

Charged-Surface Stationary Phase Improves Peptide Separations in Low Ionic Strength Mobile Phases

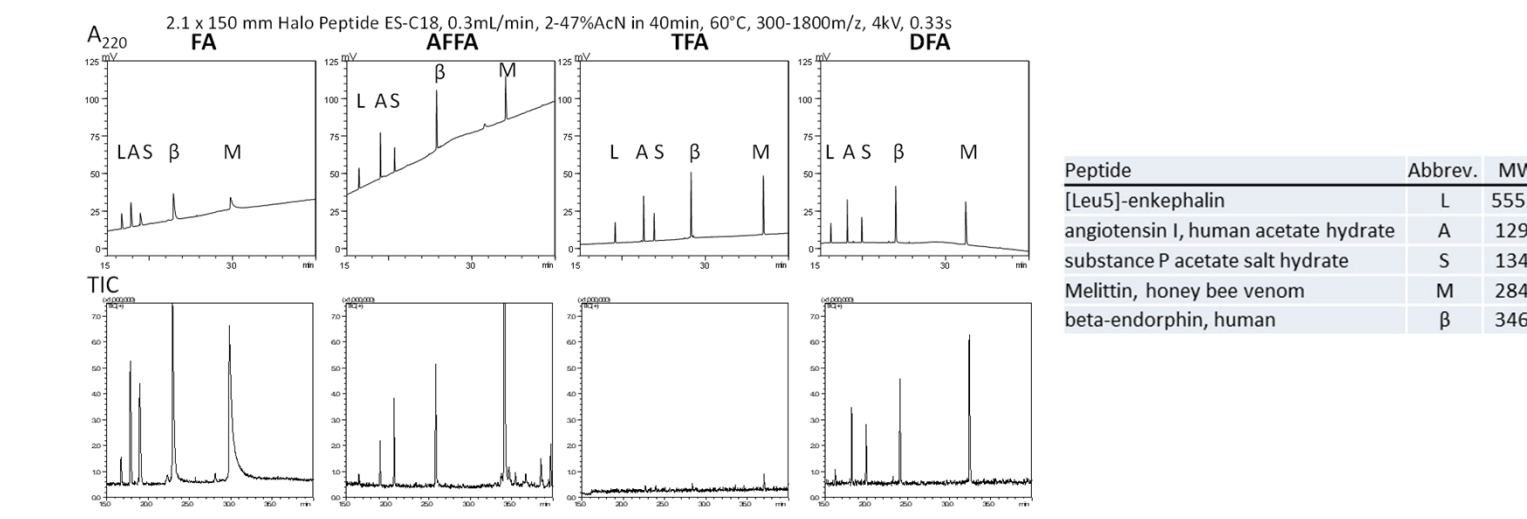
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Introduction

Superficially porous particles (SPPs) enable rapid, high-resolution separations with favorable compromise between permeability of packed beds (pressure) and excellent chromatographic efficiency. We have previously demonstrated that 160 Å pore size 2.7 µm diameter particles are favored for highly efficient separations of peptides e.g. from proteolytic digests, synthetic peptides, and therapeutic biopolymers. The use of formic acid (FA) modified mobile phases for LC/MS separations of peptides in reversed-phase HPLC (RPC) can have limitations. Peptides are variably charged in weakly acidic mobile phases, but cationic species often show load dependent tailing in weak acids. The use of perfluorinated acid modifiers, such as trifluoroacetic acid (TFA), yields superior peak shape and load tolerance in RPC, but poor LC/MS detection resulting from tight ion pair formation, leading to poor ionization efficiency. Our previous results have illustrated means to gain improvements in RPC, via mobile phase selection, while reducing ionization suppression for peptide and proteomic analyses (shown in Fig. 1; Johnson, et al., 2013 J. Biomol. Tech., 24, 187-197). Stationary phase manipulation presents another tool for method development. A novel 160Å 2.7µm SPP possessing a positive charge surface (PCS), with RPC retention afforded by the C18 stationary phase is shown for a variety of peptide and protein digest analyses..

Fig.1 Synthetic Peptide Mixture LC/MS in Several Acidic Modifiers



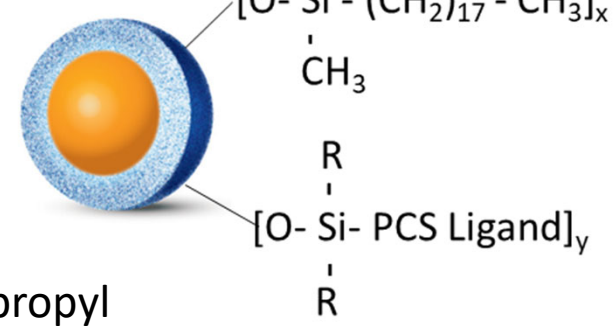
New PCS Surface Modification for Silica Surfaces

The novel reversed-phase positively charged surface chemistry modification has recently been developed by AMT, for modification of 90 Å and 160 Å pore size Spp silica particles. The controlled surface charge density supplied by this ligand is paired with suitable organosilane modified reversed-phase surfaces (in this case dimethyl-C18).

Reproducible RPC separations require total and relative density control of charged and hydrophobic surface ligands. A steric-protected positively charged ligand (diisopropyl silane) provides superior stability and performance with elevated temperature and acidic mobile phase use conditions common for peptide separations.

HALO 90 Å and 160 Å pore size, 2.7 µm PCS C18

- Excellent peak shape and increased loading capacity for basic compounds
- Alternate L1 selectivity (PCS C18)
- Built upon Fused-Core® technology for fast, efficient and reliable separations



Peptide Separations Comparing Traditional and PCS Modified Surfaces

Fig.2 Peptide mixture resolution and peak shape in Formic Acid Mobile Phase

- Gradient separation of a QA mixture including 5 variant synthetic peptides + insulin B₃₀
- Reduced retention time for HALO® PCS C18 Peptide compared to traditional Peptide ES-C18
- Improved peak widths and reduced tailing in formic acid

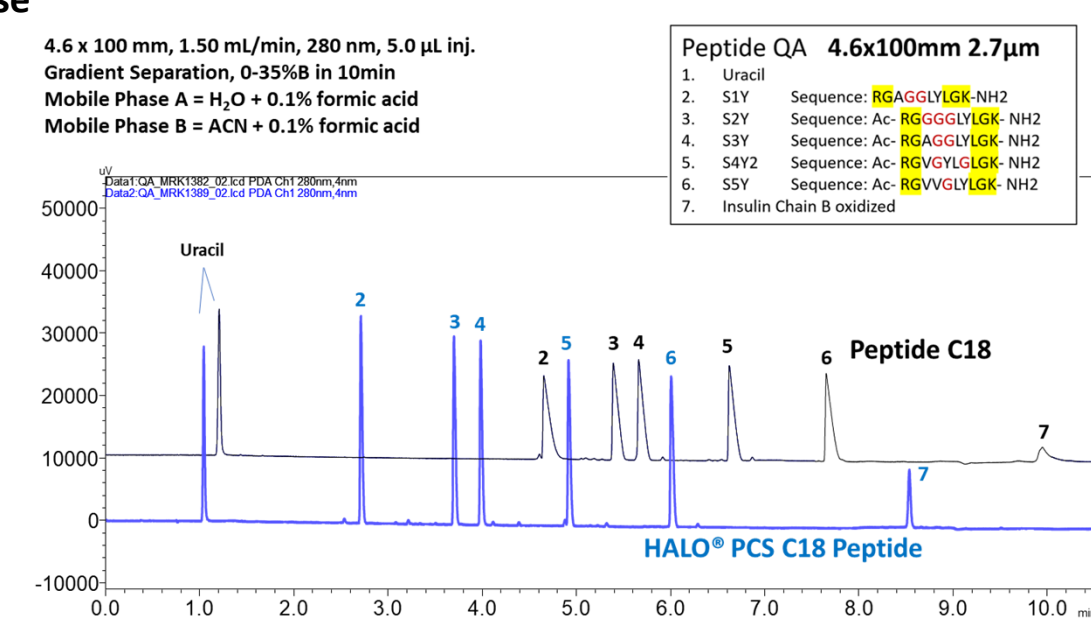


Fig.3 Peptide peak shape in Formic Acid Mobile Phase at increased load

- Varying load of a 10 residue synthetic peptide and insulin B₃₀ on a traditional and charged surface RPC material
- Notable improvement in load tolerance (peak width and peak tailing) on PCS as load increased from 0.3 to 4.5 µg on column

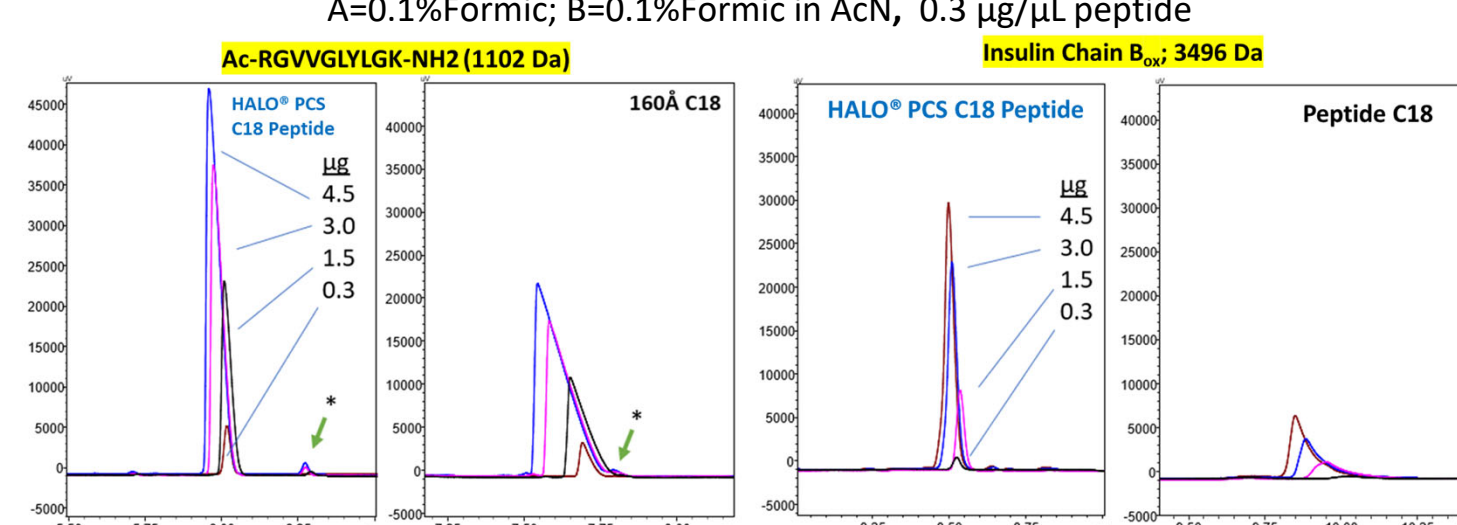


Fig.4 Tryptic digest analysis in Formic Acid Mobile Phase increases peak capacity in LC/MS

- Peak capacities (n_{pc}) measured with modest load (2 µg) of trastuzumab tryptic digest on a 2.1 mm ID column
- n_{pc} based on 12 ID peptides measured using extracted ions (XICs) PW_{1/2}, t_R and Δt_R for this specific sample set
- Decreased peak widths effect notable increase in peak capacity

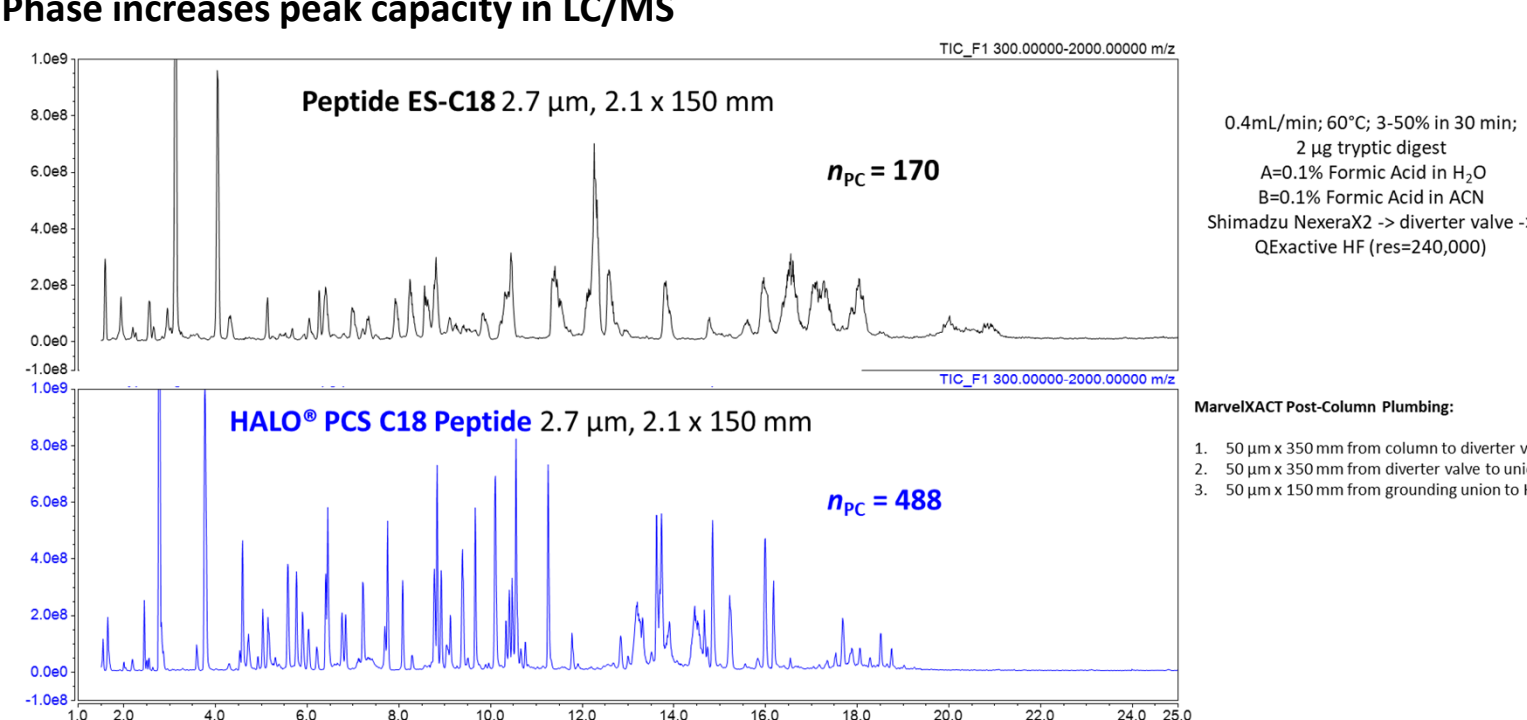
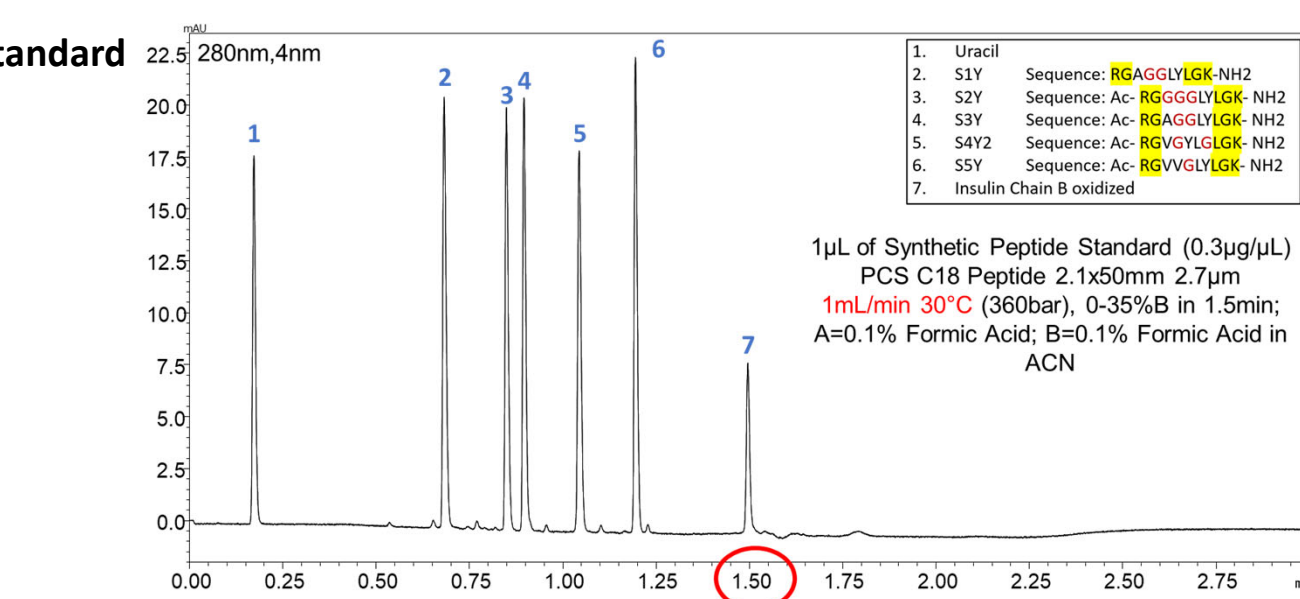


Fig.5 Very high-speed analysis of a synthetic peptide standard on the 50 mm PCS C18 Peptide Column

- The highly efficient 160 Å pore superficially porous particle permits very high throughput analysis
- The example shows separation conducted in less than 2 minutes, with modest backpressure, even at moderate temperature.



Application of PCS-C18 Peptide to Structure Analysis by LC/MS

Fig.6 Peptide/Glycopeptide Pair Impurity Analysis

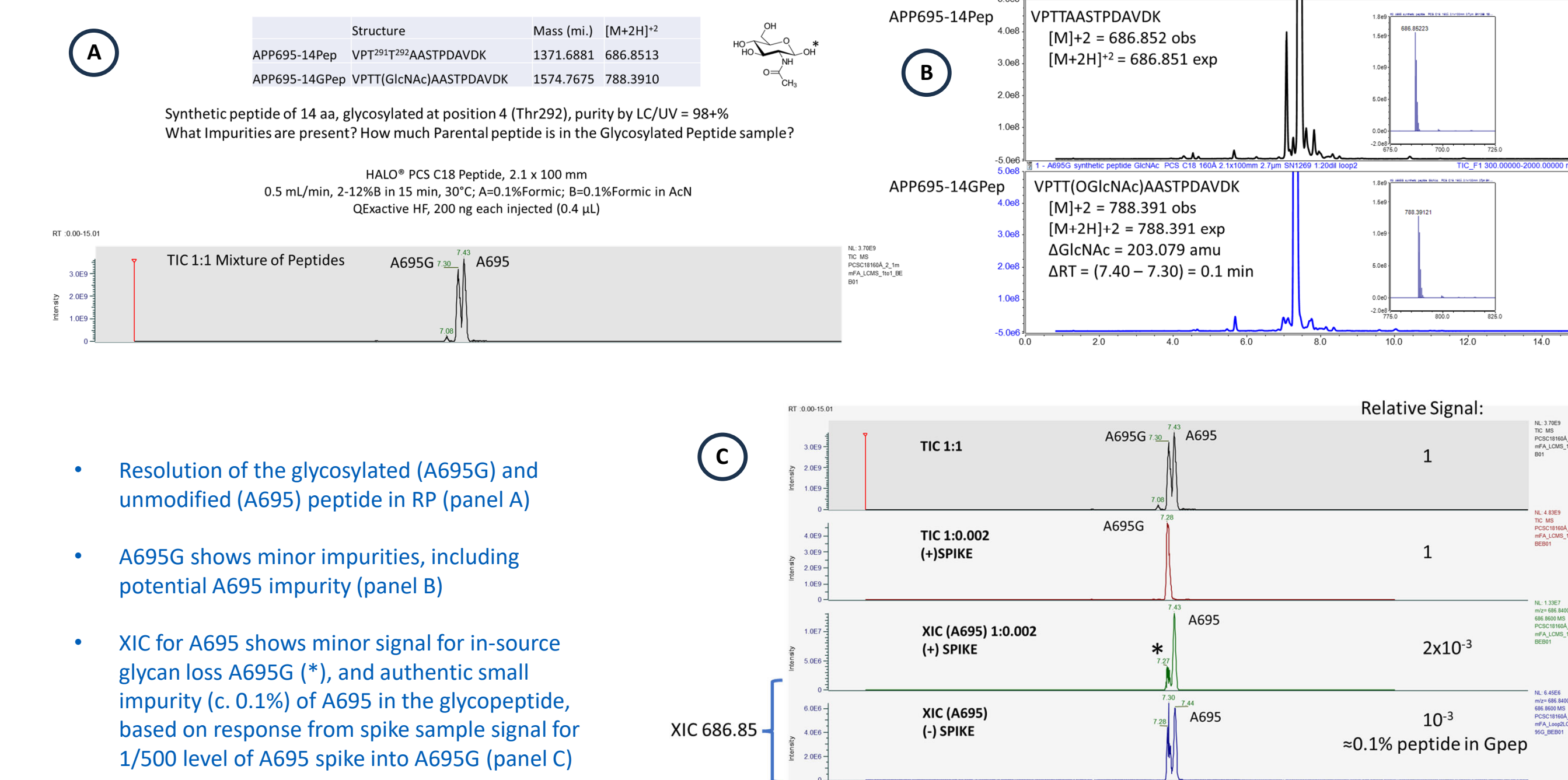
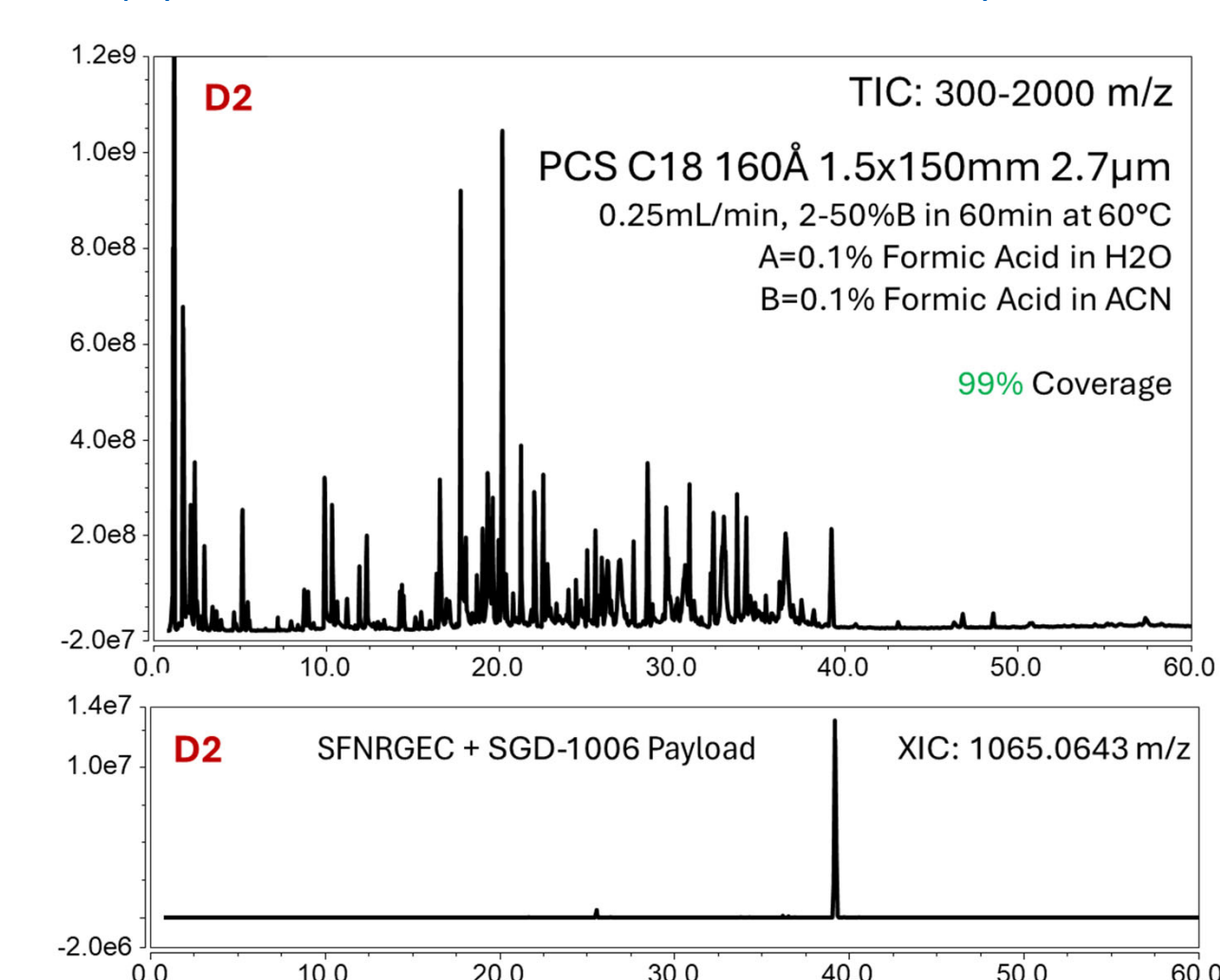


Fig.7 Tryptic digest LC/MS analyses to identify payload site for isolated DAR2 ADC

- HIC isolated mAb with 2 vedotin-efjv payload conjugated to enfortumab was digested with trypsin. A single L-chain cysteine site was occupied by the payload, with retention identified in the XIC, verified by MS/MS.



Conclusions

HALO® 160 Å PCS C18 exhibits favorable peak shape for peptides in weakly acidic or ion pairing mobile phases – expanding choice of mobile phases for effective LC/MS.

HALO® PCS phases improve load tolerance in FA vs. traditional stationary phases – useful for analysis of less abundant variants/impurities.

All HALO® PCS phases exhibit the speed and resolution advantages of Fused-Core® superficially porous particles.

New material shows high resolution for complex mixtures, as obtained from fragments of mAbs and ADCs.

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