Introduction

In the past decade, the generation of superficially porous silica particles with pore sizes in the range of 90 Å to 130 Å is the most significant advancement in LC column technology. These particles provide similar efficiency to smaller sized totally porous particles whilst generating significantly lower back pressure. Whilst chromatographers enjoy the high efficiency of these superficially porous particles, a significant challenge in analyzing complex protein digests is the separation of peptides with high efficiency and resolution. Formic acid is an MS-friendly ion-pairing agent that is often used in LC/MS methods, permitting sensitive detection. However, formic acid also produces broader peak shapes, which leads to the co-elution of peptides. In addition, poor peak capacity, peak broadening, and poor resolution of closely related peptides are limitations with the current formic acid containing mobile phase LC/MS methods. Hence the correct choice of reverse-phase column and LC/MS methods is critical in achieving a successful peptide mapping separation.

Agilent AdvanceBio Peptide Plus columns are designed for high efficiency peptide separation. The incorporation of the basic organosilane reagent provides positive charge surface on a superficially porous particle with C18 ligand. The optimum pore size (120 Å) and particle size (2.7 µm) delivers UHPLC like performance with low back column pressure. The AdvanceBio Peptide Plus columns are also compatible with mobile phases containing formic acid and provide superior peak shapes and high loading capacity.

Peptide mapping of a therapeutic monoclonal antibody (mAb) was evaluated using this novel charge hybrid C18 hybrid superficially porous column coupled to an Agilent 6545XT AdvanceBio LC/TOF system. The superior performance of AdvanceBio Peptide Plus column delivered high efficiency peptide map separation. This, in combination with high mass accuracy enabled reliable mAb peptide maps with high sequence coverage. This unique selectivity was found to be highly advantageous for peptide mapping because it greatly improves resolution of deamidated peptides versus their un-modified variants.

Experimental

The superficially porous silica particles were selected and used as the base particles. Under acidic conditions, organosilane(s) were hydrolyzed and co-condensed on silica surface and formed an organo/inorganic hybrid layer. Later, these treated particles were modified with C18 alkyl chains and evaluated in HPLC (Figure 1).

HPLC Performance Comparison for Synthetic Peptides

3 Synthetic Peptides in 0.1% FA

[Data Table]

Figure 2. HPLC Performance Comparison for Synthetic Peptides with Different Sample Loadings.

AdvanceBio Peptide Plus Column with charged surface demonstrated the best performance when using LC/MS-compatible formic acid solvent.

LC/MS Analysis of Peptide Mapping

Peptide mapping is an indispensable tool for the characterization of biopharmaceutical proteins. It is widely used to ensure product integrity and stability at various stages in the production of biopharmaceuticals. This study demonstrates the superior performance of AdvanceBio Peptide Plus column for monoclonal antibody (mAb) peptide mapping separation with a mobile phase containing formic acid. An LC/MS method was developed by investigating different particle effects including flow rate, gradient time, and temperature to obtain optimum peptide separation. Agilent AdvanceBio Peptide Mapping columns delivered reliable mAb peptide maps with high sequence coverage.

Selectivity Comparison for Deamidated vs Normal Peptides

Determination of deamidation is one of the most important goals of peptide mapping. On uncharged C18 columns, deamidated peptide variants often elute slightly later than the original form, but quite often they coelute or have poor resolution. AdvanceBio Peptide Plus column with charged surface has higher selectivity for deamidated forms of peptide over the normal form.

Conclusion

1. AdvanceBio Peptide Plus column is compatible with the mobile phase containing formic acid and provide superior peak shapes with no deterioration in performance for higher mass loads with mass spectrometry.
2. The results also demonstrated the superior performance of AdvanceBio Peptide Plus column for monoclonal antibody (mAb) peptide mapping separation with a mobile phase containing formic acid.
3. In addition, the novel charge-coating technology greatly improved resolution of deamidated peptides versus their un-modified variants.