Waters
Lab Highlights
An Internal Communication
of Applications and Techniques
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Gradient Optimization
Part 1 - Evaluation of a Commercial Software Package for Single Segment Gradients

Gradient elution is an important technique in liquid chromatography for the separation of components with a wide range of relative retention. In gradient elution, the solvent strength of the mobile phase is increased during the course of the separation. In general, gradient methods for complex samples result in shorter analysis time, sharper peaks, and increased resolution and detection sensitivity over comparable isocratic methods. Development of a gradient separation for an unknown sample can be tedious and time consuming since, in addition to the initial and final eluent composition, the rate of change of eluent composition must be optimized.

Due to the relative complexity of gradient methods development, many chromatographers have turned to optimization software for assistance. This Lab Highlight describes results from our evaluation of DryLab™ G (LC Resources, Inc.) the best known commercial software package, which runs on an IBM PC or compatible. Information presented here may be of interest to Waters customers who are considering the purchase of DryLab G software for installation on their Maxima system.

Drylab G requires as input the retention times from two experimental runs differing only in the gradient time. In the following example of a PTH amino acid separation, the retention times from a 10 minute and a 30 minute gradient are used as input. Also required are the void volume, dwell-volume (the volume of the mixer and tubing ahead of the column), flow rate, and the initial and final percentages of organic modifier. After some internal calculations, the program provides a list of options. One of the options, a relative resolution map, is a plot of the resolution of the worst resolved peak pair versus the gradient time as shown in Figure 1. From this relative resolution map, the chromatographer can pick the

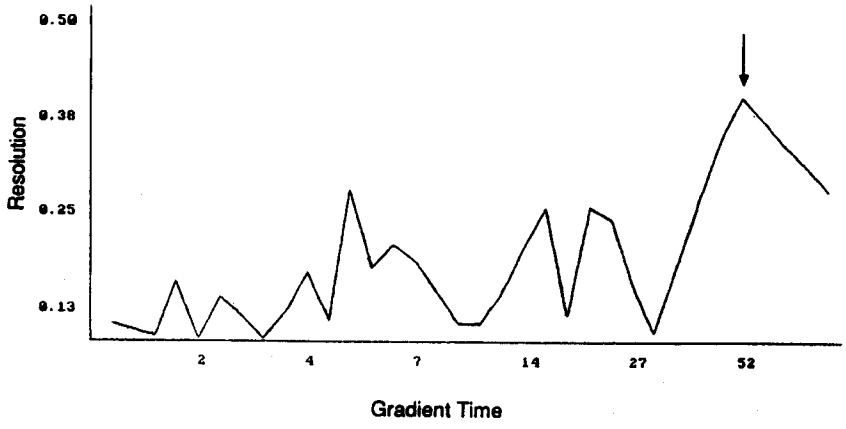

Figure 1. Relative Resolution Map for 17 PTH-amino acids.

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gradient time that should provide the best overall separation. In this example, the optimum resolution under a given set of experimental conditions(column, eluent composition, temperature) corresponds to an extrapolated gradient time of about 50 minutes. With another option, a simulated chromatogram for a selected gradient time is displayed (Figure 2a).

In this PTH amino acid example, DryLab G worked well for predicting single linear gradients from two initial experiments. In Figure 2, the retention times for the PTH amino acids in the simulated chromatogram agree to within 4% with the experimental values. However, in order to achieve this level of agreement, care must be used in selecting the initial input runs. DryLab G is capable of accepting only two input runs at a time and the program assumes a linear dependence in its calculations. In reality there is a non-linear dependence and this can lead to significant errors if the two input runs are either too close (errors due to extrapolation) or too distant in gradient time. In DryLab G, the prediction of an optimized separation cannot be refined by including additional experiments as is the case with WISE™ software for isocratic eluent optimization.

Future lab highlights will evaluate DryLab G in predicting segmented gradients, the difficulties encountered, and present alternate approaches to gradient optimization based on WISE software.

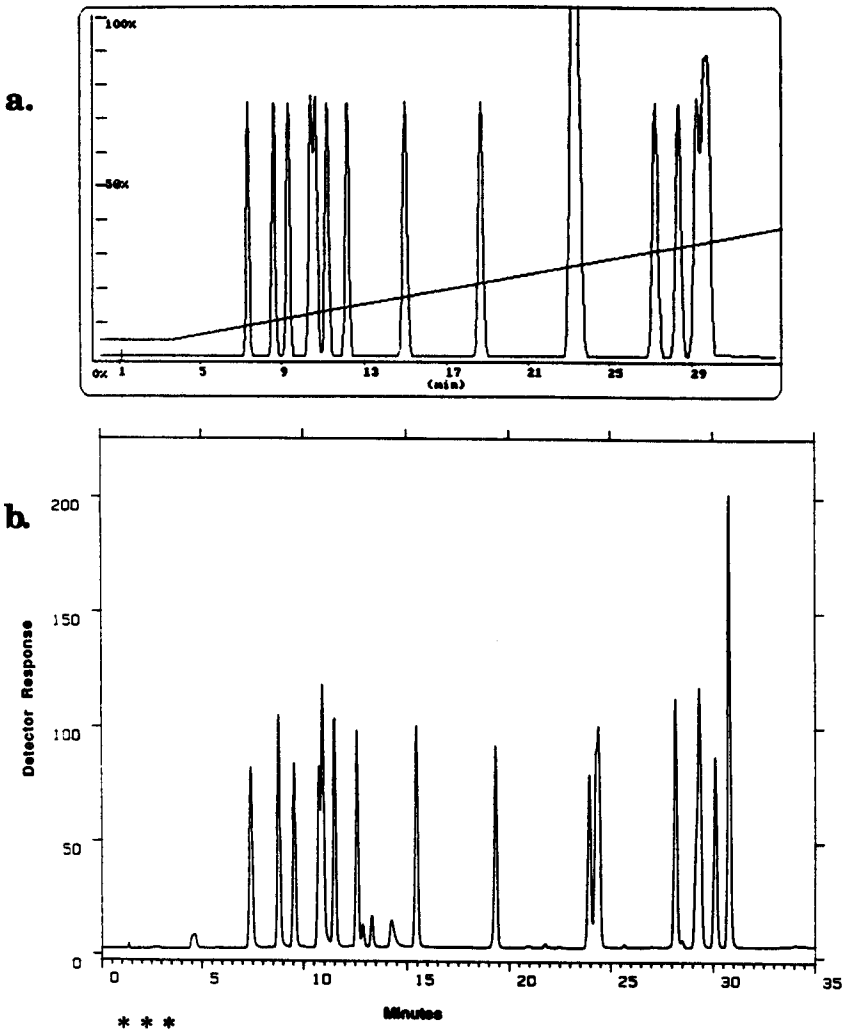

Figure 2.
a. simulated chromatogram
b. actual chromatogram
Experimental conditions:
Column: NovaPak® C_{18}, 15cm.
Eluent A: 40mM ammonium acetate,pH 4.0
Eluent B: acetonitrile
Temperature: 56°C
Flow: 1.0ml/min
50min gradient (5 to 60%B)

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