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MULTIFACTOR OPTIMIZATION OF REVERSED-PHASE LIQUID CHROMATOGRAPHIC SEPARATIONS

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SUMMARY

The single-factor "window diagram" technique of Laub and Purnell is extended to the multifactor case, and is successfully applied to the reversed-phase liquid chromatographic separation of a nine-component mixture.

INTRODUCTION

Reversed-phase high-performance liquid chromatography employing chemically bonded hydrocarbonaceous stationary phases has enjoyed widespread success primarily because of the large number of variable factors that can be adjusted in the polar mobile phase to give improved chromatographic performance¹. These factors include pH, ionic strength, polarity, dielectric constant, hydrogen bonding capability and concentration of surface-active ions². To date, most studies directed toward the improvement of chromatographic performance have optimized only one factor at a time. It is clear, however, that if the full power of eluent composition is to be realized, efficient strategies for multifactor chromatographic optimization must be developed.

As discussed previously³, optimization of chromatographic performance is especially difficult because of the existence of multiple optima over the domain of factor space. Geometric pattern search techniques such as evolutionary operation^{4,5} or the sequential simplex technique⁶⁻⁹ will find a local optimum, but cannot guarantee that the "global" or overall optimum chromatographic performance has been achieved¹⁰.

The "window diagram" technique of Laub and Purnell¹¹⁻¹⁵ has been shown to be an effective means of locating the global optimum if a mathematical functional relationship between chromatographic retention and a single variable factor is known or can be assumed. Single-factor systems for which the window diagram technique has been used successfully include variation of stationary phase composition in gas chromatography¹¹, variation of pH in liquid chromatography¹⁶⁻¹⁸, and variation of lanthanide-induced-shift reagent concentration in nuclear magnetic resonance spectrometry¹⁵.

This paper extends the single-factor window diagram technique to the multifactor case. Results are presented for a two-factor study in which values of pH and

concentration of ion-interaction reagent (IIR) are chosen to give optimal chromatographic performance.

THEORY

The existence of multiple sets of locally optimal experimental conditions in chromatographic systems arises because of the reversal of peak elution order with variation of experimental conditions^{3,9,11,16}. Laub and Purnell¹¹ have shown that plotting the relative retention (α) as a function of a single chromatographic factor (e.g., pH) for all possible pairs of compounds in a mixture gives a "window diagram" that can be used to locate the globally optimal experimental condition. The "windows" consist of the areas below the curves showing lowest relative retention. The experimental condition corresponding to the top of the tallest window gives the best possible separation of the two worst separated pairs of compounds¹¹. Measures of chromatographic response other than relative retention can also be used as optimization criteria, and might also take into account analysis time and ruggedness³.

If relative retention values for all possible pairs of compounds in a mixture are plotted as a function of two or more chromatographic factors, the "windows" become multidimensional in nature. In the case of two factors (pH and concentration of IIR, for example), as the "flat" windows in one factor dimension are projected through the second factor dimension, they would change height, width, and position because of interactions between the two factors: the "window diagram" would then take on the appearance of a mountain range. The set of experimental conditions corresponding to the top of the tallest "mountain" gives the best possible separation of the two worst separated pairs of compounds.

EXPERIMENTAL

Experimental conditions used to investigate the liquid chromatographic behavior of nine related compounds as a function of pH and concentration of ion-interaction reagent (IIR = octylamine) have been given previously¹⁹. In the present study, all calculations were carried out on a Hewlett-Packard 9830A digital computer (Calculator Products Division, Loveland, CO, U.S.A.); figures were drawn from digitized data on a Model 9862A plotter (Hewlett-Packard).

RESULTS AND DISCUSSION

Fig. 1 shows the predicted retention behavior¹⁹ of *trans*-ferulic acid (F) and phenylacetic acid (A) as functions of both pH (3.6–6.0) and concentration of ion-interaction reagent (0–6.0 mM). The intersection of the two response surfaces corresponds to conditions of pH and [IIR] at which *trans*-ferulic and phenylacetic acids have the same retention time, conditions that are clearly undesirable for separating the two compounds. Combinations of pH and [IIR] that are not at the intersection will lead to a separation of the two compounds, some combinations giving better separation than others.

Fig. 2 plots the predicted retention behavior¹⁹ of *trans*-*p*-coumaric acid (M) and phenylacetic acid (A) as functions of both pH and [IIR]. The intersection of

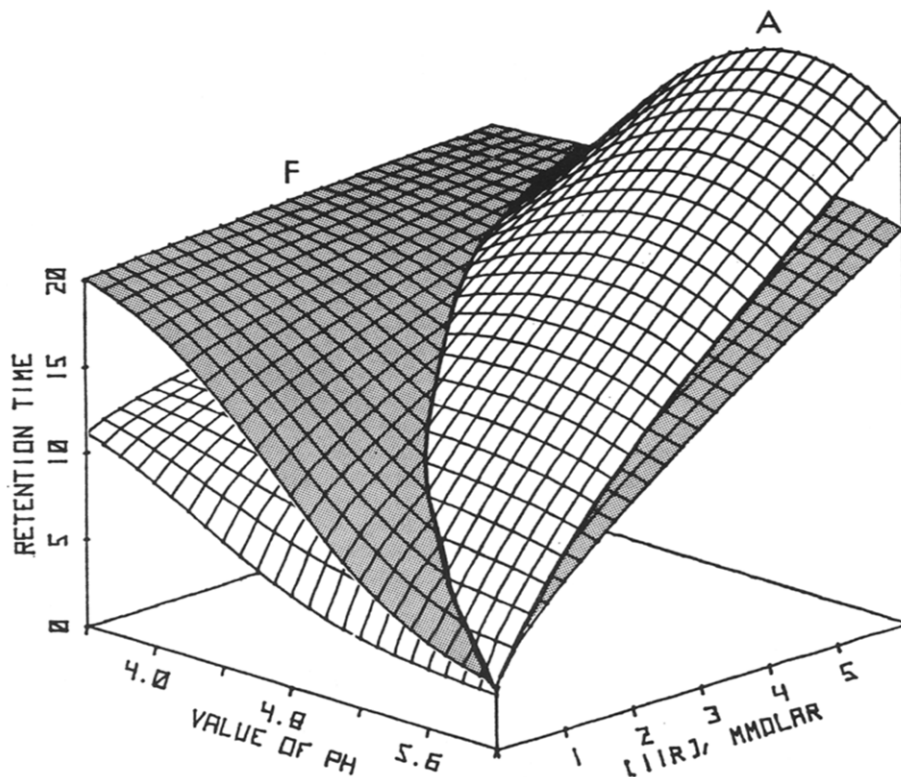


Fig. 1. Predicted retention behavior of *trans*-ferulic acid (F) and phenylacetic acid (A) as functions of pH and [IIR].

these two response surfaces occurs at different values of pH and [IIR] than the intersection in Fig. 1. Thus, while *trans*-ferulic and phenylacetic acids might be well separated at pH 4.6 and [IIR] = 1 mM (see Fig. 1), *trans*-*p*-coumaric and phenylacetic acids would be poorly separated under these conditions (see Fig. 2).

Similar information is given in Figs. 3 and 4 where relative retention (α) values are plotted for *trans*-ferulic and phenylacetic acids and for *trans*-*p*-coumaric and phenylacetic acids, respectively. As expected, α goes to unity at conditions corresponding to equal retention times (the intersections in Figs. 1 and 2). Values of α greater than 1.4 are set equal to 1.4 in Figs. 3 and 4.

Fig. 5 is a two-dimensional composite window diagram for nine compounds evaluated in a previous study¹⁹: hydrocinnamic (H), *trans*-cinnamic (N), phenylacetic (A), *trans*-*p*-coumaric (M), *trans*-ferulic (F), *trans*-caffeic (C), and vanillic (V) acids, phenylethylamine (E), and phenylalanine (P). The surface in Fig. 5 shows the α values for the worst separated pair of these compounds at each combination of pH and [IIR]; all other pairs of compounds are better separated. Again, the top of the tallest two-dimensional "window" corresponds to experimental conditions of pH and [IIR] giving the best separation of the worst separated pairs of compounds.

Fig. 5 predicts that at pH 5.6 and [IIR] = 0 mM, at least one pair of compounds should be very poorly separated. This is confirmed in the chromatogram

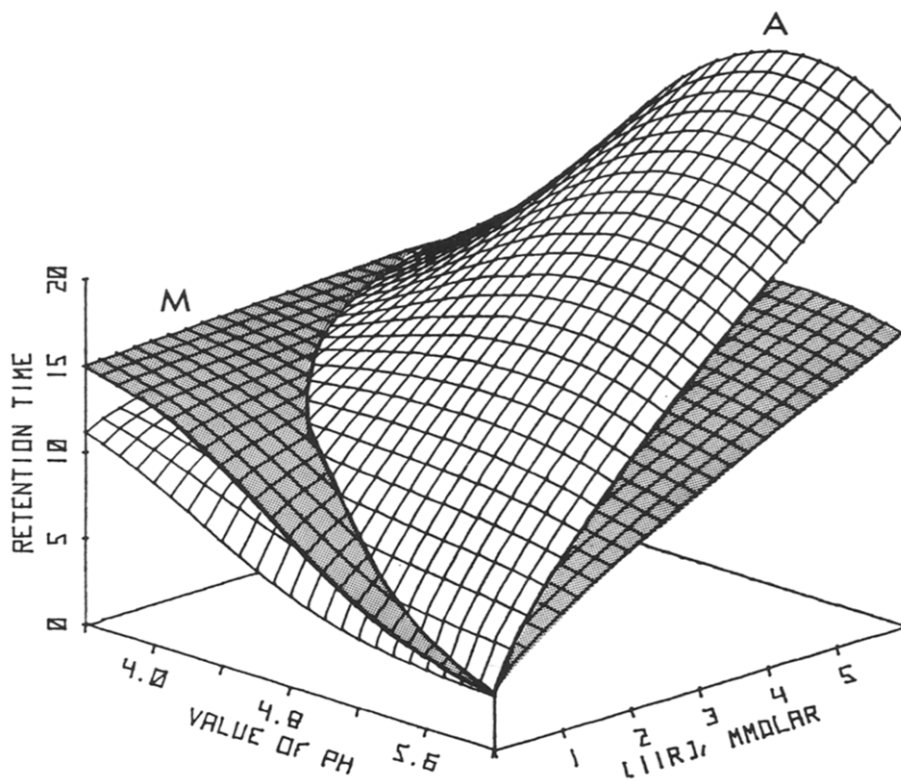


Fig. 2. Predicted retention behavior of *trans-p*-coumaric acid (M) and phenylacetic acid (A) as functions of pH and [IR].

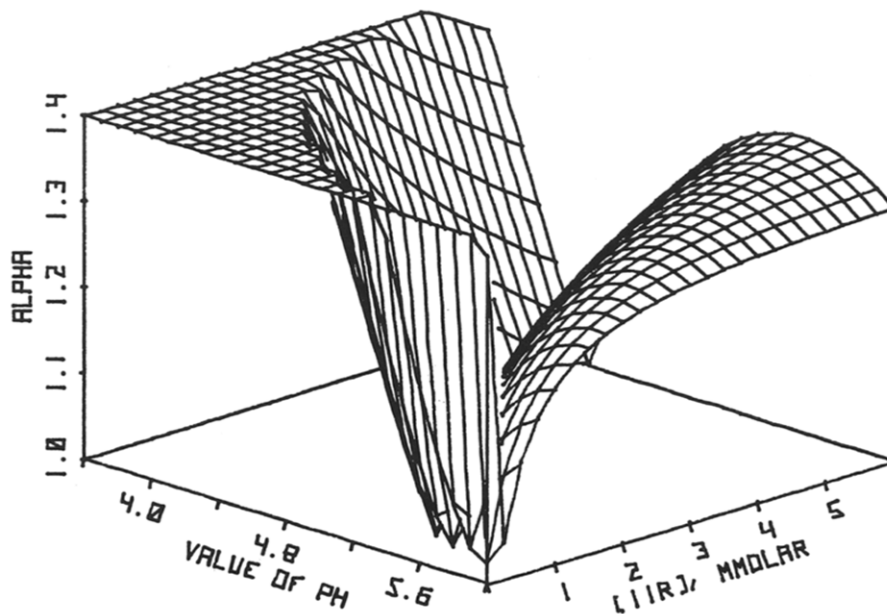


Fig. 3. Predicted relative retention (α) values for *trans*-ferulic and phenylacetic acids as a function of pH and [IR]. Values of α greater than 1.4 set equal to 1.4.

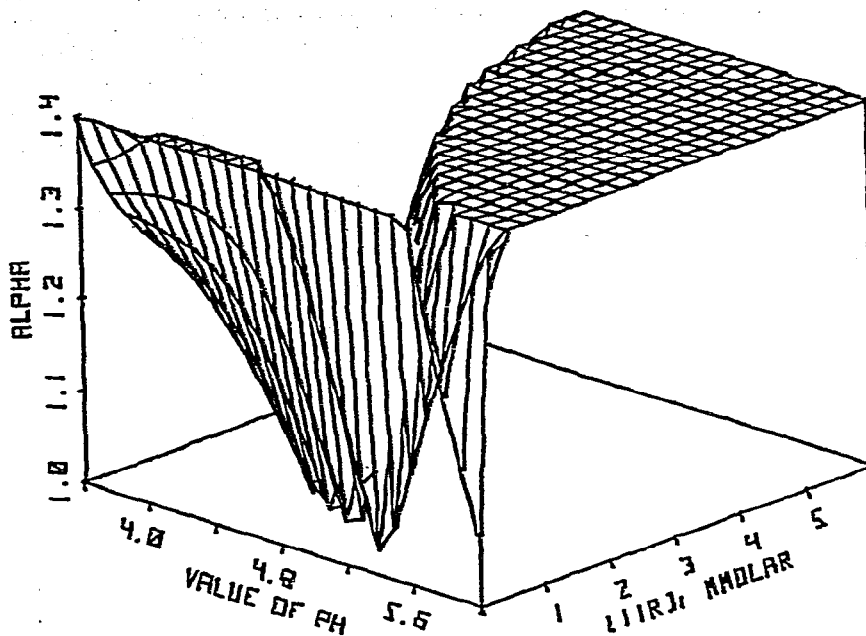


Fig. 4. Predicted relative retention (α) values for *trans-p*-coumaric and phenylacetic acids as a function of pH and [IR]. Values of α greater than 1.4 set equal to 1.4.

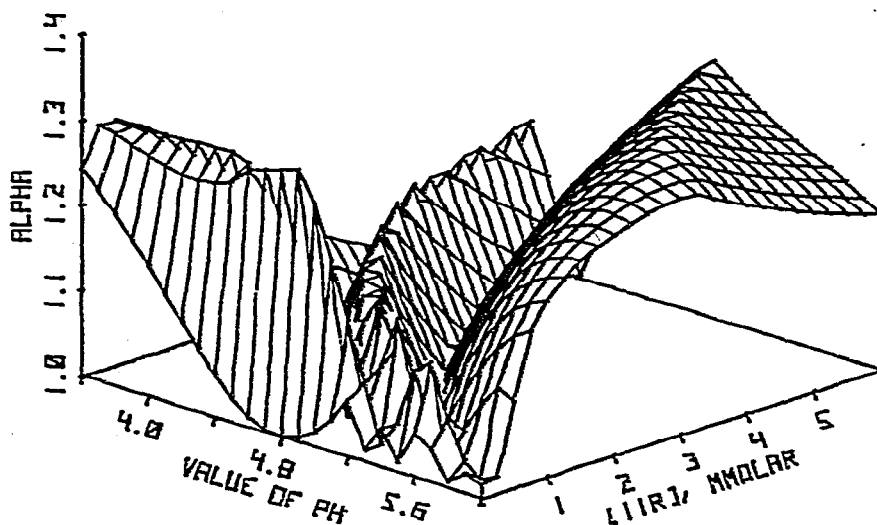


Fig. 5. Two-dimensional window diagram for nine-component mixture. See text for discussion.

shown in Fig. 6: a mixture of the nine compounds was injected, but only six peaks are observed. Note that the retention times are all small, as expected, because of the high pH (the compounds are all charged and relatively eluophilic) and because of the absence of ion-interaction reagent.

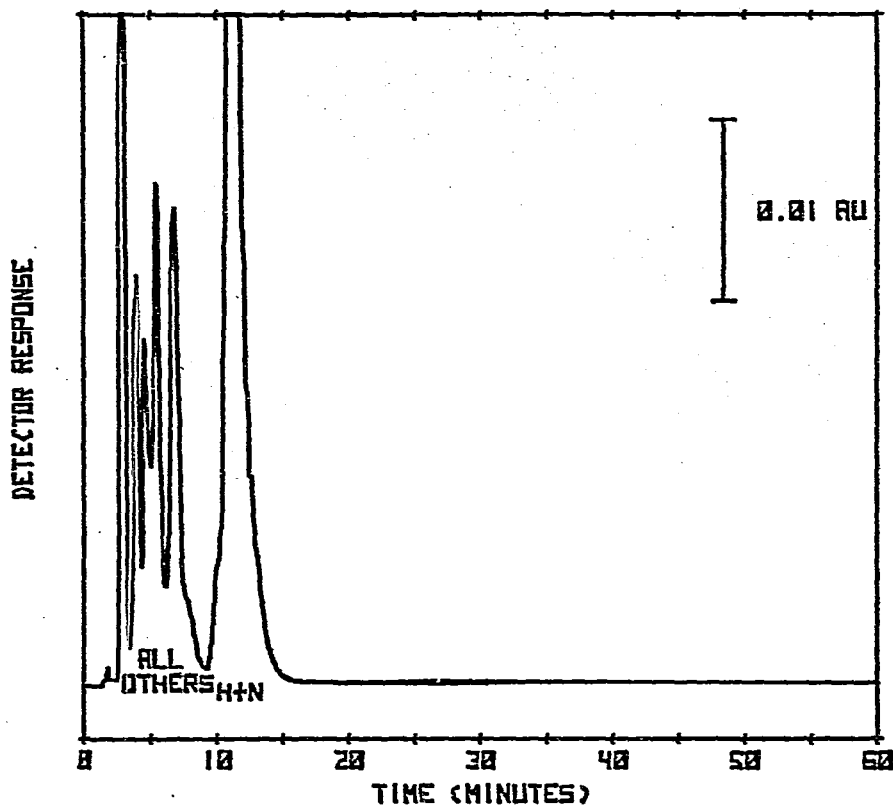


Fig. 6. Chromatogram of nine-component mixture at pH 5.6 and $[IIR] = 0 \text{ mM}$. Eluent: methanol-water (20:30), 0.010 M acetate buffer. Ultraviolet detector, 254 nm. H = Hydrocinnamic acid, N = *trans*-cinnamic acid.

Fig. 5 predicts good overall separation in a broad region near pH 5.8 and $[IIR] = 3.2 \text{ mM}$ which is confirmed in the chromatogram of Fig. 7. The nine compounds are all well separated. *trans*-Caffeic acid (C), however, exhibits extensive tailing and overlaps with *trans*-*p*-coumaric (M), *trans*-ferulic (F) and phenylacetic (A) acids. The retention times are generally larger because of the relatively high concentration of ion-interaction reagent present in the eluent.

Good overall separation is also predicted in a narrow region near pH 3.7 and $[IIR] = 0.75 \text{ mM}$ (see Fig. 5). Fig. 8 shows the resulting chromatogram. Some of the retention times are large, not because of the ion-interaction reagent concentration (which is relatively low), but rather because of the low pH which causes the weak acids to exist primarily in their protonated (uncharged) form. *trans*-Caffeic acid still exhibits extensive tailing, but only phenylacetic (A) and *trans*-*p*-coumaric (M) acids are affected.

Given this extended view of the separation process, selected areas of experimental conditions can be further evaluated with respect to other responses such as analysis time and ruggedness. For example, shorter analysis time with adequate separation could probably be achieved at pH 6.0 and $[IIR] = 2 \text{ mM}$ (see Fig. 5).

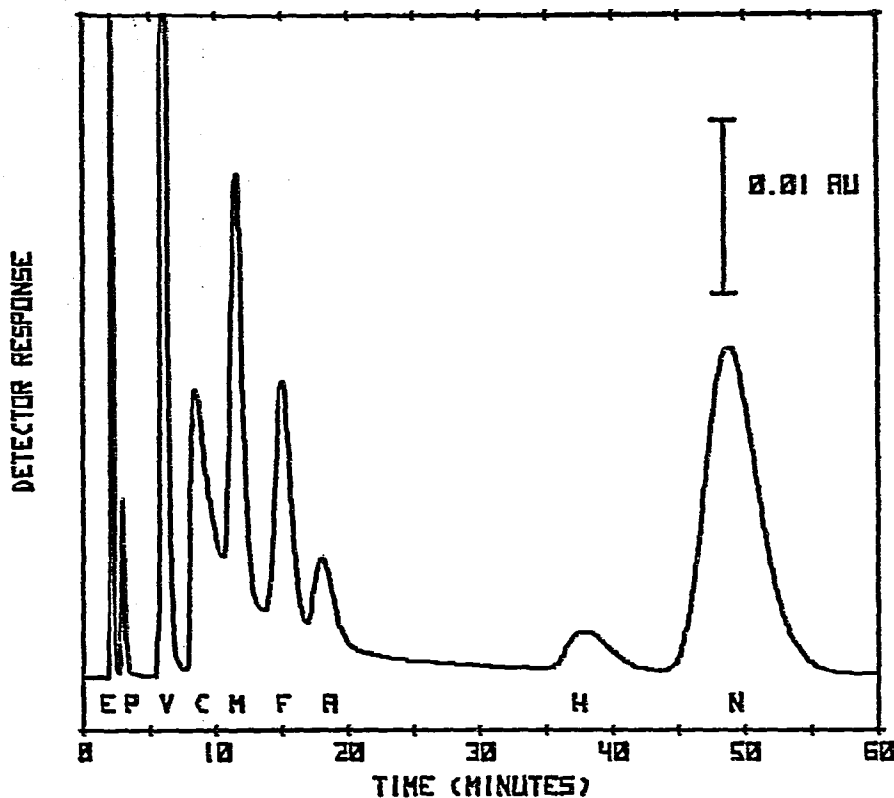


Fig. 7. Chromatogram of nine-component mixture at pH 5.8 and $[IIR] = 3.2$ mM. Other conditions as in Fig. 6. E = Phenylethylamine, P = phenylalanine, V = vanillic acid, C = *trans*-caffeic acid, M = *trans*-*p*-coumaric acid, F = *trans*-ferulic acid, A = phenylacetic acid; other abbreviations as in Fig. 6.

Similarly, with regard to ruggedness, it might be impractical to prepare an eluent with the tight tolerances required to correspond to conditions along the ridge that runs through the middle of Fig. 5; larger factor tolerances²⁰ could be obtained in the region of the plateau at pH 5.6 and $[IIR] = 5.0$ mM.

The retention order of *trans*-*p*-coumaric, *trans*-ferulic, and phenylacetic acids in Fig. 7 (M-F-A) and in Fig. 8 (A-M-F) is the order predicted by Figs. 1 and 2. The observed retention order of all nine compounds is consistent with the retention order observed in a previous study¹⁹.

CONCLUSION

Multifactor window diagrams are a useful technique for finding eluent compositions that give optimal separation in reversed-phase liquid chromatography. Although the present study has involved only two factors, the concepts are entirely general and can be extended to higher dimensional factor space. Visual presentation of 3- or more-factor window diagrams is difficult, however, and computer selection of feasible regions will probably be desirable.

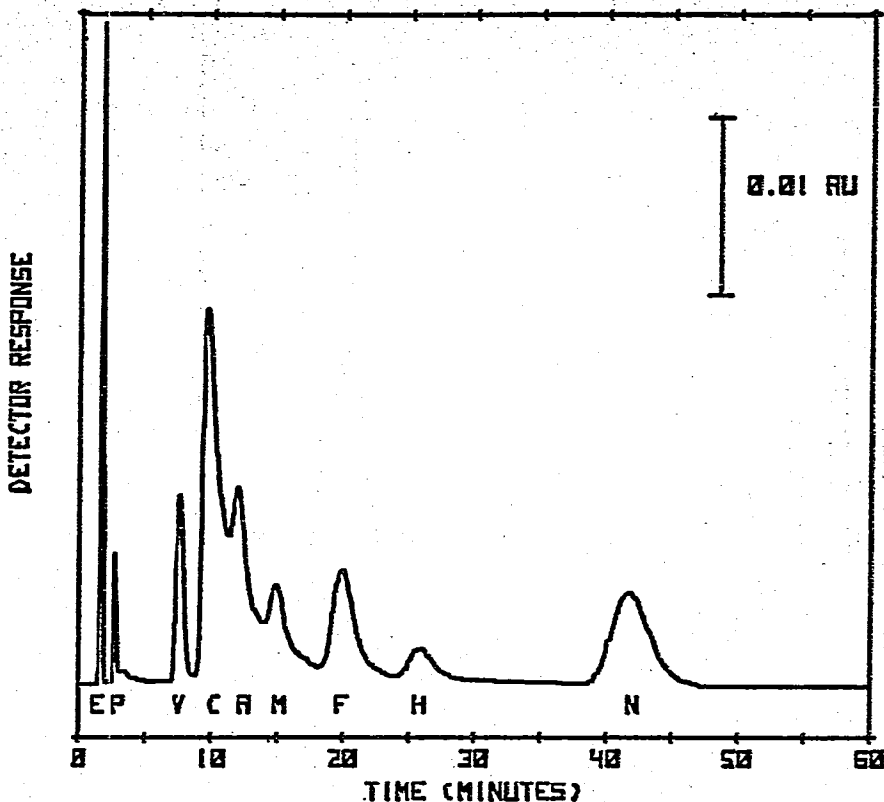


Fig. 8. Chromatogram of nine-component mixture at pH 3.7 and $[IIR] = 0.75 \text{ mM}$. Other conditions as in Fig. 6. Abbreviations as in Figs. 6 and 7.

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