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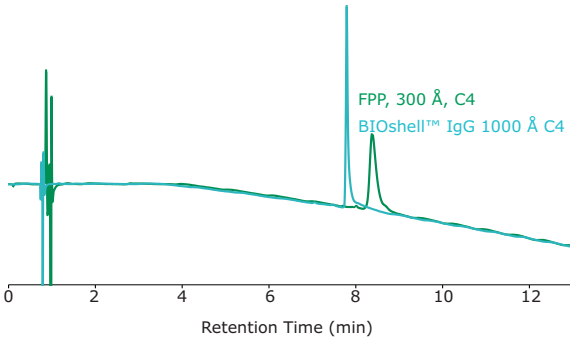
Lower Pore Diameters Result in Broader Peaks and Decreased Sensitivity

column:	BIOshell™ IgG 1000 Å C4, 10 cm x 2.1 mm I.D., 2.7 µm; FPP 300 Å C4, 10 cm x 2.1 mm I.D., 1.7 µm
mobile phase:	[A] water (0.1% (v/v) difluoroacetic acid); [B] acetonitrile (0.1% (v/v) difluoroacetic acid)
gradient:	Hold at 22% B for 2 min; 22% B to 52% B in 15 min
flow rate:	0.3 mL/min
column temp.:	75 °C
detector:	UV, 215 nm
injection:	5 µL
sample:	IgG4, 100 µg/mL, water

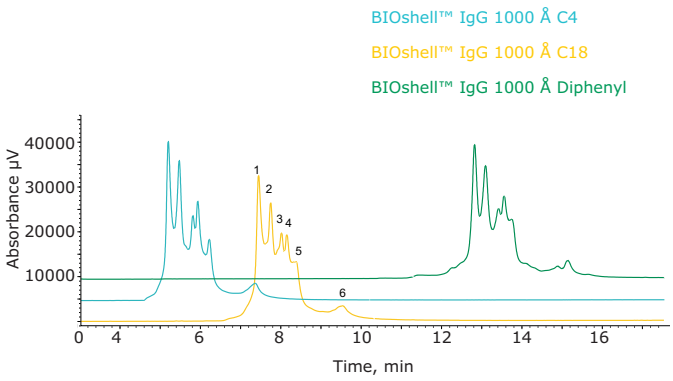
The large pores of the BIOshell™ IgG 1000 Å C4 column allows improved access to the stationary phase resulting in narrower peaks.



Effect of Phase Chemistry on Protein Selectivity

column:	BIOshell™ IgG 1000 Å C4, 15 cm x 2.1 mm I.D., 2.7 µm; BIOshell™ IgG 1000 Å C18, 15 cm x 2.1 mm I.D., 2.7 µm; BIOshell™ IgG 1000 Å Diphenyl, 15 cm x 2.1 mm I.D., 2.7 µm
mobile phase:	[A] 2:10:88 n-propanol/acetonitrile/water (0.1% v/v difluoroacetic acid); [B] 70:20:10 n-propanol/acetonitrile/water (0.1% v/v difluoroacetic acid)
gradient:	16% B to 26% B in 20 min
flow rate:	0.2 mL/min
column temp.:	80 °C
detector:	UV, 280 nm
injection:	2 µL
sample:	Denosumab, 2 mg/mL, water (0.1% v/v trifluoroacetic acid)

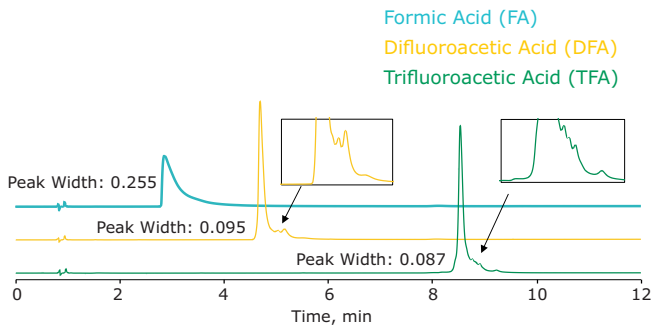
Monoclonal antibodies are unique molecules and therefore can interact differently with different phase chemistries.
The numbered peaks correspond to IgG2 disulfide bond variants.



Choice of Ion-Pairing Reagent Is Crucial to Enhanced Resolution and Sensitivity

column:	BIOshell™ IgG 1000 Å C4, 15 cm x 2.1 mm I.D., 2.7 µm
mobile phase:	[A] Water (0.1% v/v formic acid, difluoroacetic acid, or trifluoroacetic acid, as indicated) [B] 20:80 Water:Acetonitrile (0.1% v/v formic acid, difluoroacetic acid, or trifluoroacetic acid, as indicated
gradient:	35% B to 47.5% B in 12 min
flow rate:	0.4 mL/min
column temp.:	80 °C
detector:	UV, 280 nm
injection:	2 µL
sample:	Trastuzumab, varied concentration, 70:30 Water:Acetonitrile

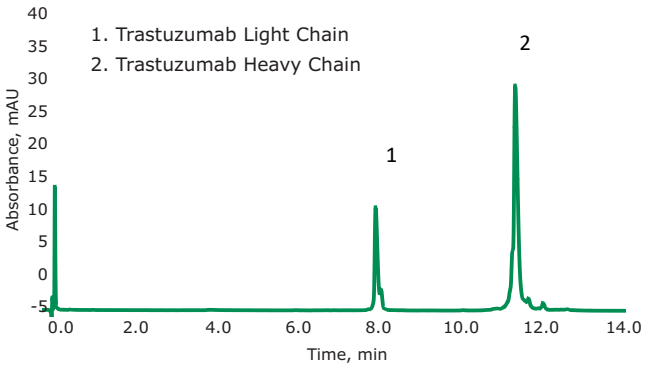
Choosing the right ion-pairing reagent can lead to better resolution of structural variants of mAbs and other proteins as well as better sensitivity.



Optimized Middle-Up Analysis of Reduced IgG1 Using the BIOshell™ IgG 1000 Å Diphenyl Column

column:	BIOshell™ IgG 1000 Å Diphenyl, 15 cm x 2.1 mm I.D., 2.7 µm
mobile phase:	[A] Water (0.1% v/v TFA); [B] Acetonitrile (0.1% v/v TFA)
gradient:	30% B to 40% B in 14.0 min
flow rate:	0.4 mL/min
column temp.:	80 °C
detector:	UV, 280 nm
injection:	2 µL
sample:	Reduced Trastuzumab, 400 µg/mL, water

The BIOshell™ IgG 1000 Å Diphenyl column allows for resolution of minor structural variants of the light and heavy chains of mAbs.



Pore Size Mismatch Can Lead to Significant Losses in Efficiency

column:	BIOshell™ A160 Peptide C18, 15 cm x 2.1 mm I.D., 2.7 µm; BIOshell™ A400 Protein C18, 15 cm x 2.1 mm I.D., 3.4 µm; BIOshell™ IgG 1000 Å C18, 15 cm x 2.1 mm I.D., 2.7 µm;
mobile phase:	[A] Water (0.1% v/v trifluoroacetic acid); [B] 20:80 Water:Acetonitrile (0.085% v/v trifluoroacetic acid)
gradient:	27% B to 60% B in 15 min
flow rate:	0.4 mL/min
column temp.:	60 °C
detector:	UV, 280 nm
injection:	4 µL
sample:	Proteins, varied concentration, water (0.1% v/v trifluoroacetic acid)

Higher efficiencies and better sensitivity can be realized with proper pore diameter selection. Here, the 1000 Å pore diameter is the only one capable of providing good peak of the mAb (peak 3) analyte.

1. Ribonuclease A (13.8 kDa)
2. Lysozyme (14.4 kDa)
3. SILu™ Lite SigmaMAb Antibody (~150 kDa)
4. Enolase (46.7 kDa)