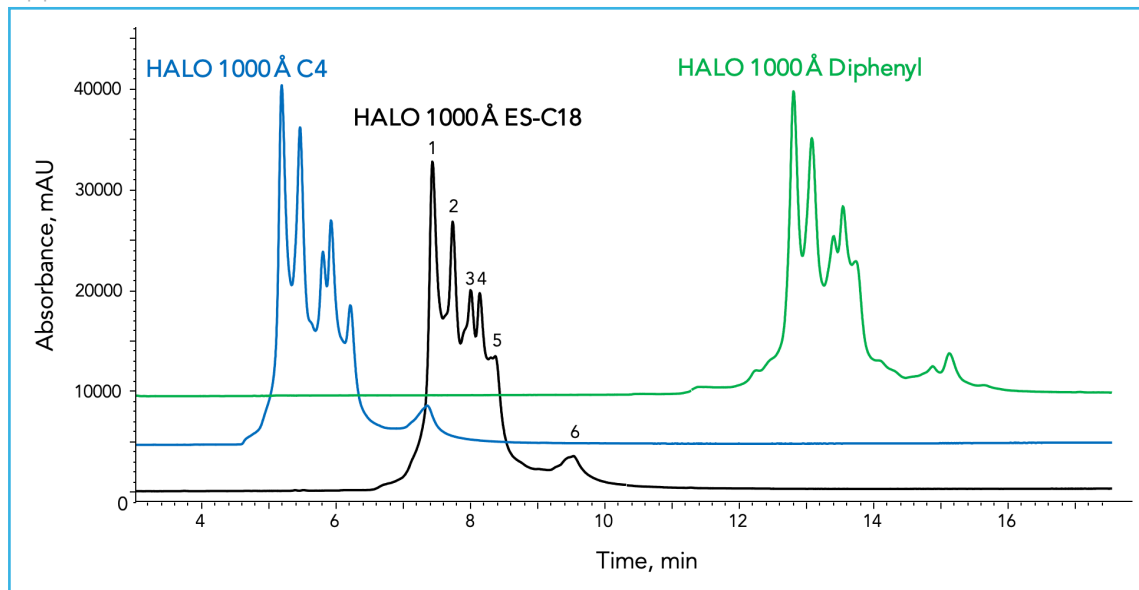




### IgG2 Comparison on HALO 1000 Å C4, ES-C18, and Diphenyl

Application Note 174-PR



There are currently three bonded phases available on HALO 1000 Å Fused-Core® particles – C4, ES-C18, and Diphenyl. Each shows unique selectivity for the separation of monoclonal antibodies. In this example, denosumab isoforms are resolved using a shallow gradient with the addition of n-propanol. Diphenyl phase is the most retentive phase, followed by ES-C18, and then C4. All three phases are recommended to be screened to determine which one yields the optimum separation for mAbs under investigation.

#### PEAK IDENTITIES:

- 1. IgG2-B
  - 2. IgG2-B
  - 3. IgG2-A/B
  - 4. IgG2-A/B
  - 5. IgG2-A
  - 6. IgG2-A\*
- } Disulfide bridge isoforms of IgG2

Note: Labels on ES-C18 chromatogram also apply to C4 and Diphenyl chromatograms.

#### TEST CONDITIONS:

##### Columns:

- 1) HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm  
Part Number: 92712-714
- 2) HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm  
Part Number: 92712-702
- 3) HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm  
Part Number: 92712-726

##### Mobile Phase:

- A: 2/10/88 n-propanol/ACN/H<sub>2</sub>O + 0.1% DFA
- B: 70/20/10 n-propanol/ACN/H<sub>2</sub>O + 0.1% DFA

**Gradient:** 16-26% B in 20 min

**Flow Rate:** 0.2 mL/min

**Temperature:** 80 °C

**Detection:** 280 nm, PDA; 350 nm reference

**Injection Volume:** 2.0 µL of 2 mg/mL denosumab

**Sample Solvent:** Water (0.1% TFA)

**LC System:** Shimadzu Nexera

