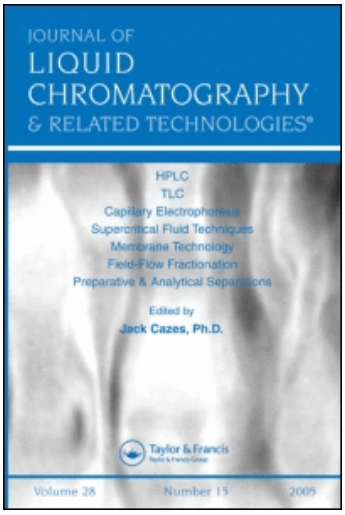

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Computer Program for the Selection of Gradient Elution in High Performance Liquid Chromatography

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To cite this Article Issaq, Haleem J. , McNitt, Karen L. and Goldgaber, Nina(1984) 'A Computer Program for the Selection of Gradient Elution in High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 7: 13, 2535 — 2543
To link to this Article: DOI: 10.1080/01483918408067023
URL: http://dx.doi.org/10.1080/01483918408067023

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(13), 2535-2543 (1984)

A COMPUTER PROGRAM FOR THE SELECTION OF GRADIENT ELUTION IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A computer program is presented for the selection of a gradient mobile phase that will give the same resolution for all the component pairs in the mixture. Each pair is treated as a separate experiment. The computer is used to compile these experiments and to recommend an optimum gradient.

INTRODUCTION

Many studies, using statistical (1-4) and graphical (5) approaches for the selection of an isocratic mobile phase that will give optimum separation have been published. Most of these methods require the use of computers

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for data handling, and storage which makes the chromatographers' job faster and easier. In HPLC this means calculation of the area under the peaks, the width of the peaks at any specified height, the retention times and the resolution between the peaks. Also, computers have been used for the prediction of peak elution order (6,7) and for the sequencing of experiments with different parameters for each. The parameters that could be changed are mobile phase composition, flow rate and temperature.
In this study we extend the use of computers for the selection of a gradient mobile phase that will give optimum separation.

DISCUSSION

Gradient elution is mainly used in two cases, (a) when an isocratic mobile phase fails to give adequate resolution of all the components in a mixture, due to the component's properties; and (b) when an isocratic mobile phase gives satisfactory resolution of all the components but those peaks eluting last are wide and far from each other. In the first case (a) gradient elution is used to facilitate the separation while in the second case (b) it is used to bring the late eluting peaks closer together, which results in better sensitivity and shorter analysis time. For detailed discussion of gradient elution see ref. (8). The gradient shapes generally used are linear, convex or concave, see fig. 1. Although these gradient shapes may improve resolution for each adjacent pair of components, if not impossible. Gradient separations that result in the same resolution between the adjacent pairs will be defined as optimum gradient, figure (2). To achieve an optimum gradient the chromatographer should treat each adjacent pair of components as a separate experiment, to optimize the separation between the first pair, then the second pair and so on, so that the sum of these series of pair optimization experiments is the

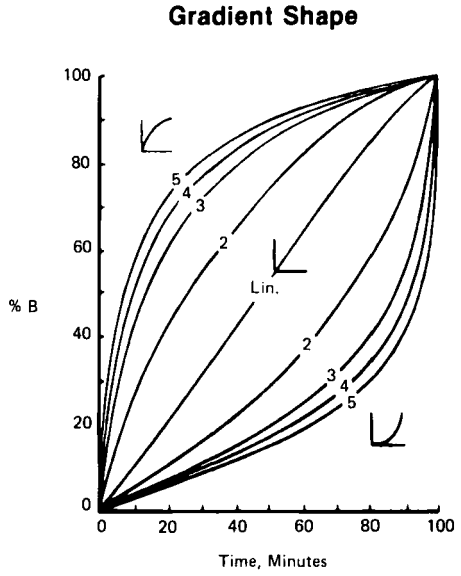

Figure 1. Gradient shapes used in high performance liquid chromatography

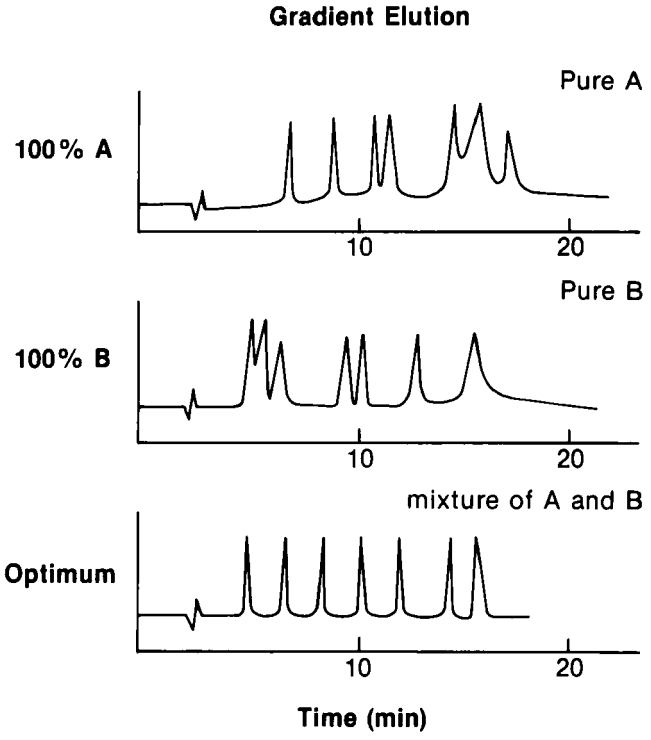

Figure 2. Gradient elution of a mixture using pure solvent A and B and optimum gradient mixture of solvents A and B.

optimum gradient. This seems to be very complex, but it is not if a computer is used to store the results of these series of experiments and to select the mobile phase for each pair based on the empirical data and to compile the final mobile phase. It is clear from the above that the gradient shape will be different from those shown in figure 1. The gradient shape predicted will consist of a series of straight lines (Figure 3). Each straight line represents a mobile phase composition that will result in the same resolution between all the pairs in the mixture. These straight lines may or may not have the same slope. To achieve an optimum gradient, a series of experiments are needed, the number of which depends on the complexity of the sample (number of components) and difficulty of separation. An average procedure will require five runs. The runs are of two types and it is up to the chromatographer to select the one he prefers. The first type is shown in figure 4, whereby the mobile phase composition is constant but the time period is not. Assume that the gradient is linear and will run from 100%A (the weak solvent) to 100%B (the strong solvent) in 10 min., the next gradient will run from 100%A to 100%B in 20 min., the third one from 100%A to 100%B in 30 min., and so on until all the components in the mixture are resolved. Solvents A and B should be miscible. Figure 5 shows the second type of gradient mobile phase composition experiments needed to determine the optimum gradient. In this case the mobile phase remains constant. The linear gradient is run from 0%B to 20%B (100%A to 80%A) in 30 min., the next time it is run from 0%B to 40% in 30 min., the third run from 0%B to 60%B in 30 min, and so on. In each case the computer will calculate the resolution between the peaks and print it in a table at the end of the experiment. The chromatographer will specify in the computer program the resolution needed between the adjacent pairs. The computer will at the end of the run search for the mobile phase composition that will give the predetermined resolution between each

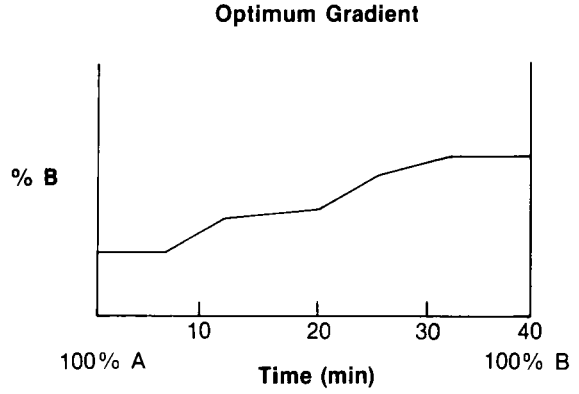

Figure 3. A graphical representation of an optimum gradient curve.

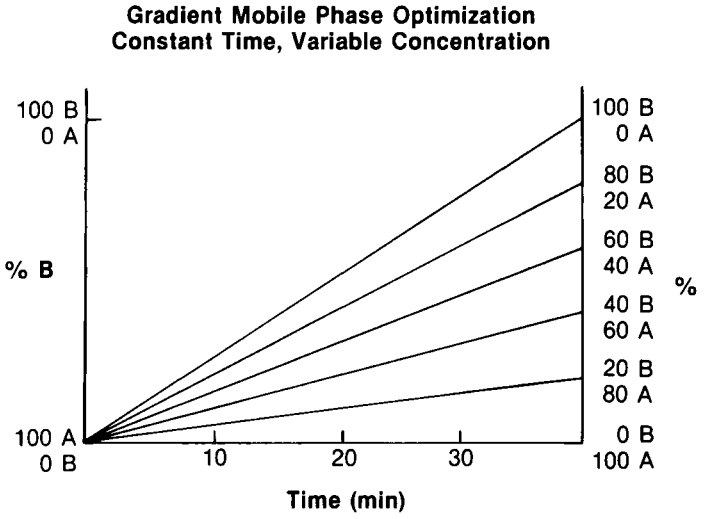

Figure 4. Gradient mobile phase optimization using constant time, 40 min., but variable mobile phase composition.

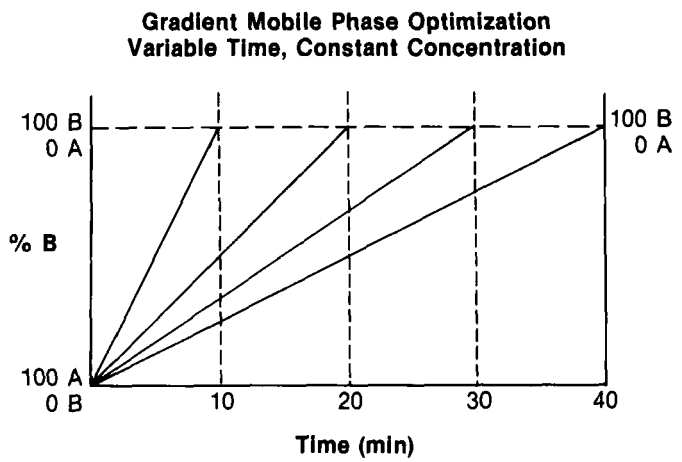

Figure 5. Gradient mobile phase optimization using variable time but the same gradient, 100%A (0%B) to 100%B (0%A).

adjacent pair and print it in a table and in a graphical form as shown in figure 3.

COMPUTER PROGRAM

The computer program is available on request from the authors. It is written in Lab Basic for a Hewlett Packard 3354 Lab Data System. A flow chart of the program is presented in figure 6. The program assumes the peak areas and retention times are saved on the system's processed data file and the signal from the instrument is available on the system's raw data files. Due to the limitations of 32K of core on the HP3354, the program is actually split into two sections, though this split is invisible to the user.

The program operates as follows: for each run the user enters the processed data file name, the peak's elution order, in case of peak crossover and the final concentration of solvent A where the starting

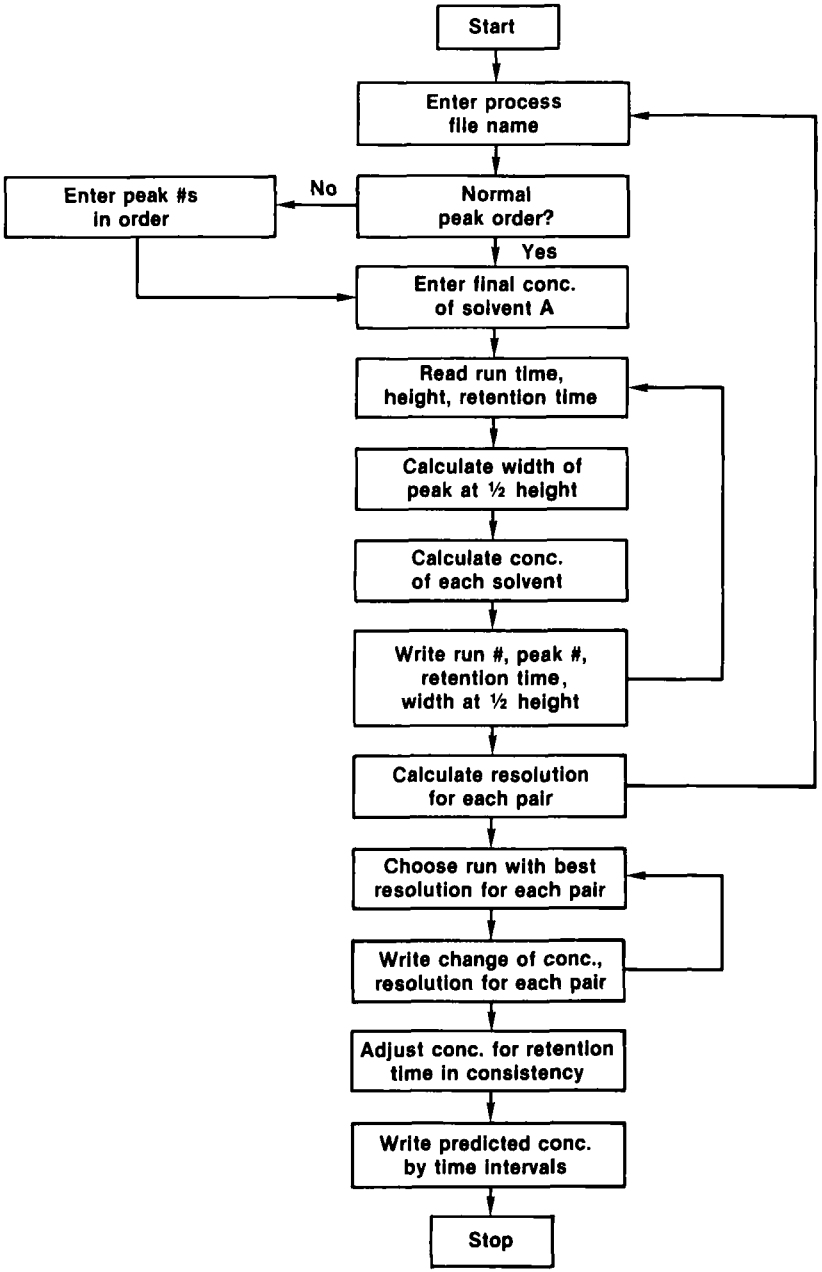

Figure 6. Flow chart of the computer program.

mobile phase composition is 0%A and 100%B. The processed data file gives the runtime and the peak retention times and heights. The peak width at half height is calculated from the raw data file. The concentration for each solvent at the peak retention time is computed and the resolution between each peak pair is calculated. Data for up to 10 runs with 10 peaks per run can be processed, this can be easily expanded to a larger number of peaks. For each peak pair the retention times and mobile phase compositions which produced the best resolution are selected as recommended at those times. If there are retention time conflicts between adjacent peaks (i.e. the best resolved retention times between peak 1 and 2 overlap the retention times for peaks 2 and 3) then a regression is fit to the four time-concentration points to smooth the data. However, no corrections are done on the mobile phase compositions if there are no retention time conflicts, which may result in inconsistant composition recommendations.

ACKNOWLEDGEMENTS

This work was supported by Contract No. NO1-CO-23910, with the National Cancer Institute, NIH, Bethesda, MD 20205.

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