Reverse Phase Column Choice Affects Peptide Purity

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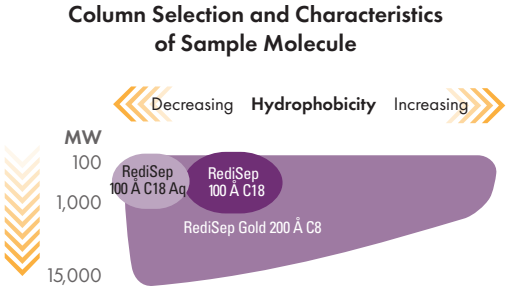
Abstract

Reverse phase (RP) column choice can have a significant impact on peptide purity. For example, Teledyne ISCO's 200 Å C8 columns, primarily intended for large or hydrophobic peptides, can also be used for smaller peptides. The selectivity of the C8 column is sufficiently different, in some cases, such that the peptide can be better resolved from impurities. This allows higher throughput from increased sample loading in addition to the improved purity. The Teledyne ISCO C18Aq column is also an excellent choice for peptide purification and is used to improve resolution for hydrophilic compounds.

Overview

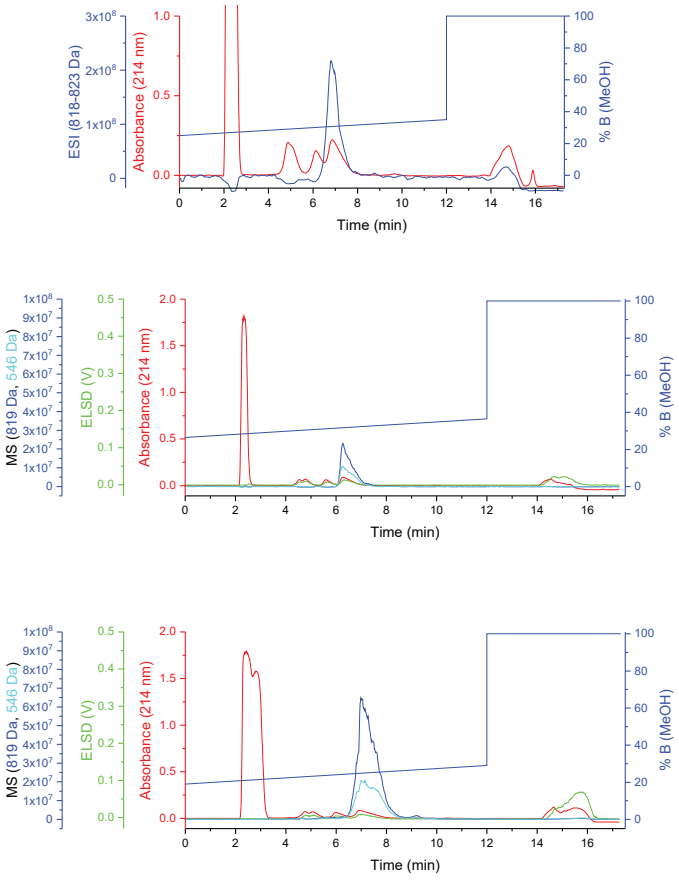


Reverse phase choice as a function of peptide size and hydrophobicity

The above illustration is a practical guide for selecting columns for peptide purification. However it is only a general guide. A quick test run will provide an opportunity to improve the purification beyond these suggested guidelines.

Experiments and results

The peptide used for these experiments is EGFRviii with a molecular weight of 1635 Da. A PurIon L mass spectrometer (PN 68-5237-084) was used for detection using the $[M+2H]^{2+}$ or $[M+3H]^{3+}$ ion. The solvent system for all runs was water (50 mMol ammonium acetate, pH 3.5): methanol. Columns used were 20x150 mm Resi*Sep*® Prep C18 (PN 69-2203-810), C18Aq (PN 69-2203-818), or C8 (PN 69-2203-858). UV detection was 214 nm, and some runs used the evaporative light scattering detection (ELSD) option.



Comparison of C18, C18Aq, and C8 purification of EGFRvii

All three columns purified the peptide very well. However, the mass spectrometer trace shows a slight overlap with an impurity for the C18 run. The C18Aq column used the same gradient method, but shows improved resolution from impurities. Improved resolution in mostly aqueous solvents systems is typical of C18Aq columns because the resistance to phase collapse improves resolution with compounds to be purified. The C8 column showed reduced retention, as shown by the weaker solvent system; the focused gradient uses a lower concentration of methanol. This is expected for a small peptide due to the larger pores in the C8 column that reduce surface area and interaction with the peptide. The shorter carbon chains also contributed to the reduced peptide retention. However, the C8 column performed as well as the C18Aq column with respect to resolution from impurities.

Conclusion

Resi*Sep* Prep columns in general provide excellent performance for peptide purification. In certain circumstances, however, one column type may provide higher purity than the others. In the case presented here, C18Aq and C8 columns provided improved purity over the C18 column.

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