Selectivity Choices for Larger Pore Superficially Porous Particle UHPLC Columns

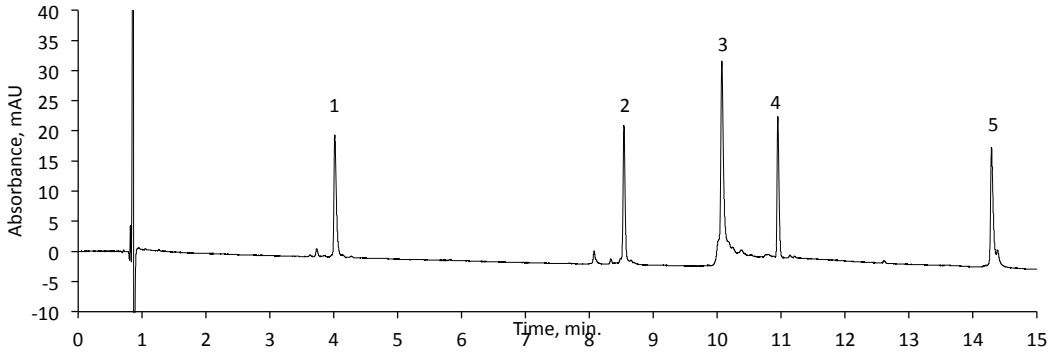
Depending on the requirements of a given biomolecule separation, one may want to use either ES-C18 or C4 phase on HALO 1000Å Fused-Core silica. Both of these phases are stable at elevated temperature and low pH, which makes them suitable for reversed-phase UHPLC separations of proteins and antibodies. When increased retention is needed or when a wide range of molecular sizes is present, choose the ES-C18 phase. Figure 1 shows the separation of a wide range of proteins using a HALO Protein 1000Å ES-C18 column.

Figure 1. Example protein separation using a HALO 1000Å ES-C18 column.

Testing Conditions:
Column: 2.1 x 150 mm HALO 1000Å ES-C18, 2.7 µm
Mobile Phase A: Water/0.1% TFA
B: 80/20 ACN/Water/0.085% TFA
Gradient: 27–60 %B in 15 min.
Wavelength: 280 nm
Injection: 2 µL
Temperature: 60 °C
Flow Rate: 0.4 mL/min.

Peak Identities:

1.	Ribonuclease A	13.7 kDa
2.	Lysozyme	14.3 kDa
3.	SigmaMAb	~150 kDa
4.	α-Lactalbumin	14.2 kDa
5.	Enolase	46 kDa monomer



When you are developing a new method for the analysis of monoclonal antibodies (mAbs), screening different stationary phase columns can be an effective approach to optimize the resolution of minor components. For example, Figure 2A shows the comparison of the separation of NISTmAb using a HALO 1000Å ES-C18 column to that using a HALO 1000Å C4 column. Increased retention is observed with the ES-C18 phase compared to the C4 phase under identical gradient conditions. This additional retention may prove valuable for additional separation space of small molecule compounds which may be intentionally or unintentionally present in drug development.

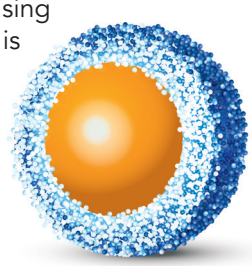


Figure 2. (A). Comparison of ES-C18 and C4 bonded phases for the separation of NISTmAb. (B). Zoomed-in region showing the minor components of the NISTmAb.

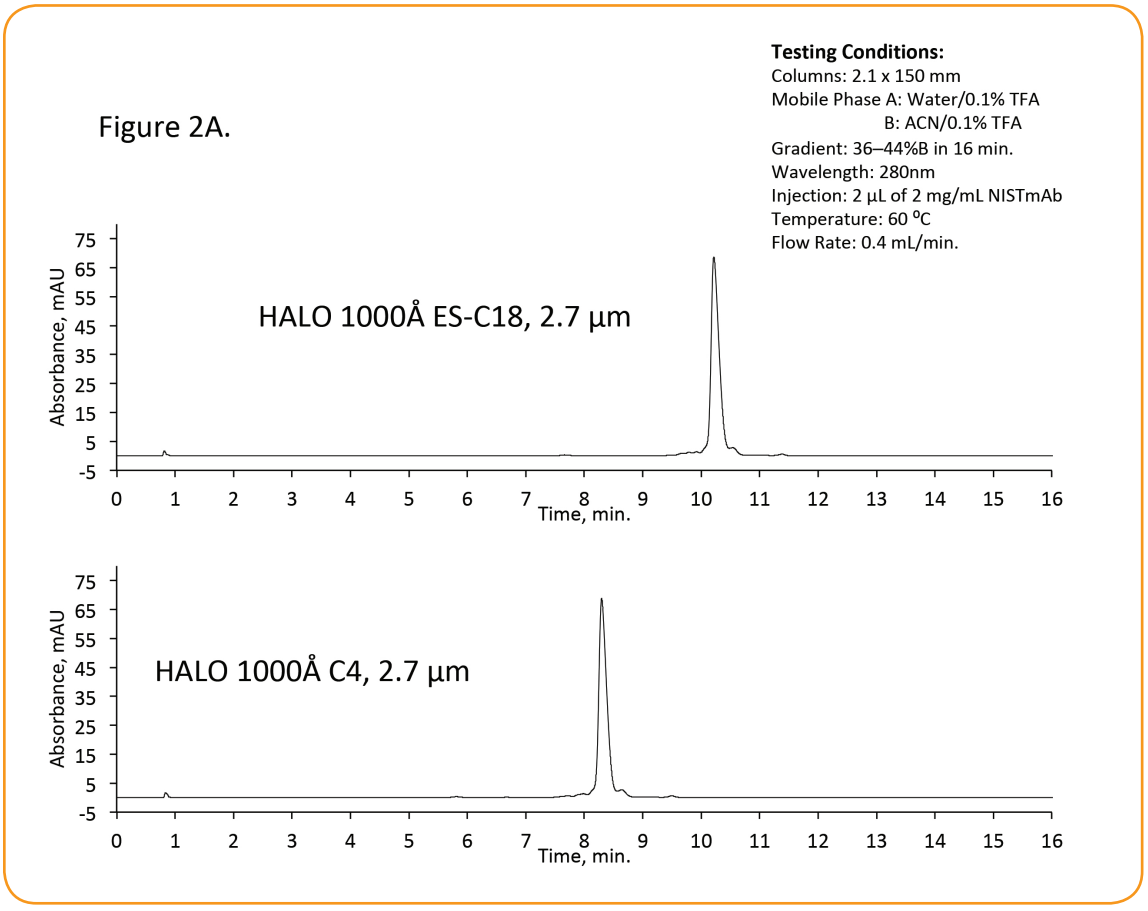
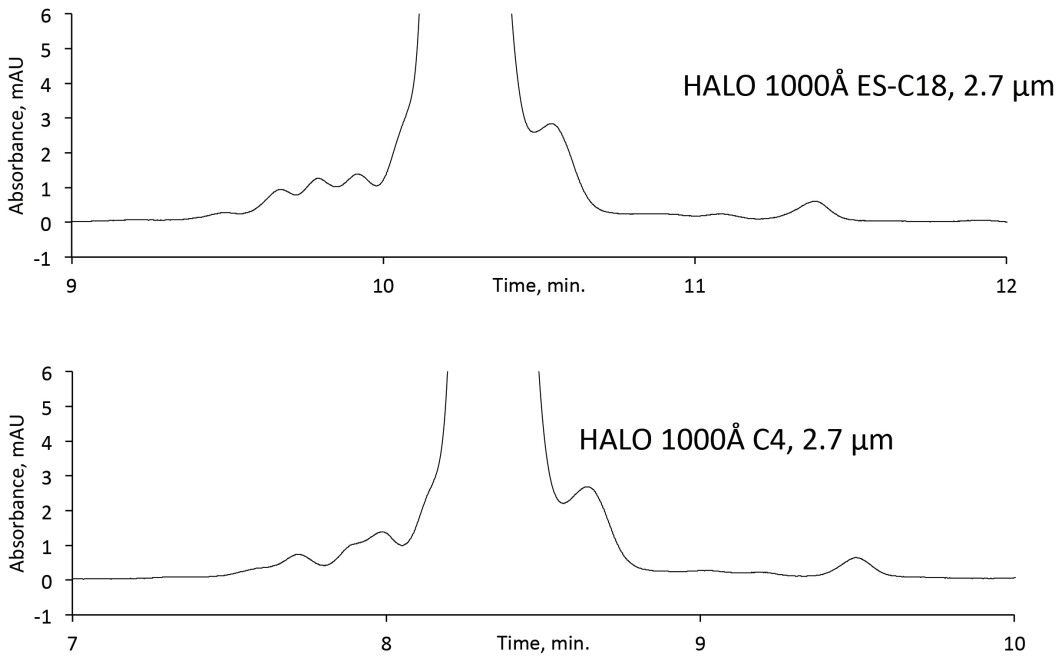


Figure 2B. Zoomed in region of Figure 2A.



In Figure 2B, increased resolution of the minor components eluting before the major component of the NISTmAb is observed with the HALO 1000Å ES-C18 column compared to the HALO 1000Å C4 column. Identification and quantification of these minor components are often part of the quality control protocol used for the manufacturing of biotherapeutics.

Conclusions

Both the ES-C18 and C4 bonded phases on HALO 1000Å are well suited for the analysis of large biomolecules. The differences in selectivity and retention can be explored during method development in order to have the most robust and efficient separation.