INTRODUCTION

The ability to effectively separate peptides is critical to the success of many different workflows, ranging from the peptide mapping of biotherapeutics to the purification of synthetic therapeutic peptides. Reversed-phase (RP) chromatography using silica based C18 is the separation mode of choice for these applications, as it offers relatively high resolving power and can provide outstanding quantitative (UV) and qualitative (ESI-MS) information. Interestingly, modification of the surface charge of the base particles can have significant impact on the quality of peptide separations. Charged Surface Hybrid (CSH) C18, for example, is an evolution of the Bridged Ethyl Hybrid (BEH Technology) C18 stationary phase in that its surface is modified to contain a low-level positive charge in addition to the C18 bonded phase. This modification has already been shown to improve the peak shapes, loading behavior, and in turn peak capacities of small, ionized molecules.

Here, we demonstrate the benefits Charged Surface Hybrid (CSH) C18 delivers for both analytical and preparative peptide separations. The following work shows that this novel stationary phase can facilitate challenging analytical separations as it exhibits greater peak capacity, unique selectivity, and less dependence on MS signal suppressing, strong ion pairing agents when compared to current state-of-the-art peptide columns.

The data also demonstrate that CSH C18 is well suited to preparative separations involving acidic acid modified mobile phases that can be exploited to streamline the preparation of peptide salts, which are preferred for pharmaceutical formulation.

METHODS

Columns—Analytical Separations

ACQUITY UPLC® Peptide Column CSH C18, 130Å, 1.7 µm, 2.1 x 150 mm (Porous) (p/n 186002956)
ACQUITY UPLC® Peptide Column CSH C18, 130Å, 1.7 µm, 2.1 x 150 mm (p/n 186002955)
CSH C18, 5 µm, 2.1 x 250 mm, Porous, 50A (Competitor Product)
XSelect® Peptide CSH C18, 130Å, 5 µm, 4.6 x 150 mm (p/n 186007077)
XSelect® Peptide CSH C18, 130Å, 5 µm, 4.6 x 150 mm (p/n 186002957)
BEH130 C18, 1.7 µm, 4 x 6.6 mm (Porous) (p/n 183600938)
BEH130 C18, 1.7 µm, 2.1 x 150 mm (Porous) (p/n 183600356)
CSH C18 1.7 µm, 2.1 x 150 mm (Superficially Porous C18 1.7 µm, 2.1 x 150 mm) (p/n 186005576)

Columns—Preparative Separations

Porous Silica C18 5 µm
Superficially Porous C18 1.7 µm
BEH130 C18 1.7 µm
CSH C18, 1.7 µm, 4 x 6.6 mm (Porous) (p/n 186000593)
XSelect® Peptides CSH C18, 130Å, 5 µm, 4.6 x 150 mm (p/n 186007077)
Superficially Porous 1.7 µm C18

 LC Conditions

System: Waters ACQUITY UPLC® H-Class Bio System Detection: Waters ACQUITY UPLC® TUV Detector with 500 nL Analytical Flow Cell (Waters ACQUITY ZQ G2 QToF Mass Spectrometer) Wavelength: 214 nm/250 nm Column Temp: 40 °C Flow Rate: 0.3 mL/min (2.1 mm ID), 1 mL/min (4.6 mm ID) Acid modifiers: trifluoroacetic acid (TFA), formic acid (FA), and acetic acid (HAc)

Gradient—Analytical Separations

0.5% ACN for 1 min, then to 50% ACN over 60 min (Acid modifier varied, Figures 2-5)

Gradient—Preparative Separations

0.45% ACN for 1 min, then to 54% ACN over 60 min (Acid modifier varied, Figure 6)

Analytical Separations—Acetic Acid

Preparative Separations—Acetic Acid

To investigate the performance of CSH130 C18 in preparative separations, load studies were performed with a number of different peptides and mobile phases that are typically used in manufacturing, namely those containing either TFA or HAc. Analytical (4.6 mm I.D.) columns packed with 5 µm particles were used for these method development, namely those with modified surface charge, tends to deliver better peak shapes in acidic mobile phases with little to no ion pairing, such as those modified with acetic acid.

High sample loads were also investigated using a synthetic, low purity peptide of the sequence DFGVYIKDPGVGVRK (MW=1,766 Da, pI=6). Separations were performed using focused gradients, to reduce run times, and low sensitivity wavelength (250 nm) detection, to assess full peak shapes. Figures 3 and 4 demonstrate the effect of using different mobile phases and hybrid column chemistries on target peptide peak shape and resolution from impurities. The analytical column chemistries with different selectivity and optimal additive concentrations, such as BEH130 C18 and CSH130 C18, can be of benefit when developing challenging preparative separations. For the parameters screened in this loading study, the CSH130 C18 column with a 1% TFA mobile phase appeared to provide both the narrowest target peptide peak and least co-elution with monitored impurities. Nonetheless, a comparable separation could be achieved with the BEH column and a 0.1% HAc mobile phase, given the peak shape afforded by a zwitterionic, anti-Langmuirian isomer.

Figure 1. Charged Surface Hybrid Technology

Figure 2. Analysis of the MassPREP peptide mixture at semi-preparative sample loads (500 µg) with BEH130 and CSH130 C18, 5µm, 4.6 x 100 mm columns.

Analytical Separations—Peak Capacity/MS Signal

CSH130 C18 delivers improved performance over current state-of-the-art peptide columns. Figure 4A shows the relationship between acid modifier composition and the peak capacities obtained with each column included in this study. With 0.1% TFA, the CSH130 C18 column provided 20% higher peak capacity than the other 1.7 µm C18 columns. However, when the mobile phase contained 0.1% FA, the CSH130 C18 column provided 90% greater peak capacity compared to the other 1.7 µm columns. Figure 4B demonstrates that the use of TFA instead of FA caused a 12-fold decrease in MS signal during these analyses. CSH130 C18 does not require much, if any, TFA for optimal peak capacity, making it ideal for LC-MS applications in which high sensitivity is desired.

CONCLUSION

A novel reversed phase column chemistry, modified to have a positive surface charge, is shown to be useful for both analytical and preparative peptide separations. CSH130 C18 provides:

- Improved loadability and greater peak capacity vs. current state-of-the-art peptides columns.
- Excellent peak shape with both TFA and FA mobile phases and thus excellent compatibility with ESI-MS.
- Unique selectivity, albeit with slightly less retention. Excellent resolution for preparative peptide separations with 1% HAc mobile phases, a fact that can be exploited to obtain peptides with a pharmaceutically acceptable counter ion in fewer steps.

References