INTRODUCTION
Peptides are used in basic research as well as in biopharmaceutical development. It is common to use synthetic peptides rather than isolating natural products. Such synthetic products may not be sufficiently homogeneous for use in experiments requiring bioreactivity and specificity. Isolation and purification, therefore, become essential steps in a peptide synthesis facility. We have examined the factors that influence purification protocols. This includes selection of a column, operating parameters for the method, and the type of solvent used for the intended use of the product. A panel of synthetic peptides covering a range of properties including size, hydrophobicity, and isoelectric point is used for these evaluations. These peptides were chosen to measure the importance of pore size and particle size. Under these controlled conditions, there was little difference between 130Å and 300Å up to 40 residues. More resolution was observed with 5μm particles than with 10μm. The uses of the purified peptides may constrain the mobile phase constituents. The suitability of biocompatible mobile phases, including acetic acid in place of TFA and ethanol instead of acetonitrile affected the selectivity of the separation. In some comparisons, selectivity effects could be related to the type of solvent used for elution, thus the need for careful interpretation. An additional set of experiments has been performed investigating conditions for classes of peptides that have extreme or special properties. We show the impact of elevated temperature as well as the use of high pH or alternative solvents for peptides that are very difficult to dissolve in common chromatographic solvents. These experimental observations are combined into a suggested protocol for developing an isolation method.

METHODS

RESULTS

DISCUSSION

Peptide Separation Technology columns can be used for synthetic peptides with a wide range of properties. Their ability to separate peptides with a variety of properties and the differences are consistent with estimated selectivity without deterioration of peak shape.

CONCLUSION

Peptide Separation Technology columns can be used for synthetic peptides with a wide range of properties. Their ability to separate peptides with a variety of properties and the differences are consistent with estimated selectivity without deterioration of peak shape.

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