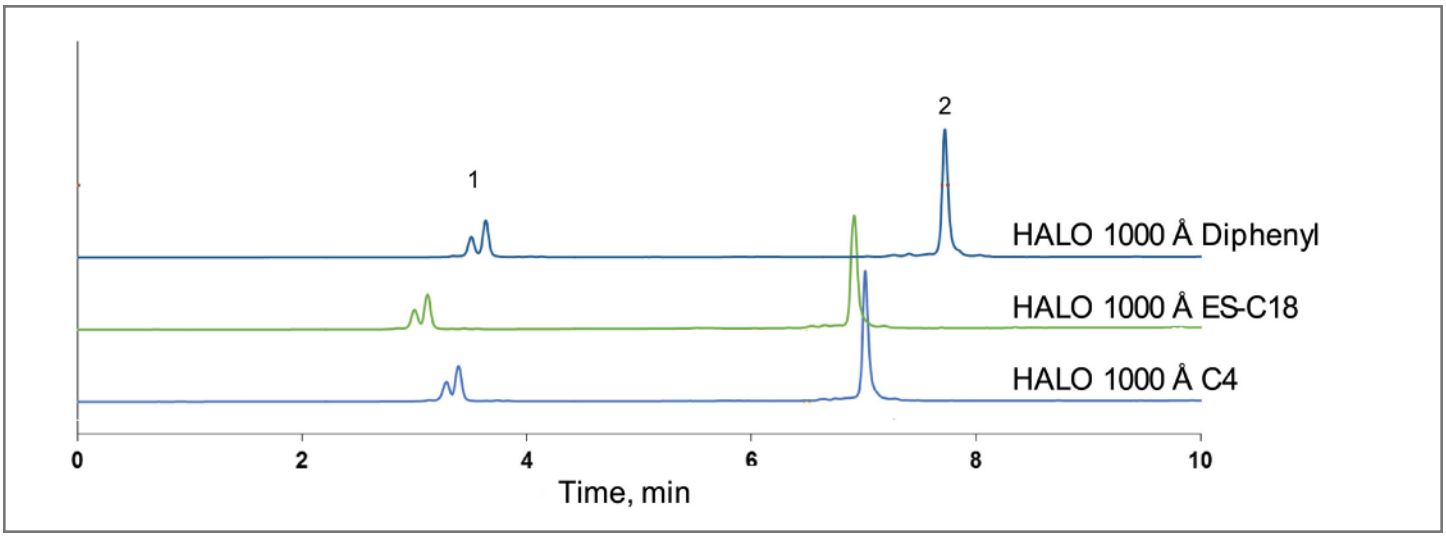
HALO®

BIOPHARMACEUTICALS

Comparison of an IdeS Digested mAb on Different HALO 1000 Å Phases

271-PR



PEAK IDENTITIES:

1. Fc/2
2. $F(ab')_2$

TEST CONDITIONS:

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

Mobile Phase A: water/0.1% TFA
Mobile Phase B: ACN/0.1% TFA
Gradient: 30-45% B in 10 min
Flow Rate: 0.4 mL/min
Temperature: 80 °C
Detection: Fluorescence (280 nm ex, 350 nm em)
Injection Volume: 0.5 µL
LC System: UPLC, I-Class

The characterization of mAbs is critically important for protein biotherapeutic drug development. Although the analysis of the heavy and light chain can provide important information, often times site specific information is more critical, and allows for a more thorough characterization of the mAb. IdeS, a cysteine protease, is often used to do a partial digestion of the mAb, and by site specific cleavage, provide heterogeneity information about the structure. Two Fc fragments (Fc/2) and one $(Fab')_2$ fragment are produced, which allows for a thorough characterization of the Fc fragment. The separation of IdeS digested Cetuximab was run on the three stationary phases that are available on the 1000 Å HALO® particle. Slightly different selectivity and retention were observed for the Diphenyl, ES-C18, and C4 with all of them providing excellent resolution and peak shape for the fragments of Cetuximab.

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