Liquid chromatography is chosen as the analysis technique for complex samples where separation is required to provide information about qualitative and quantitative composition. The suitability of a method is defined by the certainty of peak identification and by demonstration that only a single entity contributes to the quantitative detector response. Development of a chromatographic method that meets these criteria exploits many physical and chemical parameters. In the experimental examination of the separation space, the sample components usually change their retention, and often give different detector response. Systematic method development becomes more complicated because it is not easy to map each sample component across different separation conditions. Component selectivity recognition, Information-rich detectors, such as, photodiode array detection and mass spectrometry, can aid peak tracking and assessment of peak homogeneity. Algorithms for matching spectra and for and judging peak purity have been used independently for LC-PDA and for LC-MS in earlier experiments. Here, the combination of the two detectors is considered as a tool for methods development. The integrated information helps to ensure that all components will be recognized and tracked. Further, the integrated information makes it easier to detect and quantitative chromatographic tools for mobile phase modification. Selectivity changes have been hard to define because it is time-consuming to prepare the solvents and pH/ion strength modifiers. To exploit the variables controlling selectivity, the data analysis must consider changes in both theoretical plate as a function of mobile phase, including both organic solvent and pH. The interpretation of mass spectra must include background, isolopes, multiple charging, and fragmentation. Instrumental functions are generally explained with solvent management algorithms to permit continuous adjustment of pH while using acetate and organic solvents. The combination of mobile phase manipulation with information-rich component identification makes chromatographic method development more complete and efficient.

**RESULTS**

- **Figure 3. Programming Separations with Auto•Blend Plus™.** The chromatographic system is based on a four-solvent, low pressure mixing gradient system. Each solvent line contains either a pure solvent or a combination of solvents. The calculation of delivered volumes is based on the relative proportions. The three spectra in both detectors are identical.
- **Figure 4. Improving Control with Multi-component Buffers.** The delivered pH was measured as a function of pH. In the original experiments, the simple two-component combination of formic acid and ammonium hydroxide created a useful pH control, but it showed a gap where the pH rose steeply with small changes in composition. By adding acetic acid to the A and triethanolamine to the B, a four-component buffer system gave a smoothed pH-control curve, reducing the gap from 4.25 to pH 5.0.
- **Figure 5. Peak Recognition with MS Detection.** Separation selectivity can be easily altered by changes in organic solvent or pH. When the two separations are compared with UV, it is not always obvious how many components are present in each peak or which analyte is associated with a particular peak. In the simple case where the expected peaks are known, the calculated mass can be used with MS data to recognize coeluting peaks, to identify the specific analytes, and to track peaks through separation experiments.

**CONCLUSIONS**

- **Development of a separation-based analytical method must consider efficient manipulation of the chromatographic, identification of the analytes, detection of expected components, and recognition of unexpected components.**
- **Auto•Blend Plus™ facilitates efficient use of pH for manipulation of separation activity.**
- **Combining MS and UV spectral detection provides greater certainty for peak identification and for detection of peak heterogeneity.**
- **New software tools simplify combination of UV and MS spectral data.**