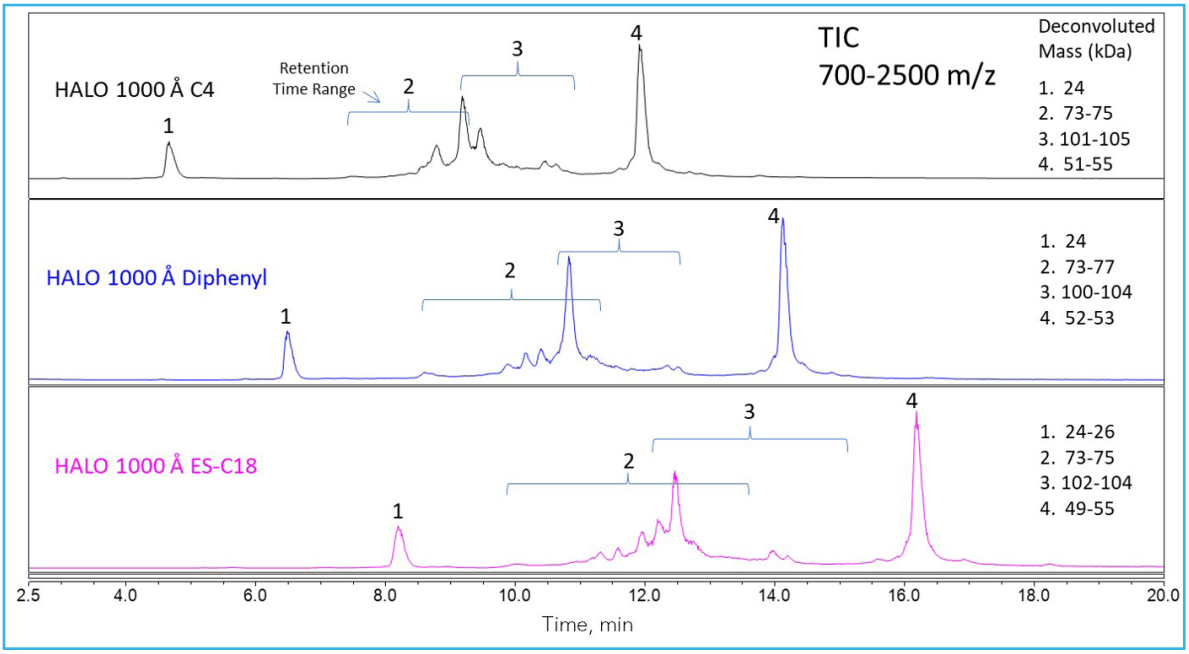
HALO®

BIOPHARMACEUTICALS

LC-MS Analysis of an ADC Mimic on HALO 1000 Å Phases

270



PEAK IDENTITIES:

MWs as labeled on the TICs

TEST CONDITIONS:

Column: HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714
Column: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-726
Columns: HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702
Mobile Phase A: Water/0.1% DFA
B: ACN/0.1% DFA
Gradient: 30-39 %B in 20 min
Flow Rate: 0.4 mL/min
Temperature: 75 °C
Detection: MS
Injection Volume: 5 µL
LC System: Shimadzu Nexera

MS Conditions:

Detection: + ESI MS/MS
ESI LCMS System: Velos Pro Orbitrap
Spray Voltage: 4 kV
Capillary Temperature: 275 °C
Source Heater: 300 °C
Sheath Gas: 35
Aux Gas: 10
RF lens: 70

SigmaMAb Antibody Drug Conjugate (ADC) Mimic was analyzed using LCMS and reversed-phase conditions. Due to the nature of cysteine-linked ADCs, the ADC Mimic does not remain intact when run using low pH and elevated temperature. The deconvoluted masses of the fragments are listed on the TIC of the results for the three stationary phases available for HALO 1000 Å: C4, Diphenyl, and ES-C18. The most retention is observed with ES-C18, followed by Diphenyl, and then C4. Different selectivity is shown for the peaks with~75 kDa MW (area 2 of the TIC). This demonstrates the importance of phase screening for ADC method development.

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