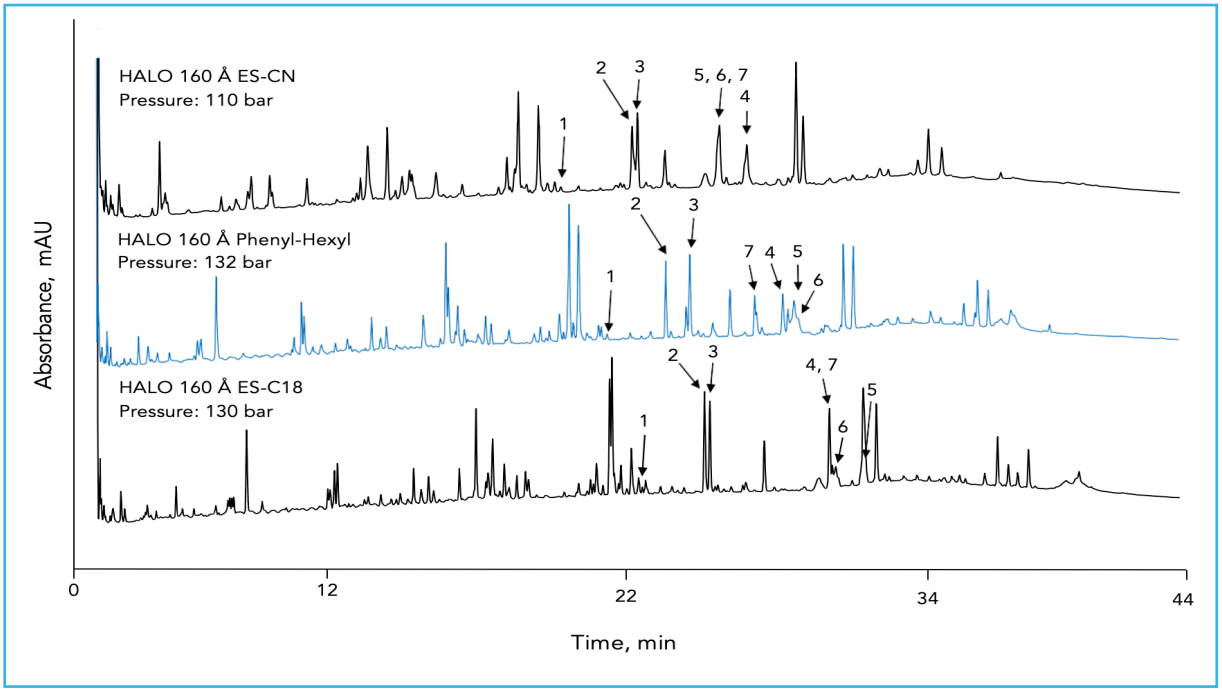
HALO®

BIOPHARMACEUTICALS

Enhanced Selectivity with HALO 160 Å Phenyl-Hexyl for a Tryptic Digest using LC-MS

Application Note 166-PE



TEST CONDITIONS:

Column:
1) HALO 160 Å ES-CN, 2.7 µm, 2.1 x 100 mm
Part Number: 92122-604
2) HALO 160 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92112-606
3) HALO 160 Å ES-C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92122-602

Mobile Phase:
A: Water + 10 mM difluoroacetic acid (DFA)
B: ACN + 10 mM difluoroacetic acid

Gradient: 2 to 50% B in 60 min
Flow Rate: 0.3 mL/min
Temperature: 60 °C
Detection: UV 220 nm, VWD
Injection Volume: 5.0 µL of 0.2 mg/mL digest
Sample Solvent: 50 mM Tris-HCl/1.5 M Guanidine-HCl with 0.25% formic acid
Response Time: 0.15 sec
Data Rate: 10 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Nexera

PEAK IDENTITIES: (using one-letter amino acid abbreviations):

1. FTISADTSKNTAYLQMNSLR (754 m/z)
2. LScAASGFNIKDTYIHWVR (747 m/z)
3. GFYPSDIAVEWESNGQPENNYK (849 m/z)
4. LLIYSASFLYSGVPSR (592 m/z)
5. SGTASVVcLLNNFYPR (899 m/z)
6. ScDKTHTcPPcPAPELLGGPSVFLFPPKPK (834 m/z)
7. VVSVLTVLHQDWLNGKEYK (1115 m/z)

The HALO 160 Å Phenyl-Hexyl column provided improved resolution between tryptic digest fragments 2 and 3 compared to the 160 Å ES-CN column and the 160 Å ES-C18 column. Peptide identification was accomplished by using MS-MS fragmentation spectra.

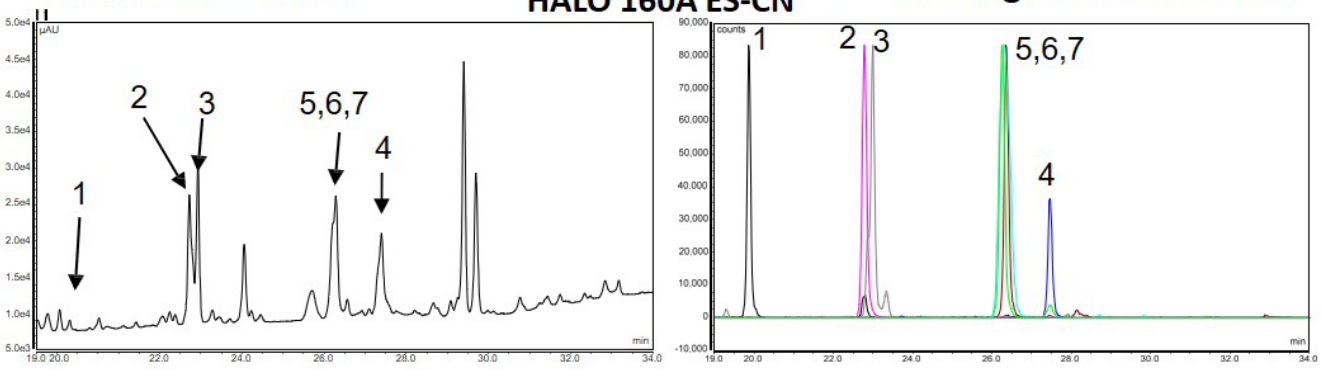
HALO®

BIOPHARMACEUTICALS

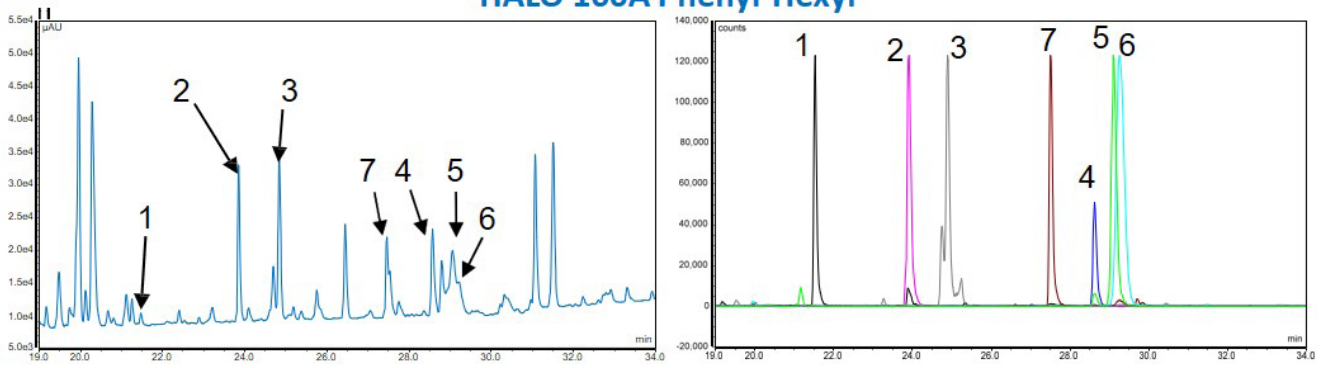
UV 220 nm traces

HALO 160Å ES-CN

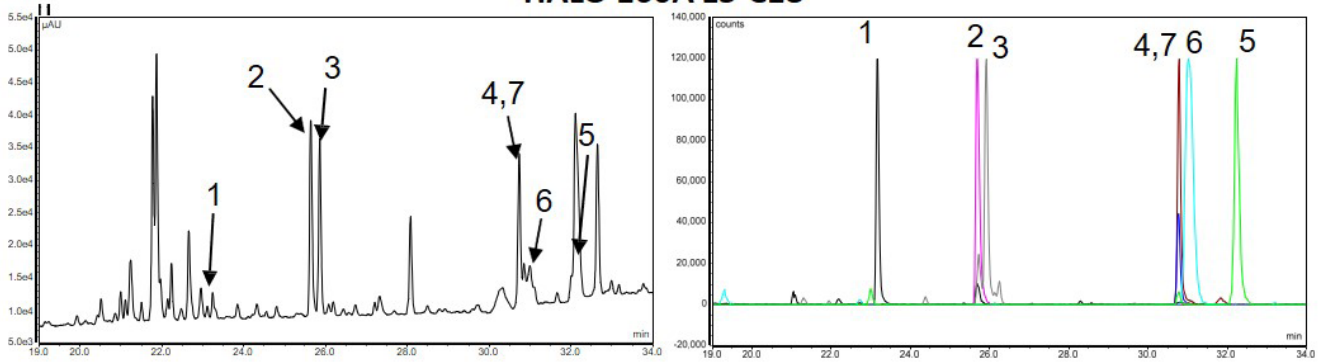
EIC signals normalized



HALO 160Å Phenyl-Hexyl



HALO 160Å ES-C18



The HALO 160 Å Phenyl-Hexyl column also provided improved resolution between tryptic digest fragments 4 and 7 compared to the 160 Å ES-C18 column. The extracted ion current chromatogram (EIC) and the mass spectrum,corresponding to each peptide fragment, are shown. The use of difluoroacetic acid (DFA) in the mobile phase facilitates symmetrical peak shape and good retention, while enabling good ionization efficiency and sensitivity.

MS System: Thermo Fisher Orbitrap VelosPro ETD
ESI: +3.5 kV
Scan Range: 50-2000 m/z
Scan Rate: 2 pps
Capillary: 225 °C
Sheath Gas: 35
Auxiliary Gas: 10
Scan Time: 2 µscans/200 ms max inject time