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INTRODUCTION

The reversed-phase LC peptide maps used forcharacterizing biopharmaceutical proteins must resolve all the peaks representing the entire sequence of the protein. This separation must be established in such a way that all modifications of the protein can be recognized and measured. Analysis of such samples requires the best possible chromatographic resolution. To achieve these separations, maps are often developed with very long, shallow gradients on the order of one to three hours. Even with these methods, additional resolution is often required. The application of 1.7μm packing materials has been shown to improve resolution in peptide mapping. Investigation of the mechanisms underlying this improved resolution suggests that improved resolution can be obtained in shorter run times by reducing dispersion. The application of 1.7μm packing materials has been shown to improve resolution in peptide mapping by reducing dispersion. The selectivity patterns are, however, indistinguishable. This indicates that the optimum linear velocity, or flow rate, is lower for molecules of similar size in solution. ACQUITY UPLC™ BEH packing materials are becoming a standard for peptide mapping, but there are some obvious differences in selectivity that are not well understood.

MATERIALS AND METHODS

Separations were performed using an ACQUITY UltraPerformance LC™ and Waters Acquity UPLC BEH (1.7μm) columns. Mobile phase A was 0.1% trifluoroacetic acid in water. The MSSystem was Waters ZQ™ Mass Spectrometer; Electrospray Ionization (+). LC System: Waters ACQUITY UPLC™ Solvent Delivery System Operating Pressures from 5000–13000 psi).

RESULTS

Figure 1: Comparison of HPLC and UPLC—Constant Gradient Time

The comparison of HPLC and UPLC peptide maps used for characterizing biopharmaceutical proteins must resolve all the peaks representing the entire sequence of the protein. This separation must be established in such a way that all modifications of the protein can be recognized and measured. Analysis of such samples requires the best possible chromatographic resolution. To achieve these separations, maps are often developed with very long, shallow gradients on the order of one to three hours. Even with these methods, additional resolution is often required. The application of 1.7μm packing materials has been shown to improve resolution in peptide mapping. Investigation of the mechanisms underlying this improved resolution suggests that improved resolution can be obtained in shorter run times by reducing dispersion. The application of 1.7μm packing materials has been shown to improve resolution in peptide mapping by reducing dispersion. The selectivity patterns are, however, indistinguishable. This indicates that the optimum linear velocity, or flow rate, is lower for molecules of similar size in solution. ACQUITY UPLC™ BEH packing materials are becoming a standard for peptide mapping, but there are some obvious differences in selectivity that are not well understood.

CONCLUSIONS

• UPLC produces higher resolution peptide maps than HPLC
• UPLC peptide maps can be developed by manipulating the same parameters used in standard HPLC peptide maps
• Short UPLC columns may be useful for rapidly screening the separation conditions in the best selectivity
• UPLC peptide maps can be selected to maximize resolution for a given selectivity
• UPLC with UV detection has sufficient sensitivity and linear range for quantitative peptide mapping

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