Journal of Chromatography, 290 (1984) 311–319
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 295

AUTOMATED DEVELOPMENT OF OPTIMUM TEMPERATURE PROGRAMMES FOR GAS CHROMATOGRAPHIC SEPARATION OF COMPLEX MIXTURES ON CAPILLARY COLUMNS

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SUMMARY

A sequential experimental optimization procedure for developing a temperature programme to separate complex mixtures using capillary gas chromatography is described. In the first part of the sequence the chromatograms are evaluated on the basis of the number of peaks. In the second part the resolution of adjacent peaks is calculated and the temperature programme is adjusted accordingly. Finally the temperature profile is compressed to save analysis time.

The procedure is performed automatically by a microprocessor-controlled gas chromatograph with autosampling. The computer program is written in BASIC. The utility of the procedure is illustrated by the automated development of a temperature programme for the separation of 38 halogenated pesticides within 10 h.

INTRODUCTION

The great analytical potential of high-performance capillary gas chromatography can be fully exploited only under the optimum working separation conditions. Once a suitable capillary column for solving an analytical problem has been selected, the development of the best temperature programme may be a time-consuming task, particularly for complex mixtures.

In the past decade several theoretical approaches to the optimization of chromatographic separations have been presented[1–5]. A critical evaluation of quality criteria for the optimization of chromatographic multicomponent separations was performed recently by Debets *et al.*[6]. They stated that all multicomponent quality criteria tested in their study were directly related to chromatographic separation. Although resolution alone does not satisfy all theoretical demands it seems to be the best criterion for practical use. Generally, chromatographers evaluate the quality of their chromatograms by means of the resolution.

In this paper we present the strategy and application of a computer program written in BASIC for an automated sequential experimental optimization of a temperature programme for analysing complex multicomponent mixtures using capillary gas chromatography. The aim was to develop on an empirical basis an optimization

0021-9673/84/$03.00

procedure that enables us to find the best separation conditions for any complex mixture on an available capillary column unattended overnight.

EXPERIMENTAL

Optimization strategy

The aims of our optimization strategy are:

(1) Separation of a maximum number of peaks that can reproducibly be recognized by a common integrator (qualitative analysis),

(2) Sufficient resolution of all peaks to obtain reproducible peak areas (quantitative analysis),

(3) Short analysis time (economic aspect).

A temperature programme in gas chromatography consists of isothermal and heating periods. This means there are the three variables: the temperature of the isotherm, the length of the isothermal period and the rate of heating.

When performing trace analysis on capillary columns with autosampling, splitless injection on a cold column is the method of choice. This means some fixed boundary conditions, namely the boiling point of the solvent, the boiling point of the most volatile component in the mixture and the maximum allowable operating temperature.

Our optimization strategy follows a stepwise approximation of the final temperature programme. The complex mixture is applied from the very beginning.

In the first part of the approximation we monitor the improvement of the separation by counting the number of peaks recognized: an increase following a change in the temperature programme is regarded as an improvement, and therefore this new part of the temperature programme is saved in the memory. On the other hand, a change that does not increase the peak number is neglected.

For the first approximation procedure we established a frame of isothermal levels with 20°C difference, three possible lengths of the isotherms and three different heating rates. After the injection at 100°C a heating rate of 30°C/min is set until the

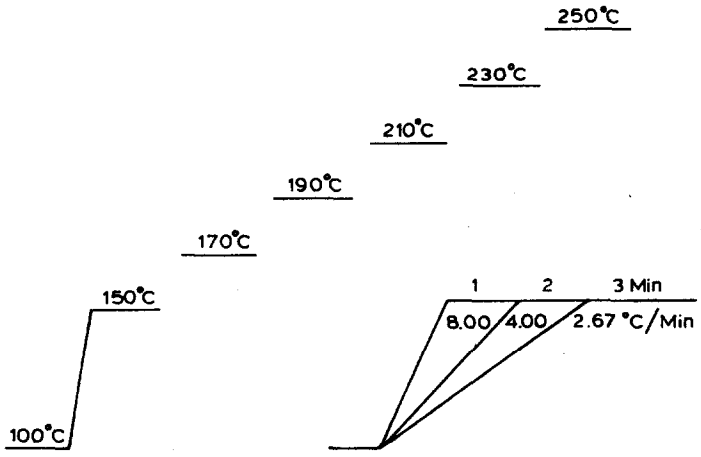

Fig. 1. Frame of the temperature program for the first part of the optimization procedure.

first isothermal level is reached (Fig. 1). The first run is performed at the highest heating rate of 30°C/min to the highest isothermal level, 250°C. This temperature is held for a fixed time to ensure the complete elution of all components of the mixture. After the peaks have been counted the computer changes the parameters: the initial heating period ends at 230°C and is followed by heating at a rate of 8°C/min to the final temperature of 250°C. The number of peaks recognized after the second run is compared with the number found in the first run. If an increase is observed the new rate of 8°C/min replaces the old one between 230°C and 250°C in the temporary temperature profile. The next parameter settings follow the same direction until no further increase of in the peak number is observed. If, for instance, a new rate of 4°C/min does not improve the separation in the third run the settings after the second run remain in the memory and the last rate will be rejected. The next parameter setting introduces an isothermal period of 1 min at 230°C, and prolongation of the latter then depends on the result of the preceding trial.

In this way the designed frame of the temperature profile is tested and each new parameter setting is determined by the result of the preceding experiment. The first part of the approximation process is finished when the predetermined frame has been filled up.

In the second part of the optimization the chromatogram is checked for resolution between all peaks. The resolution (R) of two adjacent peaks with retention times t_1 and t_2 and peak widths at half-height w_1 and w_2 is calculated and compared with a given value:

$$R = (t_2 - t_1)/(w_2 + w_1)$$

In the present example R was set to 1.5. If the test is positive, it means that the slope of the temperature programme is too steep in this particular region. Therefore, new sections have to be introduced in order to slow down the rate of the temperature increase. This is executed as follows. The computer calculates the oven temperature at the retention time of the unresolved peaks. Two cases are possible. (1) If the retention temperature indicates that the temperature programme is on a ramp, the difference between the calculated retention temperature and the temperature at the start of this ramp is halved. A new isotherm lasting for 2 min is introduced at the calculated level. (2) If the temperature indicates that the programme is on an isotherm, the preceding rate is halved, and an isotherm of 2 min is introduced at the calculated temperature. The former isothermal level is reached with a new calculated rate.

In the second approximation the computer is programmed only for achieving a sufficient resolution. The aim is attained by slowing down the temperature increase, which results in an extension of the analysis time. Therefore, we added a third section to the optimization procedure in order to reduce the time of analysis. Again, the resolution between all peaks is checked and if a resolution between two adjacent peaks is found to be higher than 2.0, the temperature programme is accelerated. This is executed in a way analogous to the second approximation step. If the retention time of the considered peaks corresponds with a rate, the slope is increased and if it corresponds to an isotherm the length is shortened.

Instrumentation

All gas chromatographic analyses were carried out on a gas chromatograph HP 5880 A (Hewlett-Packard, Avondale, CA, U.S.A.) equipped with a splitless injector for capillary columns and an electron-capture detector. The signal from the detector was processed on the built-in microprocessor–integrator, which can be programmed in BASIC. Injections of 3 μl were executed by an autosampler HP 7671, which is controlled by the HP 5880 A gas chromatograph.

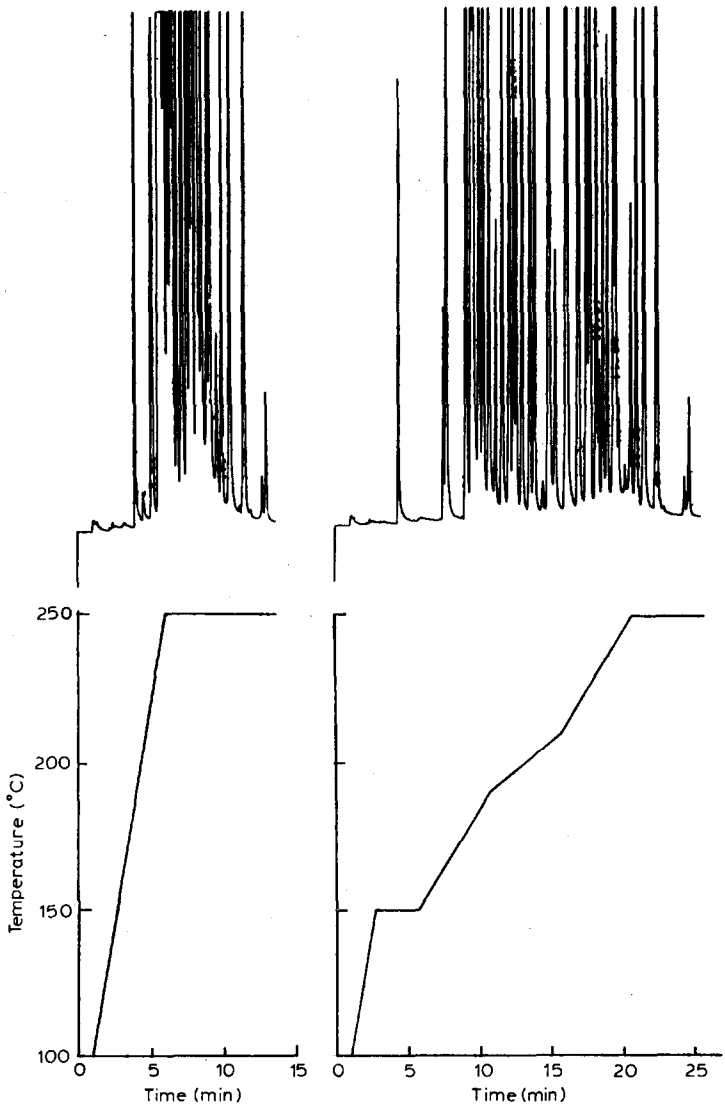

Fig. 2. Chromatograms of the first (left) and the last experiment (right) in the first part of the optimization procedure: 28 peaks were registered after the first run, 36 after the last run.

Gas chromatography

The analyses were performed on a fused-silica capillary column coated with "bonded phase" dimethylsilicone BP-1 (25 m × 0.2 mm I.D., Scientific Glass Engineering, Ringwood, Australia). Helium was used as carrier gas, the electron-capture detector was purged with argon containing 10% methane at 25 ml/min. Temperatures were set at 240°C for the injection port and 300°C for the detector. Splitless injection[7] into the "cold column" at 100°C was carried out with the split valve closed for 30 sec, and 60 sec after injection the temperature programme was started. The complex test mixture consisted of 38 halogenated pesticides as used in pesticide residue analysis in food[8].

RESULTS

The first run was performed with the highest heating rate of 30°C/min to the highest isothermal level, 250°C. After 10 min at this temperature the chromatogram was finished. Within *ca.* 14 min 28 peaks out of 38 compounds in the mixture were recognized by the integrator. Following the outlined frame the computer reduced the first isothermal level to 230°C and introduced a heating rate of 8°C/min for the final 20°C. After 7 h and sixteen injections a temperature programme was created.

The intitial chromatogram and the last one of the first approximation part are compared in Fig. 2. The number of peaks is almost at the maximum, but the resolution is obviously insufficient. The temperature programme created is relatively simple.

In the second part only two further injections were necessary to achieve complete separation into the maximum number of 38 compounds. All peaks were separated at the given resolution of 1.5 (Fig. 3), and the temperature programme looked rather complicated. The chromatogram showed several sections of overresolution, which were reduced in the third part of the optimization procedure. After another 1.5 h work with two injections the final temperature programme was established. All 38 compounds were completely separated within 37 min (Fig. 4).

In this example the optimization process was finished within *ca.* 10 h. The first approximation contained 16 injections and lasted *ca.* 7 h. The second part was obviously the most efficient one. With only two injections the aim of separating all 38 compounds with a minimum resolution of 1.5 was achieved within 1.5 h. The final modification included two injections taking another 1.5 h and yielded a 16% saving of analysis time.

DISCUSSION

The computer program described here enables us to find the optimum separation conditions for the analysis of a complex mixture on an unknown capillary column overnight. The reason for developing this automated optimization procedure was very simple. We did not want to waste our time with trial-and-error games in the laboratory when having a gas chromatograph at hand that understands BASIC. Therefore, we transferred our thinking to the language of the microcomputer, which would do the job overnight. Starting with the "job-transfer" we recognized that we had to organize rationally the trial-and-error game into a frame of predetermined

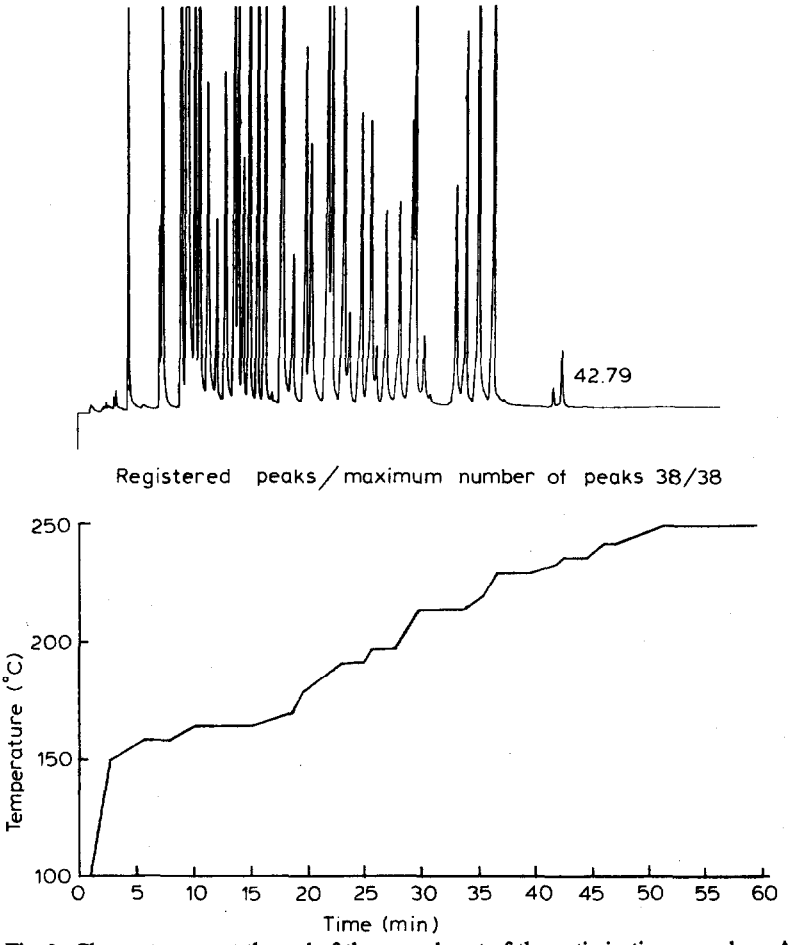

Fig. 3. Chromatogram at the end of the second part of the optimization procedure. All components are separated at a minimum resolution of 1.5.

temperature levels and to provide the computer with an optimization criterion. In the first approximation part we opted for the peak number, because in residue trace analysis the most important objective is to differentiate between a multitude of components. For quantitative analysis a better resolution is necessary, therefore we tried to improve the separation to obtain a resolution of 1.5 between all peaks. This resolution guarantees a reproducible quantitative calibration of all compounds in the mixture.

Our computer program demonstrates in its first part a very formal approach that does not make use of any information about the sample. This general form was designed to show the applicability of the program to completely unknown mixtures. Therefore, it cannot fulfill at the same time all the demands of effectiveness. Mostly, there is a considerable body of knowledge available about the chromatographic behaviour of the substances under investigation. Frequently, a temperature programme on a similar column is available. In this situation the first run may be started with

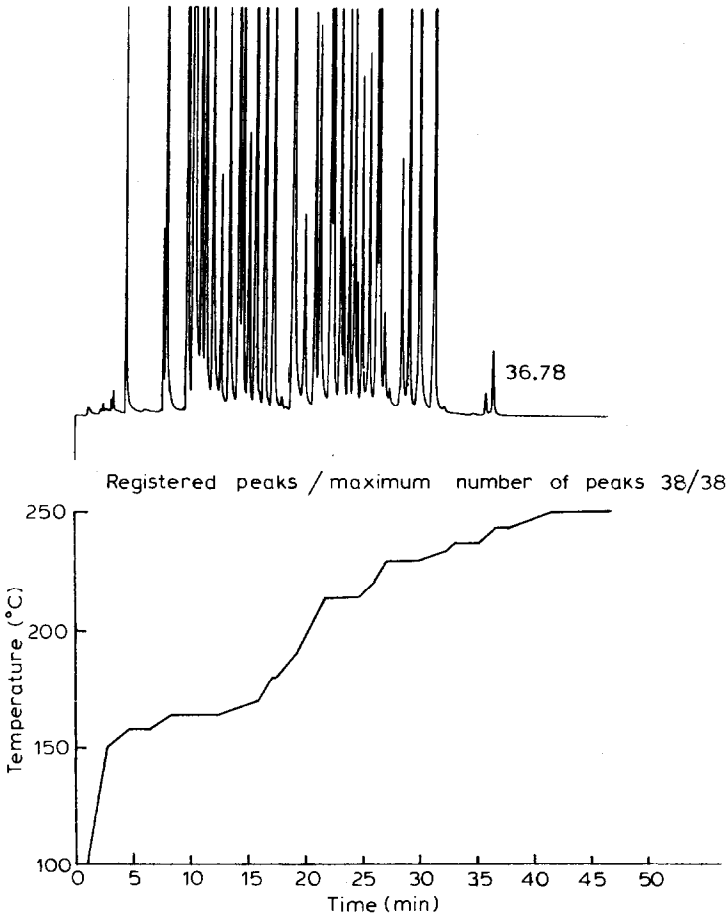

Fig. 4. Final chromatogram resulting from the automated optimization procedure within 10 h.

a casual temperature program which afterwards can be improved by applying the second and third part of the optimization procedure. This should result in a significant saving of time.

Doubtless, the second part of the optimization has proved to be the most efficient one. In the example described the required resolution was attained in 1.5 h. This may be a favourable example because it was known from previous work that all components of the mixture can be separated on the column used[8]. With a multicomponent mixture that cannot be completely separated on that column the process takes longer. In this case the computer tries to resolve the identified critical pairs by slowing down the temperature increase but without success. After a given number of such fruitless attempts the computer indicates the critical pairs and stops trying to change the temperature profile in this region.

The modification of the temperature programme designed to save analysis time is performed as the final optimization step. The present version is not very effective owing to the lack of sufficient memory capacity. The temperature profile is compressed only gradually in order to prevent the loss of necessary resolution.

Optimization of an analytical procedure is a general problem in analytical chemistry. According to Massart *et al.*[9] this can be carried out in two general ways:

(1) Using the analytical approach, with the word "analytical" in its mathematical sense meaning identify the underlying physicochemical principle and to develop a mathematical equation to describe the process.

(2) Using the black box approach, which means considering the method purely from the experimental side.

Our method is a typical black box approach using a sequently experimental design. It may be considered as a two-variable function with only one optimum. The two variables are the heating rate and the time. For two- or multi-parameter methods the so-called simplex method has been used systematically[9]. Its general theory and the planning of experiments for optimizing a chemical analytical procedure was first described by Long[10]. The simplex algorithm includes a lot of mathematical calculations, and until now it has been preferentially applied to optimize the experimental conditions in liquid chromatography[11–13]. Berridge[13] reported the unattended optimization of reversed-phase high-performance liquid chromatographic separations with a modified simplex algorithm using a microcomputer-controlled chromatograph with autosampling. This work represents an analogous approach to ours.

An early application to gas chromatography was reported by Morgan and Deming[1]. The simultaneously varied the oven temperature and the carrier gas flow-rate in order to optimize these parameters for a five-component system using packed columns.

Holderith *et al.*[2] presented an experimental approach to minimize the analysis time in gas chromatography applying the simplex algorithm. They changed alternately the initial oven temperature, the rate of the linear temperature programme and the carrier gas pressure to optimize the separation of nine components in a mixture of methylbenzenes on a packed column. The optimization criterion was the value of the peak separation for two critical pairs higher than 0.5. After seventeen experiments a reasonable approximation to the defined optimum was reached, and after 24 runs the final result was obtained.

Debets *et al.*[6] stated in their evaluation of optimization criteria that all of them are based on resolution. They do not give an optimal value of the criterion response without prior information about the number of peaks to be found in the chromatogram.

Our empirical approach differs from those that are based on the mathematical theories described by applying various optimization criteria in the successive parts of the approximation program. In the first part of the procedure the criterion is the maximum peak number. In the predetermined frame the next experiment is selected based on boolean decisions. In the second part further optimization is carried out with a fixed resolution as quality factor and afterwards with the total analysis time as optimization criterion. Limitation of the analysis time is desirable not only for economic reasons. In trace analysis a short analysis time guarantees the required detection sensitivity also for the later eluting compounds.

Applying this automated optimization programme to establish new analytical methods will be less time-consuming. This is valid not only when developing a completely new gas chromatographic system as described but also when adapting a method to another capillary or increasing the number of components in a mixture.

Furthermore the automated procedure enables the analyst to select the best column for an analytical problem, because this can be achieved with a minimum of attendance.

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