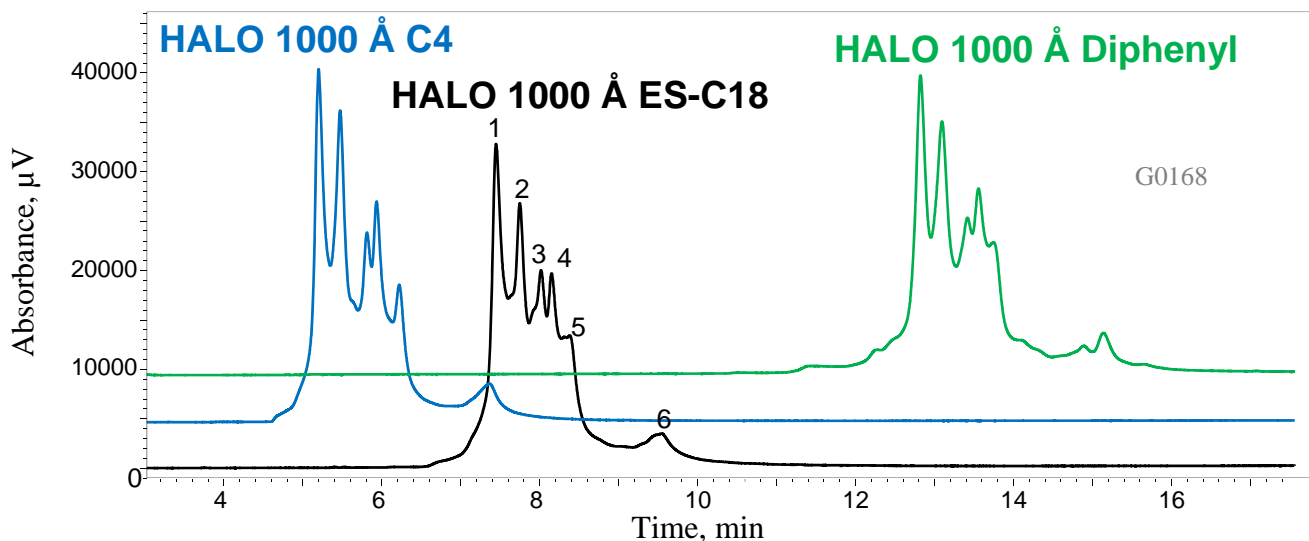


IgG2 Comparison on HALO 1000 Å C4, ES-C18, and Diphenyl



TEST CONDITIONS:

Columns:

HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm

Part Number: 92712-714

HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm

Part Number: 92712-702

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/H₂O

+ 0.1% difluoroacetic acid (DFA)

Mobile Phase B:

70:20:10 n-propanol/ACN/H₂O + 0.1% DFA

Gradient: 16–26% B in 20 min

Flow Rate: 0.2 mL/min

Temperature: 80°C

Instrument: Shimadzu Nexera

Detection: PDA 280 nm; 350 nm reference

Injection Volume: 2 µL of 2 mg/mL denosumab

Sample Solvent: water (0.1% TFA)

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.

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.