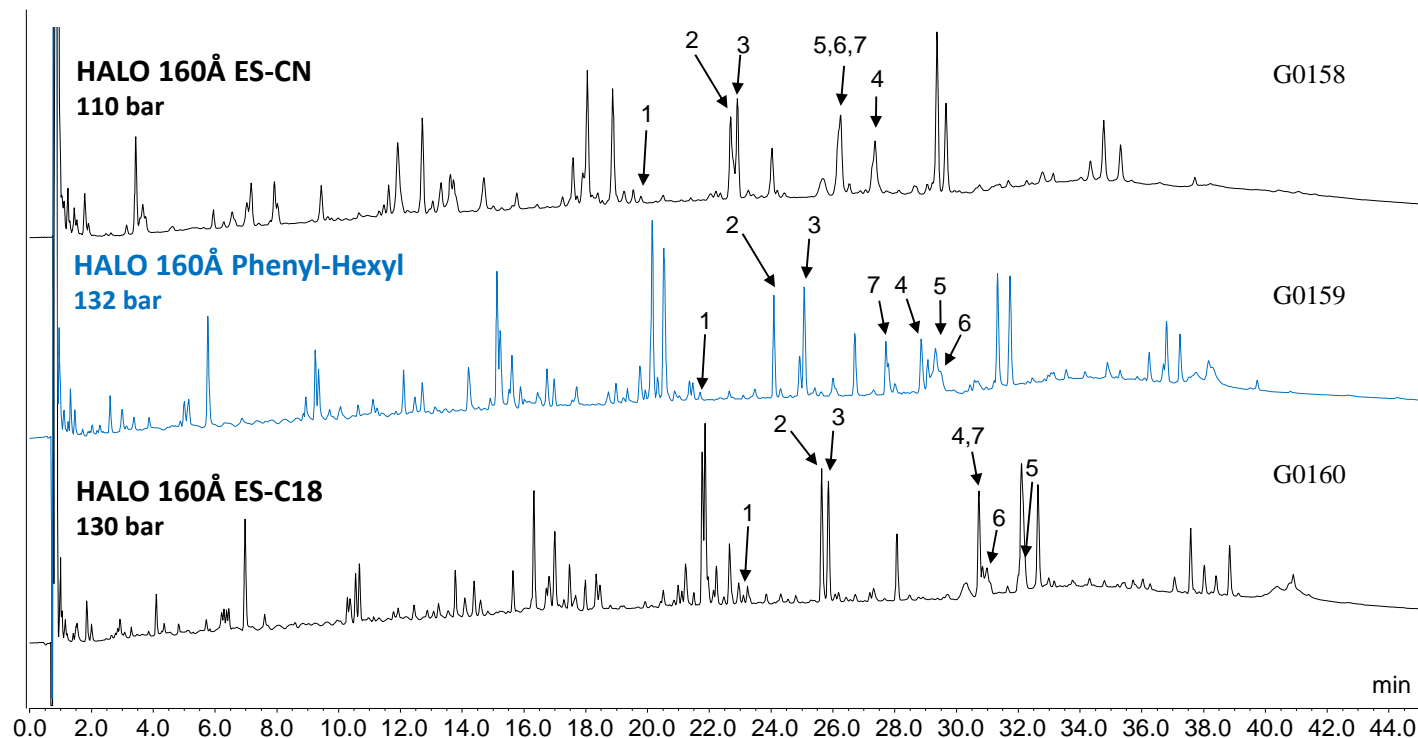


# HALO | Fused-Core® Particle Technology

Application Note: 166-PE

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



### TEST CONDITIONS:

Columns: HALO 160Å ES-CN, 2.7  $\mu$ m, 2.1 x 100 mm  
 Part Number: 92122-604  
 HALO 160Å Phenyl-Hexyl, 2.7  $\mu$ m, 2.1 x 100 mm  
 Part Number: 92112-606  
 HALO 160Å ES-C18, 2.7  $\mu$ m, 2.1 x 100 mm  
 Part Number: 92122-602

### Mobile Phase:

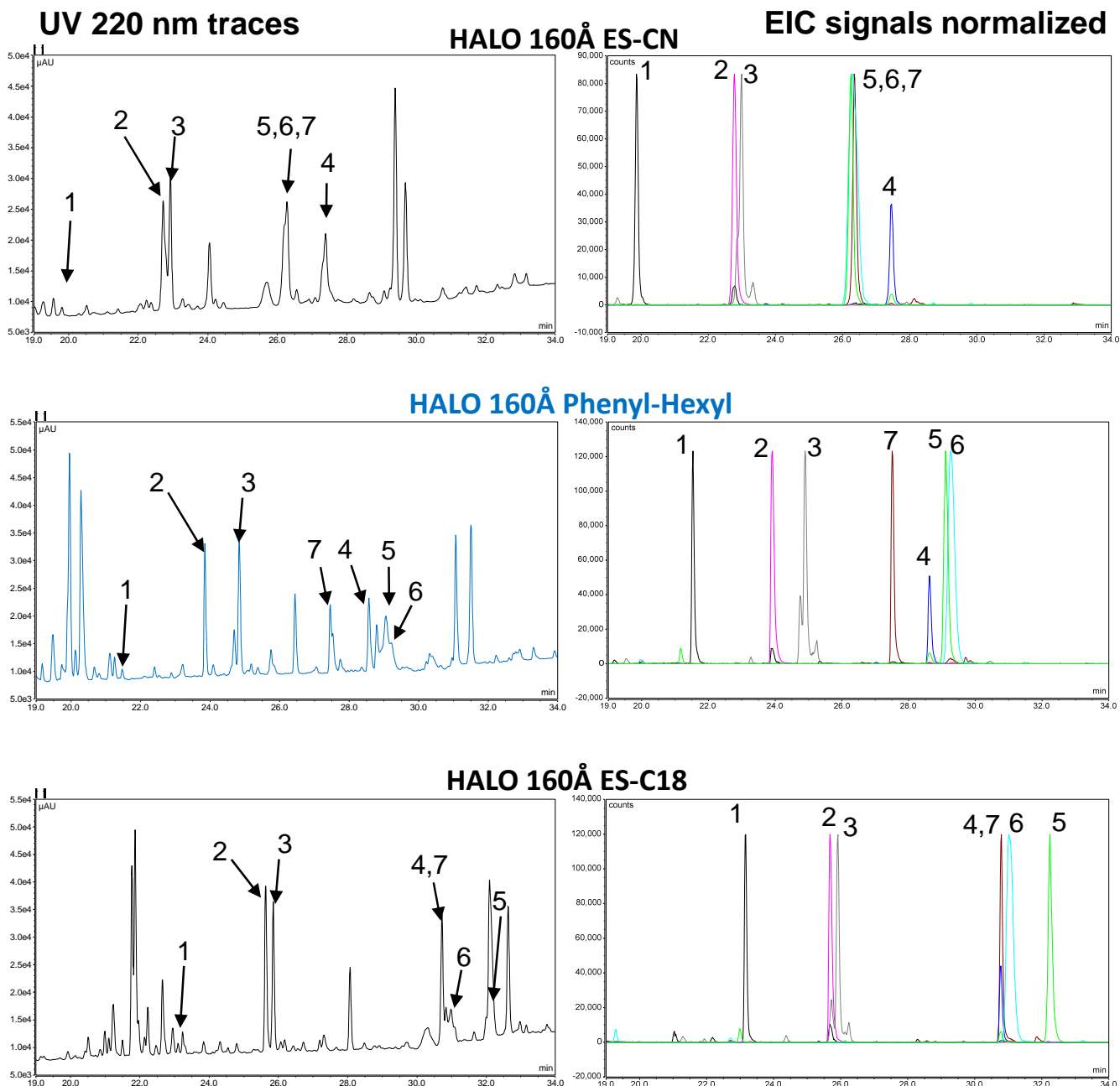
A = water + 10 mM difluoroacetic acid (DFA)  
 B = ACN + 10 mM difluoroacetic acid  
 Flow Rate: 0.3 mL/min  
 Gradient: 2–50%B in 60 min  
 Temperature: 60 °C  
 Detection: UV 220 nm, VWD  
 Injection Volume: 5  $\mu$ L of 0.2 mg/mL digest  
 Sample Solvent: 50 mM Tris-HCl/1.5 M Guanidine-HCl with 0.25% formic acid  
 Response Time: 0.15 sec  
 Data Rate: 10 Hz  
 LC System: Shimadzu Nexera  
 Flow Cell: 2.5  $\mu$ L semi-micro

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

The HALO 160Å Phenyl-Hexyl column also provided improved resolution between tryptic digest fragments 4 and 7 compared to the 160Å 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 CONDITIONS:

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