Figure 5: CE Separation of Chicken Cytochrome c Using 100 mN Phosphoric Acid

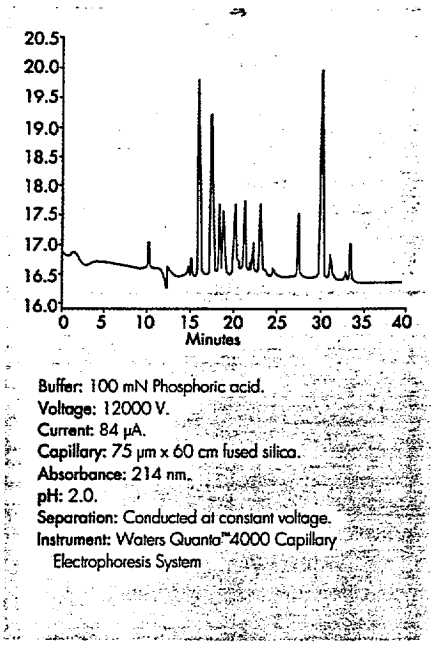
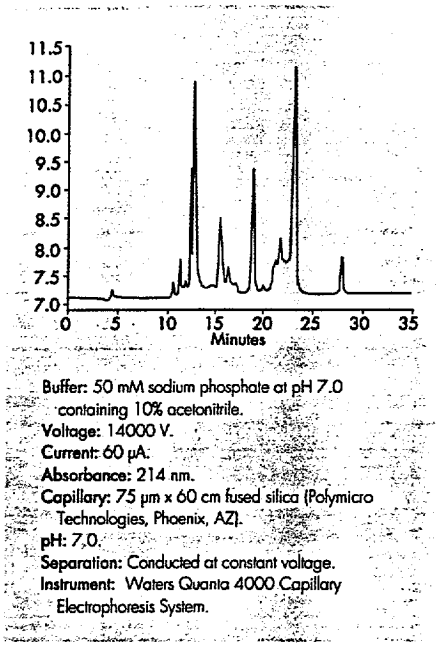


Figure 6: CE Separation of Chicken Cytochrome c using 50 mM sodium phosphate at pH 7.0



Conclusion

Capillary electrophoresis provides the pharmaceutical chemist with a powerful analytical tool. The very high separation efficiencies make it an ideal technique for solving difficult problems like chiral separations and for the determination of impurity profiles. The selectivities of CE complement those most commonly found in HPLC and as such provide the analyst with a powerful combination of techniques for the analysis of complex mixtures of molecules and the determination of absolute sample purity.

For more information on Waters new Capillary Electrophoresis system be sure and mark number 6 on the Business Reply Card.

Improved Chromatographic Selectivity Using WISE™ Software

by Vince Warren, Senior Applications Chemist

Introduction

The Waters Interactive Selection of Eluents (WISE™) software package was first introduced for the 840 Data and Chromatography Control Station at the 1988 Pittsburgh Conference in New Orleans. With the release of Expert Ease version 2.2, this software option will be available to all Waters 845 and 860 users as well. In fact, version 2.2 of Expert Ease* will include a demonstration version of WISE software, providing all users with an easy way to gain familiarity with the capabilities of this software product option.

WISE software is an interactive approach to the optimization of eluents for isocratic LC methods, with a focus on improving chromatographic selectivity to meet the chromatographer's requirements.

*Compatible with DEC/VMS® operating system.

The WISE software strategy can be applied to a wide variety of eluent blending problems, providing an extremely versatile tool which can assist with the optimization of pH, temperature, additive (e.g., buffer salt or PIC® Reagent) concentration or modifier composition for aqueous/organic eluents. Normal phase as well as reverse phase eluents can be optimized using WISE software.

In addition to the diversity of eluent variables which can be addressed, WISE software offers several advantages over other comparable approaches. To keep the optimization process efficient, the WISE strategy uses a minimal number of starting experiments (typically 2 or 3) and then moves forward in a self-correcting manner to locate the optimal blend of the starting eluents. As each new experiment is added, the new retention information is used to update the retention model from which all WISE software predictions are calculated. The total number of eluent blends which must be tested in order to achieve an accurate prediction of the optimum varies with the complexity of the separation problem, but usually it is six or fewer.

As the optimization progresses, WISE software keeps the user informed not only of the latest predictions, but of the reasoning behind these. For clarity, easily understood graphical displays are used to summarize the available information. The Eluent Selection Diagram (ESD) (refer to Figure 8) presents the current retention model along with plots of the user-selected quality criteria. In addition, simulated chromatograms are available for the predicted optimum and for the suggested next experiment. When appropriate, WISE software also advises whether the optimization process should be continued or ended. The ESD, which is central to the WISE

software strategy, does more than keep the user informed about progress during the optimization process. The ESD provides a useful addition to the documentation of the methods development process, indicating the eluent blends which were investigated and predicting the chromatographic results expected for untested blends. Because it summarizes information for the entire tested range of the eluent variable, the ESD can also be used to assess the ruggedness of the predicted optimum. That is, the vulnerability of chromatographic resolution to variation in the eluent composition can be readily determined from inspection of the ESD. This is discussed as part of the beta blockers optimization example below.

How WISE Software Works

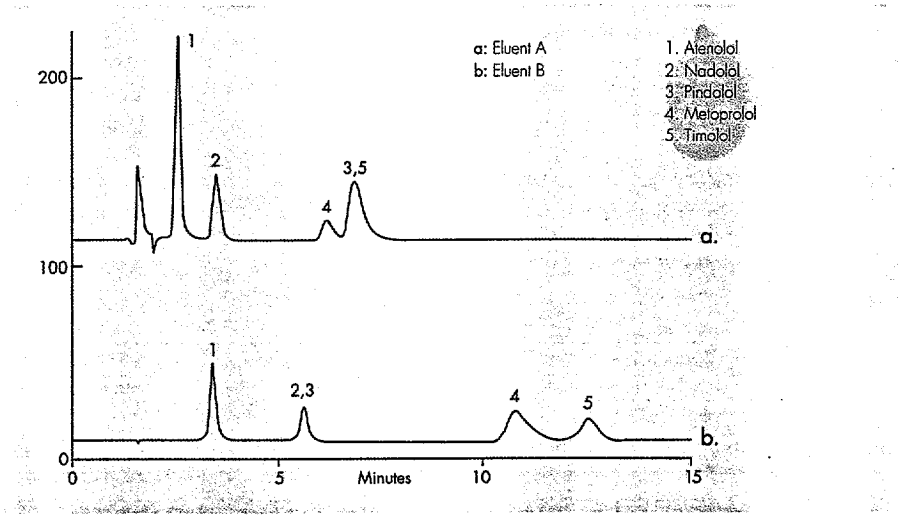
The WISE software product includes two modules to address different optimization problems for isocratic LC eluents. The Single Variable Optimization module facilitates the optimization of eluent variables such as pH, temperature, salt concentration, percentage of organic modifier (e.g., methanol) or PIC reagent concentration. The Ternary Eluent Optimization module is used when blends of an aqueous component plus two organic modifiers (typically methanol, acetonitrile or tetrahydrofuran) are to be optimized.

To illustrate the way in which WISE software works to optimize an isocratic eluent, a simple example of ternary eluent optimization will be discussed. In this example, the goal is the isocratic separation of five beta blockers, using aqueous/organic eluents and a Nova-Pak C_{18} cartridge.

Table 1: Experimental Conditions for HPLC Isocratic Separation of Five Beta-blockers

Column: Nova-Pak C_{18} Cartridge (100 x 5 mm)
Flow rate: 0.8 mL/min
Detection: UV at 254 nm
Sample: Five beta-blockers: Atenolol, Nadolol, Pindolol, Metoprolol, and Timolol
Eluent A: 780 mL H_2O, 220 mL THF, 1 Vial PIC Reagent B8 (low UV)
Eluent B: 500 mL H_2O, 500 mL MeOH, 1 Vial PIC Reagent B8 (low UV)

Figure 7: Initial Isocratic Separation of Five Beta-blockers with Binary Eluent Blends



Information obtained from the initial separations in a & b above are entered into the WISE software as part of the process in obtaining an Eluent Selection Diagram.

For ternary (three-solvent) eluent optimization, the starting point is always a set of chromatograms with binary (two-solvent) eluents. In this case, the starting binary eluents contain methanol or THF blended with water. To adequately retain these solutes, PIC B8 reagent was included in all eluents at a constant concentration. The chromatograms for the starting eluents (Figure 7) fail to provide the desired resolution of all five solutes, so it is appropriate to consider blending the starting binary eluents to form a ternary eluent (i.e., methanol/THF/aqueous). This is the point at which WISE software can provide assistance.

For each of the starting chromatograms, retention times for the five solutes are entered into WISE software. The user also enters the column void time. An Eluent Selection Diagram (Figure 8) is then produced. In this ESD, the top portion contains the retention model which permits calculation of chromatographic quality values for all possible blends of the starting binary eluents. As a preliminary retention model, ln (k') is assumed to vary linearly between the measured retention values as a function of blending proportion.

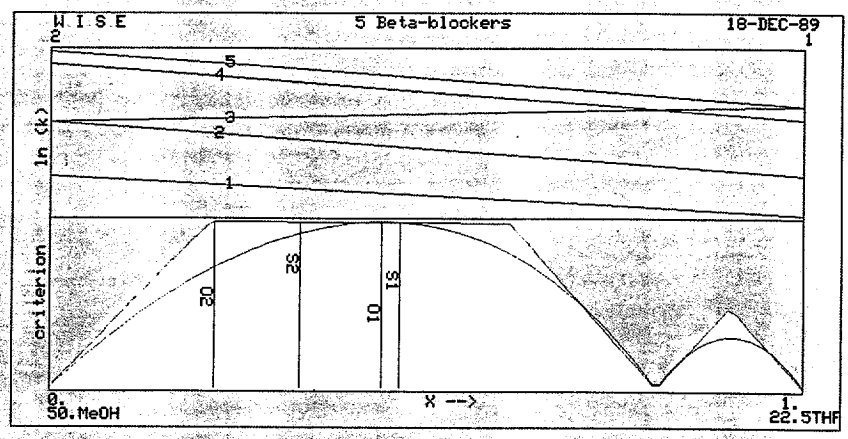
(continued on page 8)

The lower portion of Figure 8 contains a plot of the calculated values for the two quality criteria included in WISE software. The two criteria monitor different but complementary aspects of chromatographic quality. Criterion 1 aims for even peak spacing while criterion 2 monitors the resolution of the worst-resolved peak pair. For the beta-blockers example, both criteria reach their highest values near the center of the ESD, corresponding to a ternary eluent which is formed by blending nearly equal volumes of the starting binary eluents.

The full optimization report in Figure 8 shows the specific advice for each quality criterion. Both quality criteria indicate a broad optimal region near the middle of the ESD. Criterion 2 (critical resolution), in fact, varies so little in the center of the diagram that the plot in Figure 8 is a plateau in this region. This indicates an extremely rugged optimized separation, with minimal impact on critical resolution arising from variability of the blended eluent. The plot for criterion 1, while not a plateau, indicates that even peak spacing in the optimal chromatogram will be similarly rugged.

The predicted optimal eluent blends for the two criteria appear to differ substantially: A 44%/56% blend for criterion 1 and a 22%/78% blend for criterion 2. However, the broad shape of the criterion plots suggests that good chromatograms can be obtained with either eluent. To assist the chromatographer in deciding what experiment to do next, WISE software provides the capability to view predicted chromatograms corresponding to the optimum and suggested experiment for each criterion. Figure 9 presents these predicted chromatograms for the beta-blockers example. Note that these chromatograms are simulations in which all peaks have a Gaussian profile and equal peak areas. The width of each peak is established by the user-provided plate count estimate.

Figure 8: An Eluent Selection Diagram (ESD) and Optimization Report



WISE TERNARY ELUENT OPTIMIZATION REPORT FOR FILE: BETABLOCK1

Report for Criterion 1 (Even Peak Spacing)

	%A	%B	%C	THF	MeOH	****	PICb	X-coord
OPTIMUM	44.00	56.00	0.00	9.90	28.00	0.00	62.10	0.440
Sug. Exp.	46.40	53.60	0.00	10.44	26.80	0.00	62.76	0.464

Advice: Perform suggested experiment

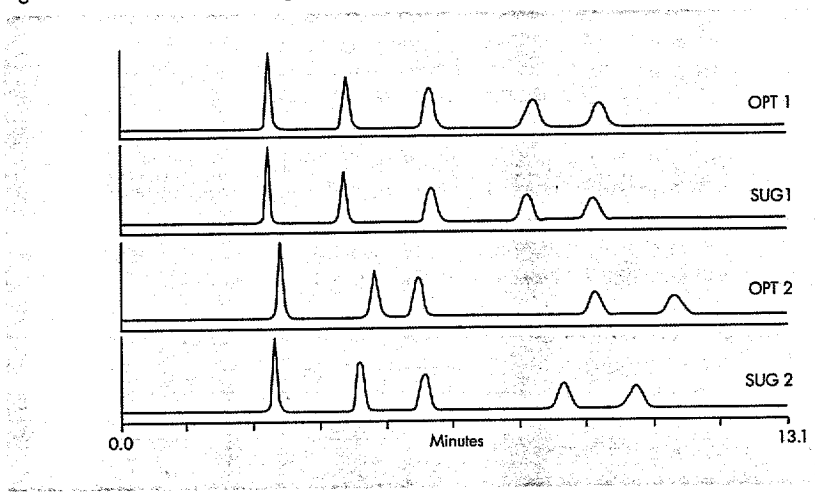
Report for Criterion 2 (Critical Resolution)

	%A	%B	%C	THF	MeOH	****	PICb	X-coord
OPTIMUM	22.00	78.00	0.00	4.95	39.00	0.00	56.05	0.220
Sug. Exp.	33.20	66.80	0.00	7.47	33.40	0.00	59.13	0.332

Advice: Perform suggested experiment

This Eluent Selection Diagram is central to the WISE software strategy. Retention times from the chromatograms collected so far are used to form a preliminary retention model in the top half of the diagram. From this model, chromatographic quality can be predicted for intermediate eluent compositions, and is plotted in the lower half of the diagram.

Figure 9: Predicted Chromatograms for the Beta-blockers as Drawn by WISE Software



The retention times corresponding to the optimal and suggested eluent blends in Figure 8 are used by WISE software to generate predicted chromatograms for each criterion.

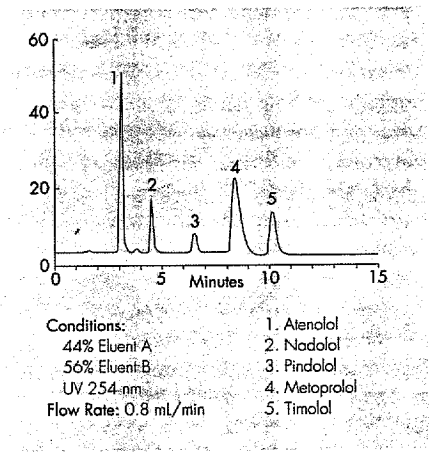
As expected, the plot of predicted chromatograms shows that the five peaks are more evenly spaced in the optimal chromatogram for criterion 1, while the worst resolved peak pair is better resolved in the optimum for criterion 2. The choice is left to the chromatographer, but both criteria provide the advice "Perform suggested experiment" in the full optimization report (Figure 8). In this example, the advice of criterion 1 was followed. Because the predicted optimum (44%A/56%B) and the suggested experiment (47%A/53%B) differ so little, the optimum was tested immediately and the chromatogram illustrated in Figure 10 was obtained.

The separation in Figure 10 is almost identical to the predicted separation of Figure 9 except for differences in peak shape. This degree of agreement between predicted and experimental chromatograms is not always obtained so quickly. Recall that the WISE software predictions were based only on two experiments, one for each starting binary eluent. The preliminary retention model assumed linear ln (k') behavior for each solute between the measured retention values. In practice, the trend in ln (k') versus solvent composition, usually follows a gentle curve rather than a linear relationship. For more accurate retention model, it is usually necessary to include the retention data of one or two additional ternary blends to the data set. The degree of success achieved here in three experiments will then require 4 or 5 experiments, still a modest investment compared to conventional trial-and-error approaches to isocratic eluent optimization.

Conclusion

Application of WISE software to the beta-blockers example resulted in the rapid optimization of a ternary (i.e., methanol/THF/aqueous) eluent blend. Since neither binary eluent was able to resolve all five solutes, this is a significant accomplishment in itself. The optimization used the results of each experiment thoroughly and efficiently to make the most rapid progress possible toward the optimum. In addition to locating the optimum, WISE software provided full documentation of the retention model and predicted separation quality for all possible blends of the starting binary eluents. Inspection of the Eluent Selection Diagram allowed an assessment of ruggedness for the optimal eluent blend, which was excellent for the beta-blockers example.

Figure 10: Chromatographic Verification of the Optimum HPLC Conditions Predicted by WISE Software



The separation above was achieved by following WISE software suggestions to obtain optimum even peak spacing (criterion 1).