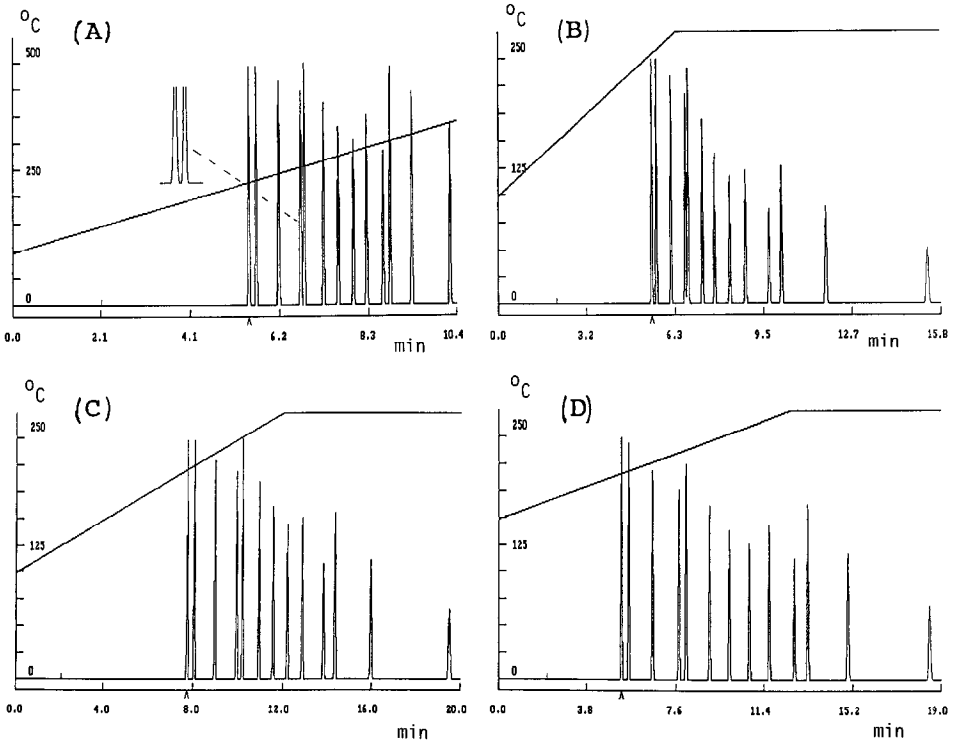

Fig. 3. Predicted separations of the herbicide sample for different conditions. (A) 100°C starting temperature, 23.5°C/min, R_s = 2.0; (B), same as (A) but with an isothermal hold after 250°C (R_s = 2.0); (C) 100°C starting temperature, 21°C/min, isothermal hold after 250°C, R_s = 5.0; (D) 150°C starting temperature, 8°C/min, isothermal hold after 250°C, R_s = 5.0.

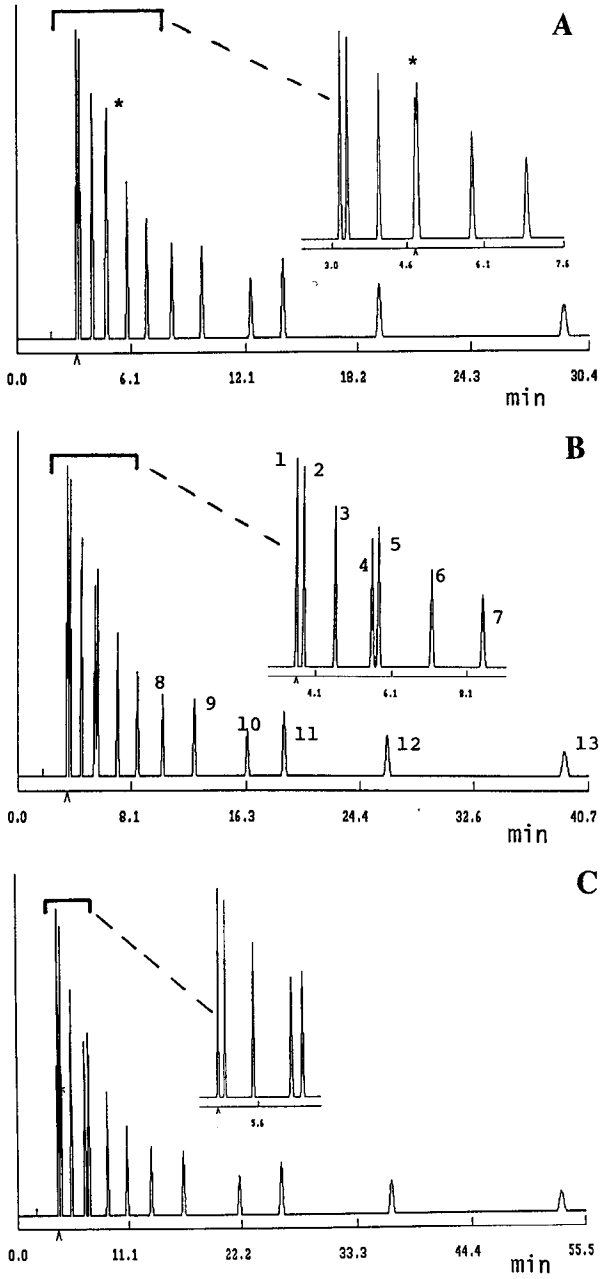

Fig. 4. Predicted isothermal separations of the herbicide sample for different temperatures: (A) 210°C; (B) 200°C; (C) 190°C. Other conditions as in Fig. 1. * = Overlapped bands.

conditions —which elutes well before 250°C— the resolution of the sample is not affected (*vs.* the separation of Fig. 3A) by the isothermal hold. The run time is increased from 10 min in Fig. 3A to 15 min in Fig. 3B, but any problem with excessive heating of the column has now been resolved.

For "easy" separations such as this, it is often attractive to aim for a sample resolution which is greater than "minimum", in order to build in a "safety factor". This allows a greater margin of error in the transfer of the method to other laboratories, and it ensures that the method will work for columns of lower plate number (*e.g.*, older columns). The resolution maps of Fig. 2 indicate that resolution increases quite rapidly with decrease in heating rate, suggesting that a major increase in R_s is possible for a modest increase in run time. This is indeed the case, as shown in Fig. 3C and D —for separation conditions that in each case yield a minimum resolution of $R_s = 5$[a]. The separation of Fig. 3D ($T_0 = 150$°C) is about a minute shorter than that of Fig. 3C, corresponding to a run time of 18.5 min. The column equilibration time will also be less for this run, because the final column temperature (250°C) needs to be lowered only to 150°C, rather than 100°C in the run of Fig. 3C.

A final consideration is the possibility of trading the extra resolution ($R_s = 5$) of the run in Fig. 3D for a much shorter run time, by shortening column length. Since resolution is approximately proportional[b] to (column length)$^{0.5}$, a reduction in column length by a factor of 4 (from 30 to 7.5 m for the present example) would still provide a resolution of bands 4/5 of $R_s = 0.5 \times 5 = 2.5$, but in a run time of only 5 min (run time is proportional to column length, other factors equal).

Isothermal separation. The possible isothermal separation of this sample is readily addressed by simulations at different temperatures. Fig. 4 summarizes predicted runs at 190, 200 and 210°C. The resolution of bands 4/5 is unacceptable at 210°C ($R_s = 0.7$ for bands 4 and 5; marked by the asterisk in Fig. 4), and the run time (55 min) is excessive for $T = 190$°C. An isothermal run at 200°C gives acceptable resolution for all bands ($R_s > 2.3$), but the 40-min run time is rather long, and the last bands are broadened to the point of diminished detectability. In short, it appears that the herbicide sample is not a good candidate for isothermal separation.

This application of computer simulation for the herbicide sample may appear trivial to practical workers, inasmuch as this sample is easily separated without much effort. However, the various computer simulations that are summarized above required less than an hour to carry out, and in the process a thorough understanding of the various separation options was achieved. Thus it is possible by using computer simulation to develop better methods in less time, even for the case of "easy" samples. As we will see, the potential advantages of computer simulation increase dramatically for more complex samples.

Phenol sample

This sample (initial experimental runs described in ref. 3) is also not difficult to separate. However, method development is somewhat more complicated, for reasons

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[a] Here and in other figures, the temperature program for a given separation is shown as an overlay in computer-simulated chromatograms.

[b] This relationship is not exact, because of changes in the pressure drop across the column for the same flow-rate.

that will become apparent. Fig. 5A–D shows the resolution maps for this sample, based on starting temperatures of 25, 50, 75 and 100°C, respectively. As in the case of the herbicide sample (Fig. 2), starting temperatures of 25–75°C result in similar changes of resolution with heating rate (Fig. 5A–C). For a sufficiently high starting temperature (100°C, Fig. 5D), however, there is a significant drop in resolution. The resolution map for T_0 = 25°C can be used to discuss the features of the other maps (for T_0 < 100°C).

In Fig. 5A it is seen that there is an optimum intermediate temperature for this separation, corresponding to r = 7.0°C/min. For higher heating rates, the resolution of bands 3/4 decreases, while for lower values of r the resolution of bands 1/2 decreases. A heating rate of r = 7.0°C/min is therefore favored for this sample (if T_0 = 25°C). A similar situation is seen for starting temperatures of 50°C (Fig. 5B) and 75°C (Fig. 5C). For T_0 = 25°C (Fig. 5A) bands 1/2 change their separation order, band 2 being eluted first for r < 2 °C/min.

If we arbitrarily select a starting temperature of 50°C and the optimum value of r (4.5°C/min, Table II), the minimum sample resolution is predicted to be R_s = 4.8

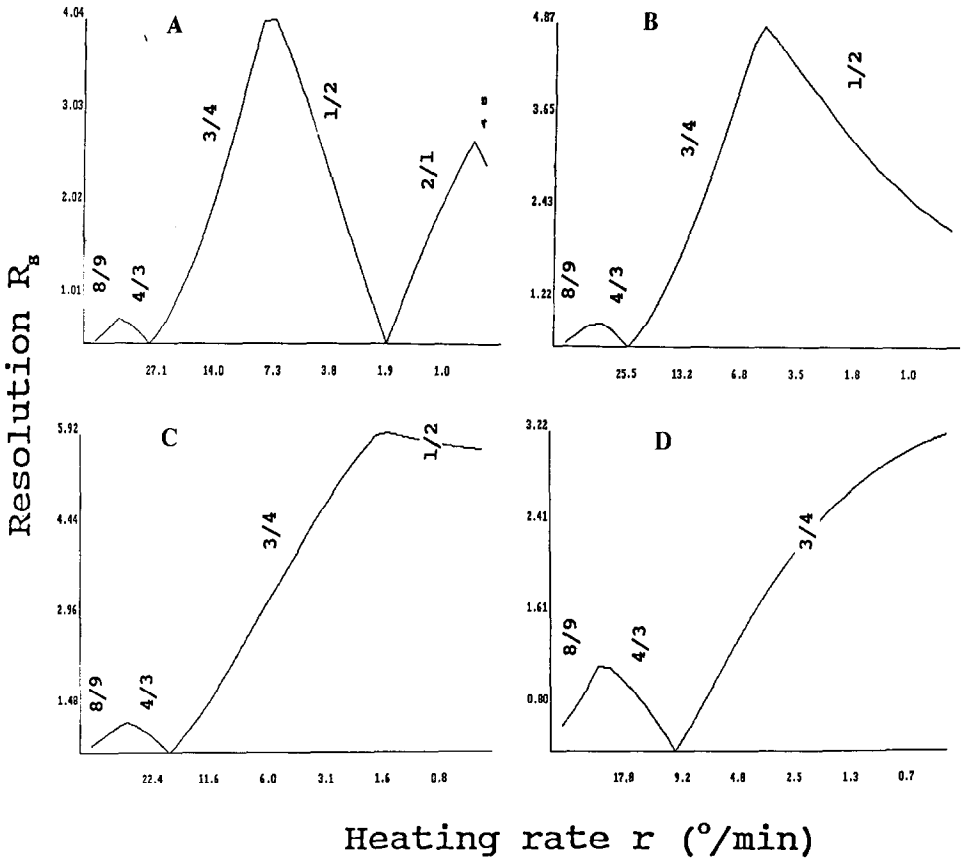


Fig. 5. Resolution maps for the phenol sample as a function of starting temperature T_0 and heating rate r. Conditions: DB-5 column, 1 ml/min. (A) T_0 = 25°C; (B) T_0 = 50°C; (C) T_0 = 75°C; (D) T_0 = 100°C.

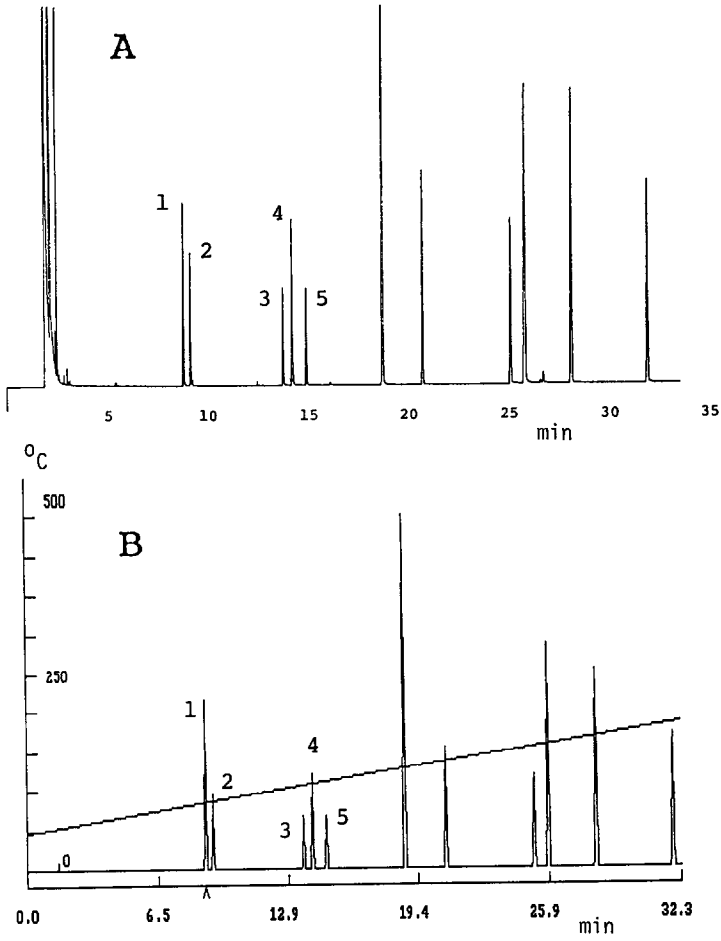


Fig. 6. Separation of the phenol sample with maximum resolution (as in Fig. 5B). Conditions: DB-5 column, 1 ml/min, 50–200°C, 4.6°C/min. (A) Experimental chromatogram; (B) predicted chromatogram.

with a run time of 32 min. This separation (experimental and predicted) is shown in Fig. 6. Note that the critical band pairs (1/2 and 4/5) occur near the beginning of the chromatogram. When this is the case, it is possible to shorten the run time significantly by increasing the heating rate after the elution of the last critical band pair (4/5). This is illustrated in Fig. 7, where run time (24 min) has been reduced by about 25%. The predicted separations of Figs. 6 and 7 agree with the experimental runs within $\pm 1.4\%$ for retention times and $\pm 5\%$ for resolution.

Table II summarizes some additional data from Fig. 5 which allow a further evaluation of the best starting temperature and heating rate for this sample. The maximum possible resolution is seen to increase with starting temperature, until $T_0 >$ 75°C. The run time increases at the same time, however, so it is not obvious which starting temperature should be preferred. If we normalize sample resolution to a value of $R_s = 2.0$, as in Table I for the herbicide sample, it is seen in Table II that any

TABLE II

SUMMARY OF RESULTS FOR PHENOL SAMPLE

Conditions: 30-m DB-5 column, 1 ml/min.

T_0 (°C)	Maximum R_s			R_s = 2.0	
	r (°C/min)	R_s	Last band t_R[a] (min)	r	t_R[a] (min)
25	7.0	4.2	27	11.5	18
50	4.6	4.8	32	10	18
75	1.5	5.9	55	7	20
100	0.5[b]	3.1	66	2.2	33

R_s = 2.0 using a shorter column (length = L)

T_0 (°C)	r[c] (°C/min)	L (m)	t_R (min)
25	7.0	11	9
50	4.5	8	9
75	1.5	5	10
100	0.5[b]	20	43

[a] Equal to run time.
[b] No maximum in R_s *vs.* heating rate map.
[c] Value for maximum resolution.

value of T_0 between 25 and 75°C will yield about the same run time (18–20 min). Since it is often undesirable to use starting temperatures near (or below) ambient, a starting temperature of 50°C with r = 10°C/min would probably be preferred for the phenol sample, if a minimum run time is important.

Run time (for R_s = 2.0) can also be reduced by reducing column length (<30 m) rather than by increasing the heating rate. Using this approach, the run time can be shortened to about 9 min (Table II, T_0 = 25–50°C). In general, when run time is quite important, it will usually be advisable to optimize column length. The use of an increase in heating rate following elution of band 5 (as in Fig. 7) could reduce run time further from 9 to about 7 min.

Because the last band of the phenol sample elutes below 250°C, there is no need to consider limiting the temperature range of this separation, unlike the case of the herbicide sample.

Spearmint oil sample

Fig. 8 shows two experimental runs with this sample for use in computer simulation; these chromatograms are quite complex, with over 100 individual bands being recognizable. The best approach for optimizing separations such as this is determined by the separation goal, as discussed in the Introduction. In some cases, only the major bands will be of interest; *e.g.*, those comprising more than 1% of the total sample. In other instances certain trace components in the sample may require quantitation.

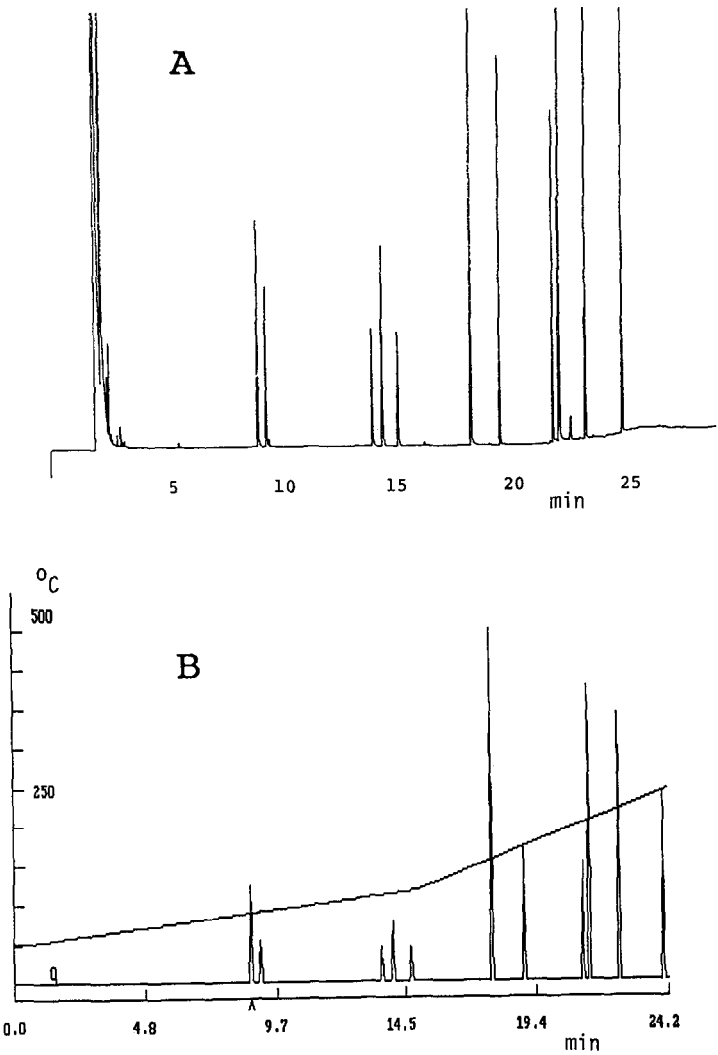


Fig. 7. Further shortening of run time in the separation of Fig. 6 by increasing the heating rate after elution of band 5. Temperature program of 50/120/250°C in 0/15/25 min; other conditions as in Fig. 6. (A) Experimental chromatogram; (B) predicted chromatogram.

Major components. Consider first the separation of the major bands in the spearmint oil sample; *i.e.*, the 19 bands with areas at least 5% as large as the largest sample band. Fig. 9 shows computer simulations that correspond to the experimental runs of Fig. 8 (same conditions), but with minor bands deleted from the chromatogram. Interestingly, all 19 bands are resolved with $R_s > 0.9$ in the run with the *higher* heating rate (8°C/min, Fig. 9B), whereas two band pairs (3/4 and 8/9) are poorly separated in the run at 2°C/min (Fig. 9A). Starting with the separation of Fig.

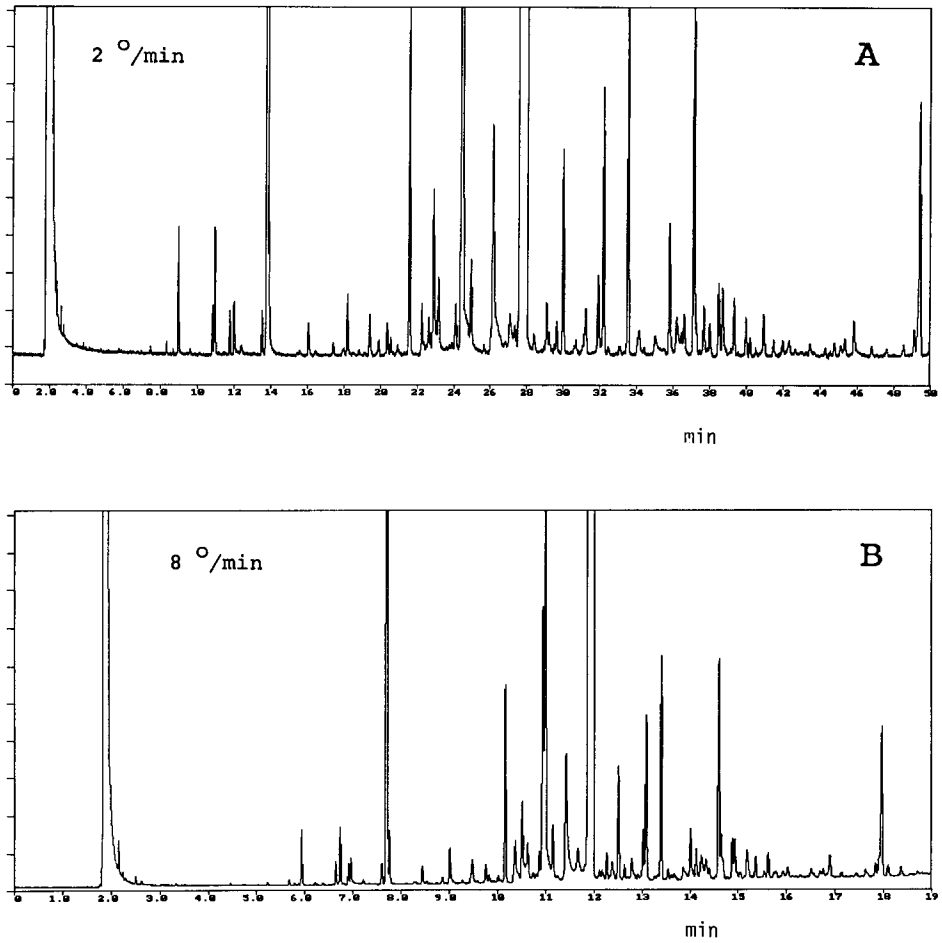


Fig. 8. Experimental runs for the spearmint oil sample. Conditions: 50–250°C, 1 ml/min, DB-5 column; (A) 2°C/min; (B) 8°C/min.

9A, conventional wisdom would suggest that a *decrease* in heating rate would be required to improve sample resolution, which in this case is not correct.

Resolution maps for the preceding 19-band sample are shown in Fig. 10A D for T_0 = 50, 75, 100 and 125°C, respectively. It is seen that the maximum possible resolution increases with T_0 from 50 to 100°C, then decreases for T_0 = 125°C (similar to the case of the phenol sample in Fig. 5). This suggests that a starting temperature of 100°C is optimum for this sample, because of the need for maximum resolution ($R_s \gg 1.0$). The optimum heating rate is r = 2.8°C/min, and the predicted separation for these conditions (minimum R_s = 1.25) is shown in Fig. 11A. Because the last critical band-pair (14/15) elutes before 12 min, it is possible to shorten the run time by using a steeper temperature ramp (30 °C/min) after 12 min. The predicted separation for these conditions is shown in Fig. 11B, with a run time (16 min) that is 30% shorter than in Fig. 11A.

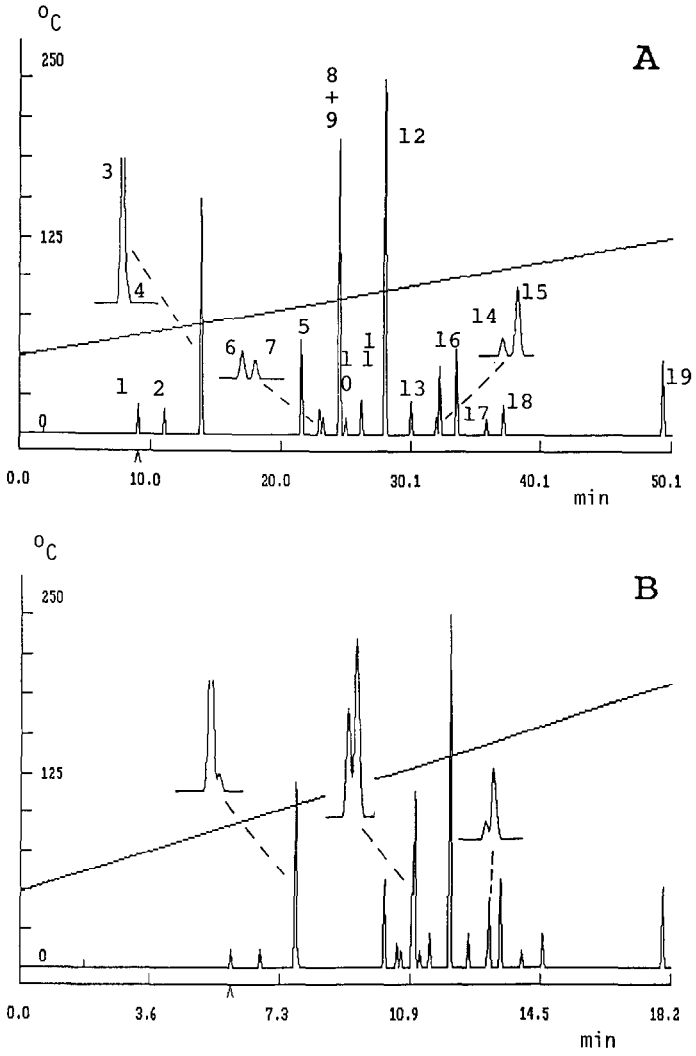


Fig. 9. Computer simulation of runs of Fig. 7 for the 19 largest bands in the chromatogram. Same conditions; (A) 2°C/min; (B) 8°C/min. Insets are magnifications of less-well-resolved band pairs.

Separations as in Fig. 11 that exhibit several critical band pairs (3/4, 8/9, 14/15; see also Fig. 10) can sometimes be improved by the use of multi-step temperature ramps. In these cases, the temperature ramp across each critical band pair is adjusted to maximize the resolution of that band pair (the similar applicability of multi-segment gradients in HPLC has been demonstrated for several samples [5,7–15]). Whether this is possible for a given sample can be quickly decided through the use of *partial resolution maps*, where the resolution of individual (critical) band pairs is studied as a function of heating rate (and starting temperature).

Fig. 12 shows such maps for T_0 = 100°C and band pairs 3/4, 8/9 and 14/15. Of

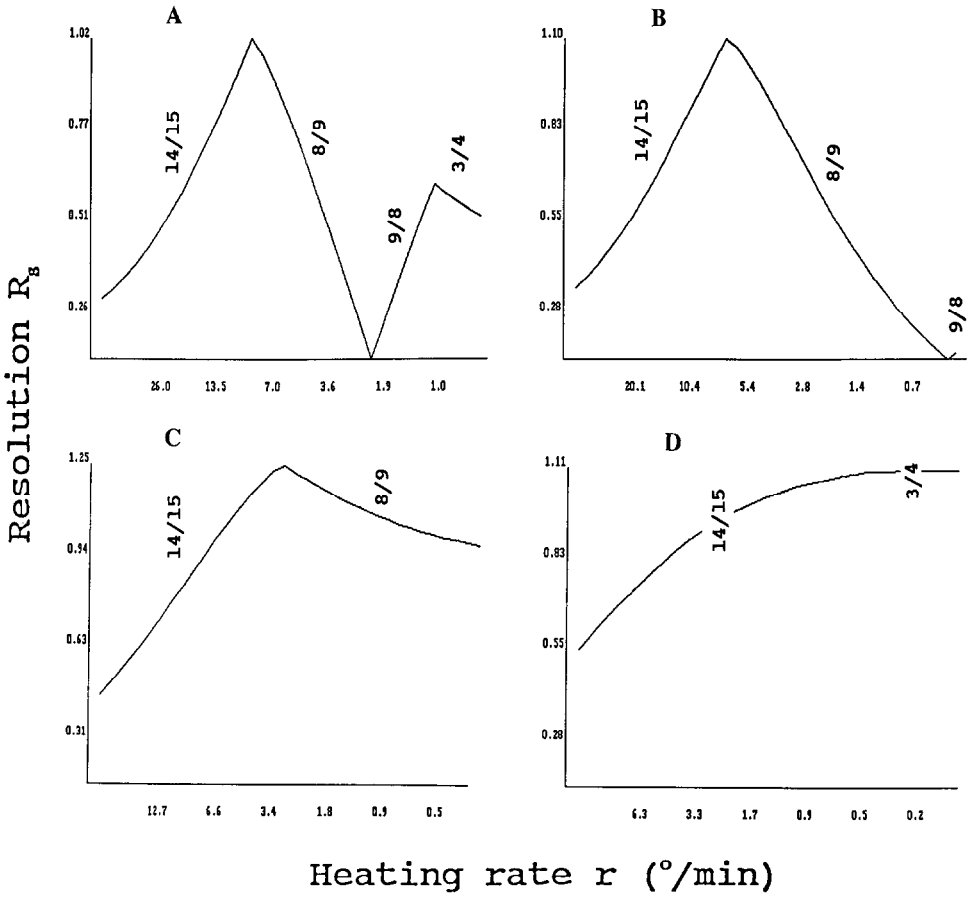


Fig. 10. Resolution maps for the 19 major bands in the spearmint oil sample, corresponding to experimental data of Fig. 8. (A) T_0 = 50°C; (B) T_0 = 75°C; (C) T_0 = 100°C; (D) T_0 = 125°C.

special interest is the map for bands 3/4, where the maximum resolution levels off at a value of R_s = 1.32. It was further established via other resolution maps that a change of T_0 does not improve this result; *i.e.*, the maximum possible resolution of bands 3/4 is R_s = 1.32 (assuming no change in stationary phase, column dimensions, flow-rate, etc). That is, no further change in the temperature program can significantly[a] increase the minimum resolution of the sample found in the linear temperature ramp of Fig. 11A (R_s = 1.25). Therefore it can be concluded that a multi-ramp temperature program will not be advantageous for the present sample.

Separation of additional bands. If bands having an area at least 2% of the largest band in the sample are included, there are a total of 47 bands to consider. We next examined optimum conditions for the separation of this 47-component sample. The

[a] A change in R_s by less than 0.1 unit is not considered significant, especially for the case of separations predicted by computer simulation.

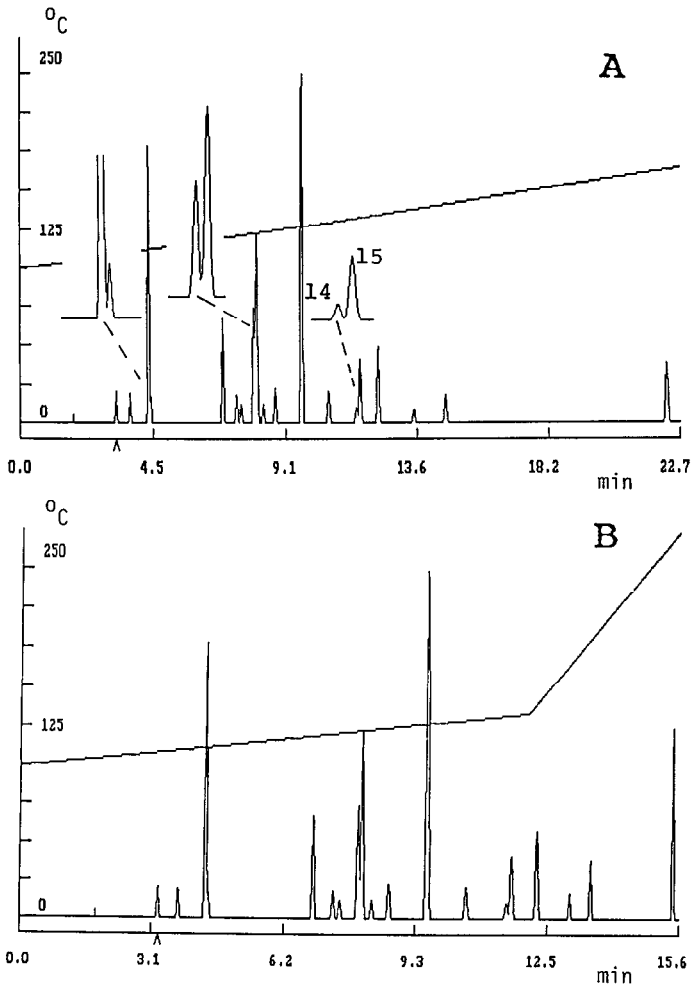


Fig. 11. Optimized separations of the 19 major bands in the spearmint oil sample. Conditions as in Fig. 7 except as otherwise noted. (A) T_0 = 100°C, 2.8°C/min; (B) same as (A), except 30°C/min after 12 min.

two experimental runs of Fig. 8 were again used as input data for computer simulation. However, for a sample this complex, it is imperative to verify that all bands have been properly matched between the two chromatograms. This is most easily accomplished by carrying out a run with an intermediate heating rate (4°C/min for the present case) and comparing the experimental and predicted retention times. If all bands have been properly assigned, there should be good agreement between these two sets of numbers. Such a comparison for a 4°C/min run with T_0 = 10°C is shown in Table III. Here it is seen that the two sets of retention times agree within ± 1.0% (average), and no individual retention-time pairs show unexpectedly large deviations.

Table IV summarizes the optimum conditions obtained from resolution maps for different values of T_0: heating rate r, value of R_s for this heating rate, and the run time. Maximum resolution can be obtained with starting temperatures of $50 < T_0 <$

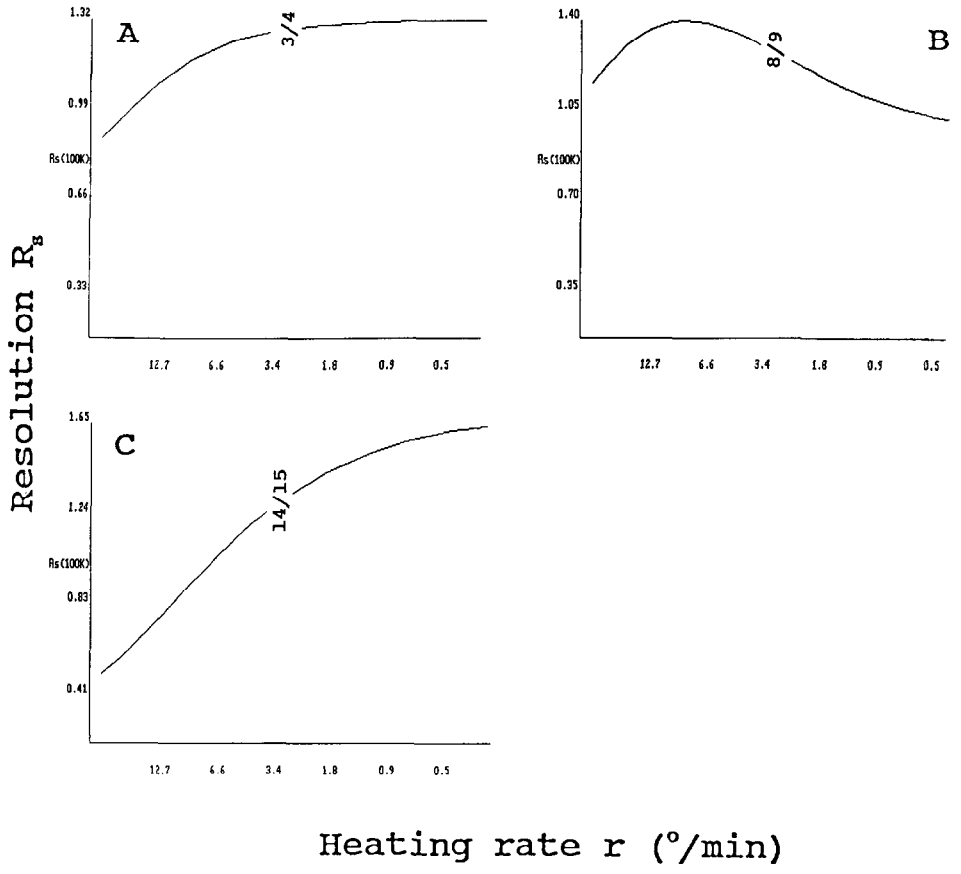


Fig. 12. Partial resolution maps for the 19 major bands of the spearmint oil sample. Conditions: T_0 = 100°C; otherwise as in Fig. 7. (A) map for bands 3/4; (B) map for bands 8/9; (C) map for bands 14/15. See text for details.

80°C, but 80°C is preferred because of the much shorter run time (21 min). Fig. 13 shows the resolution map for T_0 = 80°C.

Fig. 13 indicates that six band reversals occur (R_s = 0) for this sample, when the heating rate is increased from 1 to 20°C/min. In addition, the band spacing of several other band pairs changes significantly with variation in heating rate. Because of the complexity of Fig. 13, it is unlikely that trial-and-error changes in heating rate would lead the average chromatographer to an acceptable separation of this sample. Fig. 14 shows the predicted separation of this sample for the optimum conditions of Table IV: T_0 = 80°C and r = 4.4°C/min. The critical band pairs (4/5 and 14/15) are expanded and shown on the side of this Fig. 14 for a better picture of the resolution achieved.

Samples such as this which have a number of different critical band pairs are often better separated with a multi-ramp temperature program, because different parts of the chromatogram require different heating rates for optimum band spacing

TABLE III

COMPARISONS OF EXPERIMENTAL AND PREDICTED SEPARATIONS OF SPEARMINT OIL SAMPLE (47 bands)

Band	Retention times (min)				
	T_0 = 70°C/4°C/min[a]			Fig. 15	
	Expt.	Calc.	Error	Expt.	Calc.
1	5.23	5.21	−0.02	5.55	5.45
2	6.02	6.02	0.00	6.14	6.03
3	6.13	6.13	0.00	6.22	6.11
4	6.32	6.33	0.01	6.35	6.23
5	6.40	6.40	0.00	6.40	6.27
6	7.20	7.24	0.04	6.94	6.81
7	7.32	7.37	0.05	7.02	6.89
8	7.41	7.45	0.04	7.08	6.95
9	8.36	8.42	0.06	7.67	7.51
10	9.21	9.30	0.09	8.17	8.02
11	9.90	10.00	0.10	8.64	8.50
12	10.34	10.45	0.11	8.92	8.79
13	10.94	11.09	0.15	9.35	9.24
14	11.29	11.45	0.16	9.58	9.49
15	11.29	11.45	0.16	9.58	9.47
16	11.52	11.69	0.17	9.73	9.65
17	11.71	11.86	0.15	9.87	9.79
18	12.12	12.28	0.16	10.16	10.10
19	12.23	12.42	0.19	10.27	10.26
20	12.31	12.49	0.18	10.20	10.19
21	12.57	12.74	0.19	10.50	10.47
22	13.07	13.25	0.18	10.83	10.85
23	13.54	13.69	0.15	11.16	11.21
24	14.03	14.20	0.17	11.59	11.67
25	14.53	14.72	0.19	12.02	12.13
26	14.78	14.95	0.17	12.39	12.33
27	14.96	15.16	0.20	12.60	12.54
28	15.32	15.43	0.11	12.83	12.77
29	15.50	15.70	0.20	13.12	13.03
30	15.89	16.10	0.21	13.32	13.45
31	16.01	16.22	0.21	13.88	13.57
32	16.61	16.83	0.22	14.69	14.18
33	17.75	17.96	0.21	15.03	15.43
34	17.90	18.10	0.20	15.23	15.62
35	18.01	18.29	0.28	15.42	15.84
36	18.33	18.48	0.15	15.63	16.06
37	18.65	18.88	0.23	16.09	16.57
38	18.83	19.00	0.17	16.76	16.66
39	18.97	19.12	0.15	16.77	16.81
40	19.27	19.43	0.16	17.18	17.26
41	19.37	19.58	0.23	17.34	17.38
42	19.75	19.99	0.24	17.72	17.85
43	20.08	20.31	0.23	18.24	18.20
44	20.57	20.80	0.23	18.97	18.70
45	23.02	23.24	0.22	20.55	20.87
46	24.87	24.98	0.11	21.97	22.20
47	24.95	25.18	0.23	22.13	22.35

[a] Linear temperature program.

TABLE IV

SUMMARY OF RESOLUTION MAPS FOR 47 LARGEST BANDS OF SPEARMINT OIL SAMPLE

T_0 (°C)	Maximum resolution[a]			
	r (°C/min)	R_s	Run time (min)	Critical bands
40	5.0	0.59	28	14/15, 19/20
50	4.4	0.62	28	14/15, 19/20
60	3.7	0.63	29	14/15, 19/20
70	2.8	0.63	32	14/15, 19/20
80	4.4	0.66	21	4/5, 14/15
90	2.4	0.54	28	4/5, 14/15
100	0.5	0.37	48	3/4, 4/5

[a] For each value of T_0, a value of r giving maximum resolution was selected; the values of R_s and run time for this value of r are shown and the critical bands are identified.

and maximum resolution; *e.g.*, as in Fig. 12. The general approach for developing an optimized multi-ramp program for GC is similar to that used in the design of multi-segment gradients for HPLC, as discussed in detail in refs. 4 and 5. This involves optimizing the separation of early bands with a linear temperature program, followed by a change in heating rate for the next group of bands, and continuation of this process for the whole chromatogram. The ability (with computer simulation) to examine a large number of possible temperature programs greatly reduces the time required for this procedure (by a factor of a hundred or more).

A similar trial-and-error (plus logic) approach for the present sample (see ref. 16 for details) was successful in increasing the resolution of this 47-band sample to R_s = 1.2, corresponding to a doubling of the resolution found in the optimized

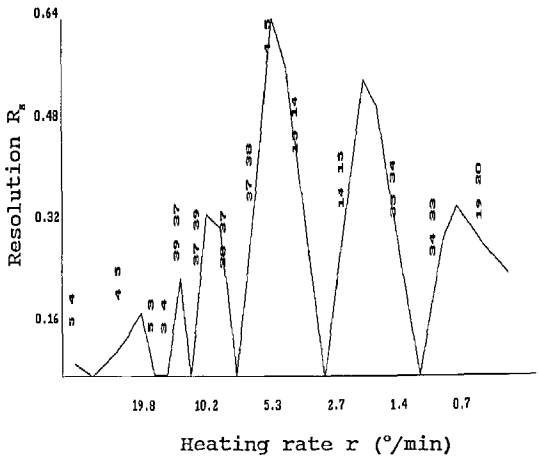


Fig. 13. Resolution map for the 47 largest bands of spearmint oil sample. Conditions: T_0 = 80°C, other conditions as in Fig. 8 unless noted otherwise.

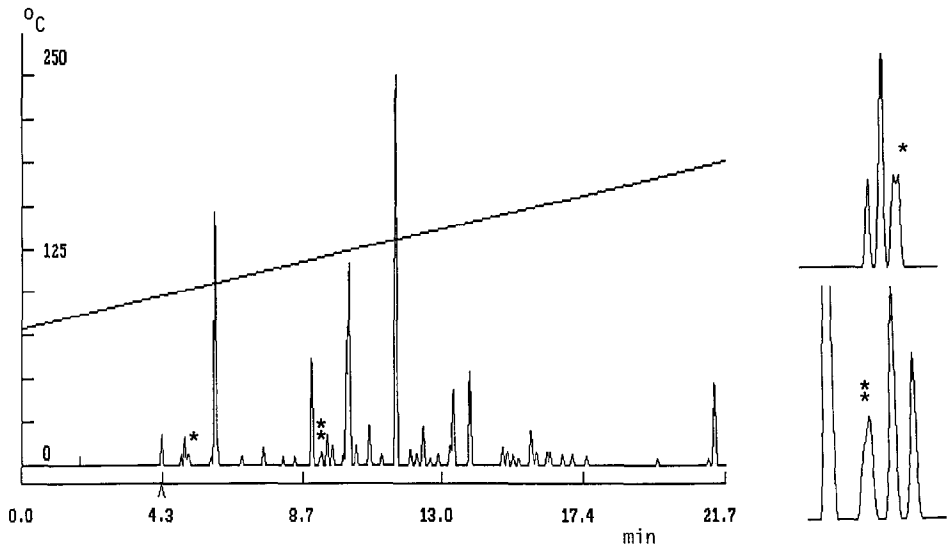


Fig. 14. Optimized separation of the spearmint oil (47 bands) based on a linear temperature program. Conditions: T_0 = 80°C, 4.4°C/min, other conditions as in Fig. 8. Critical band pairs [4/5 (*), 14/15 (**)] expanded on side.

linear-program separation of Fig. 14. The resulting separation is shown in Fig. 15, where predicted (A) and experimental (B) chromatograms are compared. The good agreement between these two separations is further documented in Table III, where retention times are compared. The average deviation between experimental and predicted retention times is only ±1.3%.

Optimizing the separation of individual compounds in complex chromatograms

For some GC assays, there may be more interest in particular compounds or in certain regions of the chromatogram, *vs.* the adequate resolution of the whole sample. In such cases we usually desire some minimum resolution of the compound(s) of interest in the shortest possible time. Computer simulation is particularly useful in optimizing a separation with respect to selected parts of the chromatogram. The general approach is similar to that pursued in Fig. 12, *i.e.*, using partial resolution maps. We will illustrate this for band 16 from the 47-band spearmint oil sample.

Partial resolution maps (as in Fig. 12) indicated that any starting temperature between 50 and 100°C would allow the adequate resolution (R_s = 2.0) of band 16. Systematic trial-and-error simulations were next used to map resolution and run time (retention time of band 16) *vs.* T_0 and r. These simulations are summarized in Fig. 16A, where resolution is plotted *vs.* the retention time of band 16 for different values of T_0 (r varying). It is apparent that a starting temperature of 100°C allows the quickest elution of band 16 with R_s = 2.

Once band 16 has left the column with adequate resolution and minimum run time, the heating rate can be increased sharply to elute the balance of the sample from the column in the shortest possible time. The resulting (optimized) separation of band 16 in the spearmint oil sample is illustrated in Fig. 16B (simulation) and compared

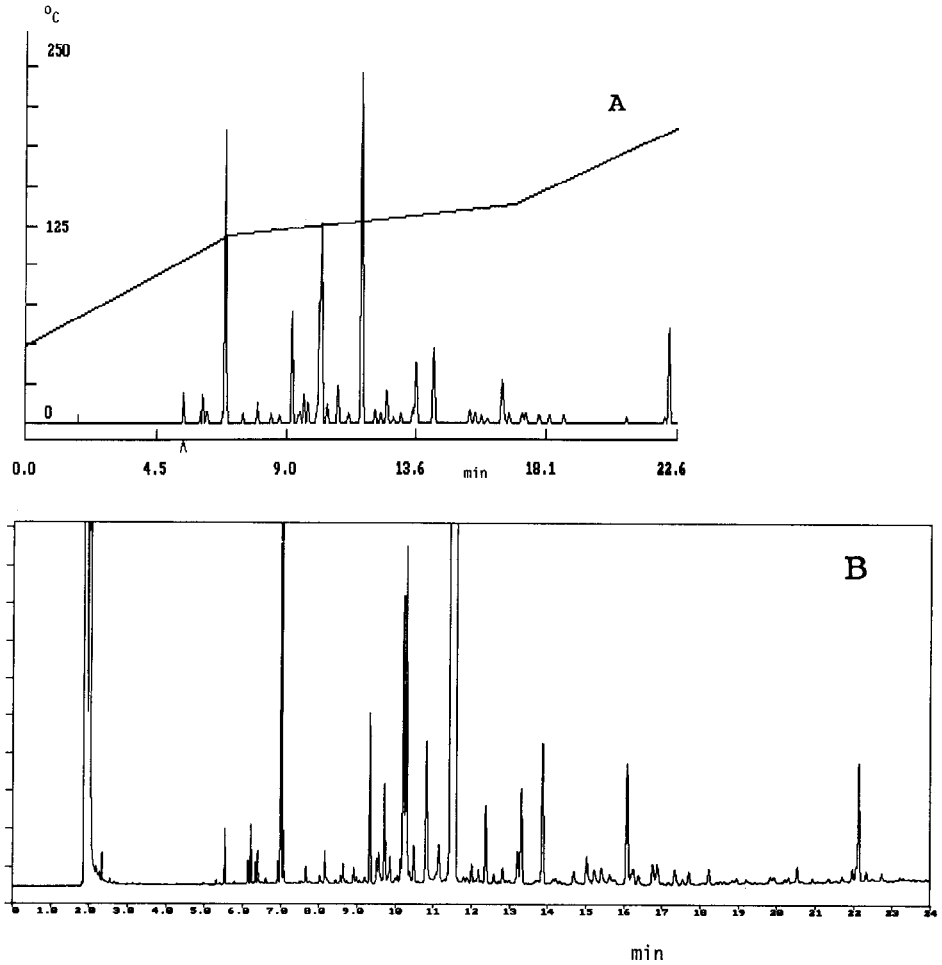


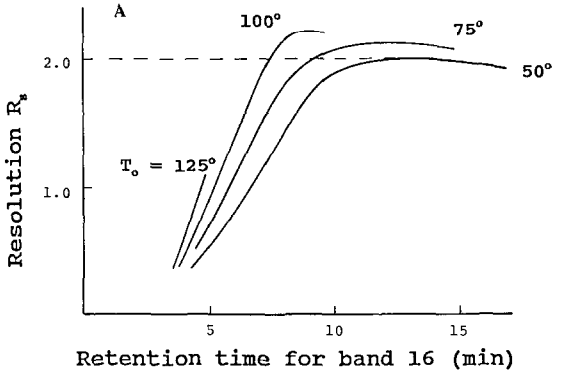
Fig. 15. Optimized multi-ramp separation of the spearmint oil (47 bands). Conditions: 50/120/140/188°C in 0/7/17/23 min, other conditions as in Fig. 8. (A), computer simulation; (B) experimental separation; (C) expansion of critical regions from (B).

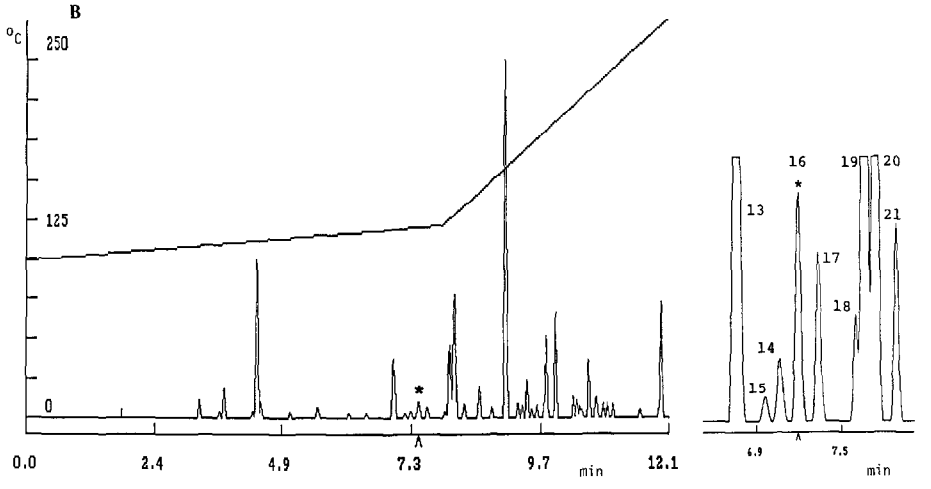
with the experimental run in Fig. 16C. Again there is reasonable agreement between the predicted and experimental separations[a].

Change in column length or flow-rate

Once the temperature program has been optimized for band spacing and maximum resolution, it may be desirable to change column length or flow-rate for either

[a] The somewhat poorer resolution of bands 13–21 in the experimental chromatogram (Fig. 16C) is probably the result of several factors: (i) the presence of other (very) small bands, in addition to the 47 largest bands of this sample (see Fig. 17 in this connection), (ii) slight tailing of the various bands compared to a Gaussian peak, and (iii) a lower plate number in the separation of Fig. 16C *vs.* the input runs of Fig. 8 (the run of Fig. 16C was carried out several months later).





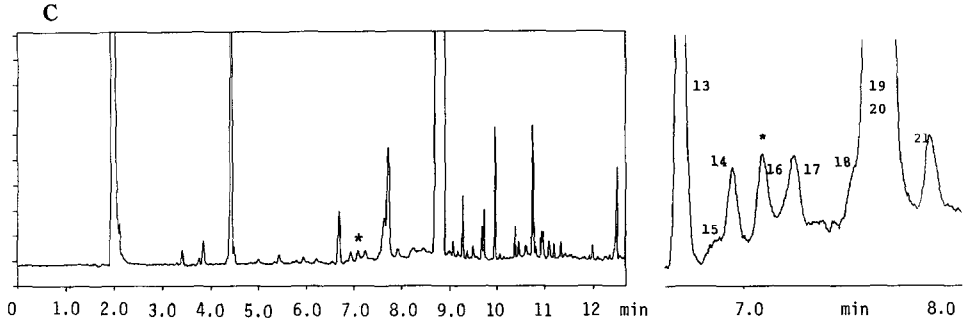


Fig. 16. Development of a GC assay for band 16 in the spearmint oil sample. Conditions as in Fig. 8 except where noted otherwise. (A) summary of computer simulations for resolution of band 16 as a function of the retention time for band 16 and the starting temperature T_0 (r varying); (B) predicted separation of band 16 (*) in minimum run time (12 min, R_s = 2.0); conditions: 100/122/250°C in 0/7.9/12 min; (C) experimental separation corresponding to (B).

an increase in resolution or a decrease in run time. This requires that the run-time be adjusted at the same time (as discussed in Part II [2]), in order to maintain b constant (eqn. 4, Part II) and preserve the optimized band spacing.

Minimizing errors in computer simulations of complex samples

Computer simulation requires accurate data, if the resulting predictions of separation are to be reliable. This means that the experimental runs used for this purpose must meet certain criteria that have been discussed in previous papers [1,3]. For complex samples such as the spearmint oil sample described in this paper, small errors in retention can result in large relative errors in predicted values of resolution. An analogous situation exists for the computer simulation of HPLC separations, as has been discussed in some detail [14]. One of the more common errors in the measurement of experimental retention times occurs for the case of severely overlapping bands, where only one retention time is reported by the data system. This is illustrated in Fig. 17A for overlapping bands 14 and 15 from the 8°C/min run used as input for the spearmint oil sample. If a single retention time (10.38 min) is used for both bands in computer simulation, significant errors (± 0.5 in R_s) can arise in the later prediction of resolution as a function of experimental conditions.

A comparison of band 14/15 in Fig. 17A (8°C/min run) with the same two bands in the 2°C/min run (Fig. 17B) shows that band 15 is eluted first in the run of Fig. 17A (note the bulge on the leading edge of this band). By making small adjustments in the estimated retention times of bands 14 and 15 in Fig. 17A, and using these estimates for the prediction of retention under other run conditions, a comparison of predicted and experimental retention can then be used to select the best retention times for bands 14 and 15 in Fig. 17A (10.39 and 10.35 min for bands 14 and 15, respectively). This procedure was used in the present study in order to refine the predictive accuracy of computer simulation for the spearmint oil sample. In this connection, note the good agreement for the predicted retention times of bands 14 and 15 in Table III (and especially the similar errors for each band).

In most cases, errors of this type (due to band overlap) will not be significant and can therefore be ignored. However, when important differences are found between subsequent predicted and experimental chromatograms, errors of this kind can be suspected and corrected as above.

CONCLUSIONS

The use of computer simulation for facilitating GC method development has been demonstrated for several different samples and applications. Method development begins by using resolution maps to define the dependence of programmed-temperature separations on the starting temperature and heating rate. For less complex samples, this usually leads directly to optimized separation conditions . Isothermal separations can also be evaluated as an alternative to temperature-programmed runs.

More complex samples can be approached in the same way, but often such samples cannot be separated by means of any linear temperature program (for any combination of starting temperature and heating rate). Using computer simulation, it is possible to identify samples of this type with minimum effort. It is also possible to

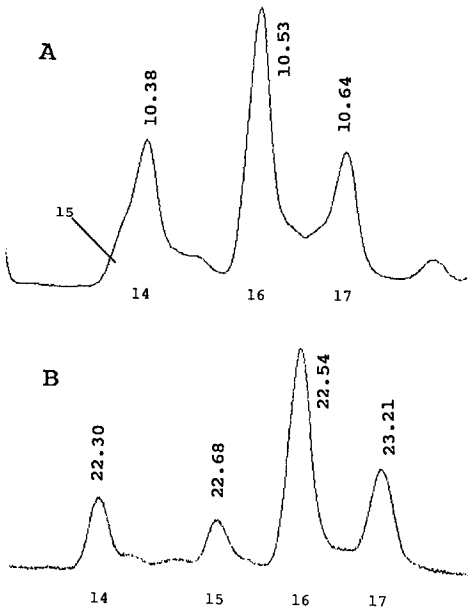


Fig. 17. Retention time errors in experimental runs due to overlapping bands (bands 14–17 shown). Bands 14 and 15 in 2 and 8°C/min runs used as input for the spearmint oil sample (47 bands). (A) 2°C/min; (B) 8°C/min. Numbers at peaks indicate retention times in min.

improve such separations significantly by means of multi-ramp temperature programs. The attempted development of optimized multi-ramp programs by the usual trial-and-error process (in the laboratory) will often prove impractical, because of the large number of experimental runs that would be required. Computer simulation can make use of partial resolution maps to reduce the number of required trial-and-error runs, while allowing such runs to be carried out (on the computer) in a small fraction of the time required in the laboratory.

Computer simulation is equally useful for the problem of optimizing the separation of one or a few bands in a complex sample, as opposed to resolving the entire sample. "Limited resolution maps" provide a systematic means of identifying optimum conditions for such cases, requiring only a few minutes of computer simulation.

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