## Mo.TI290E

Cadenza HS-C18

150 x 4.6 mm

Technical

## Relationship between molecular weight and protein exclusion in a hybrid ODS stationary phase



Cadenza HS-C18, 150 x 4.6 mm A: 100mM ammonium acetate, B: acetonitrile, 0-70%B (0-10min), 1 mL/min, 37 degC, 280 nm Cadenza HS-C18 contains a silicabased hybrid ODS stationary phase with an optimal arrangement (ODS) of hydrophobic and hydrophilic groups. By injecting blood serum directly, pharmaceutical analysis excluding pretreatment steps such as solid phase extraction is possible. Blood serum-derived proteins (albumin) exclude via the hydrophilic groups, and the hydrophobic groups retain the drug. HS-C18 most common use is blood serum-derived proteins in pharmacokinetics, but the column also provides excellent separation molecular for low weight compounds in protein samples.

The left chromatogram shows the proteins excluded by HS-C18 in order of their molecular weight. To avoid the isoelectric point (pI) influence in the experiment, we used a neutral ammonium acetate in the initial mobile phase. As for the samples used in this experiment, the chromatogram shows that a 30kDa molecular weight protein was not retained by the column. On the other hand, an under 30kDa molecular weight protein (including a peptide) was retained due to the interaction with the hydrophobic ODS in the stationary phase. It is important to take into account the elution characteristics of a protein with pI higher than the mobile phase pH. Nevertheless, a protein greater than 30kDa was already excluded from the column and you can separate low molecular weight compounds such as these sample proteins without any pretreatment. HS-C18 can serve a myriad of applications beyond blood serum.