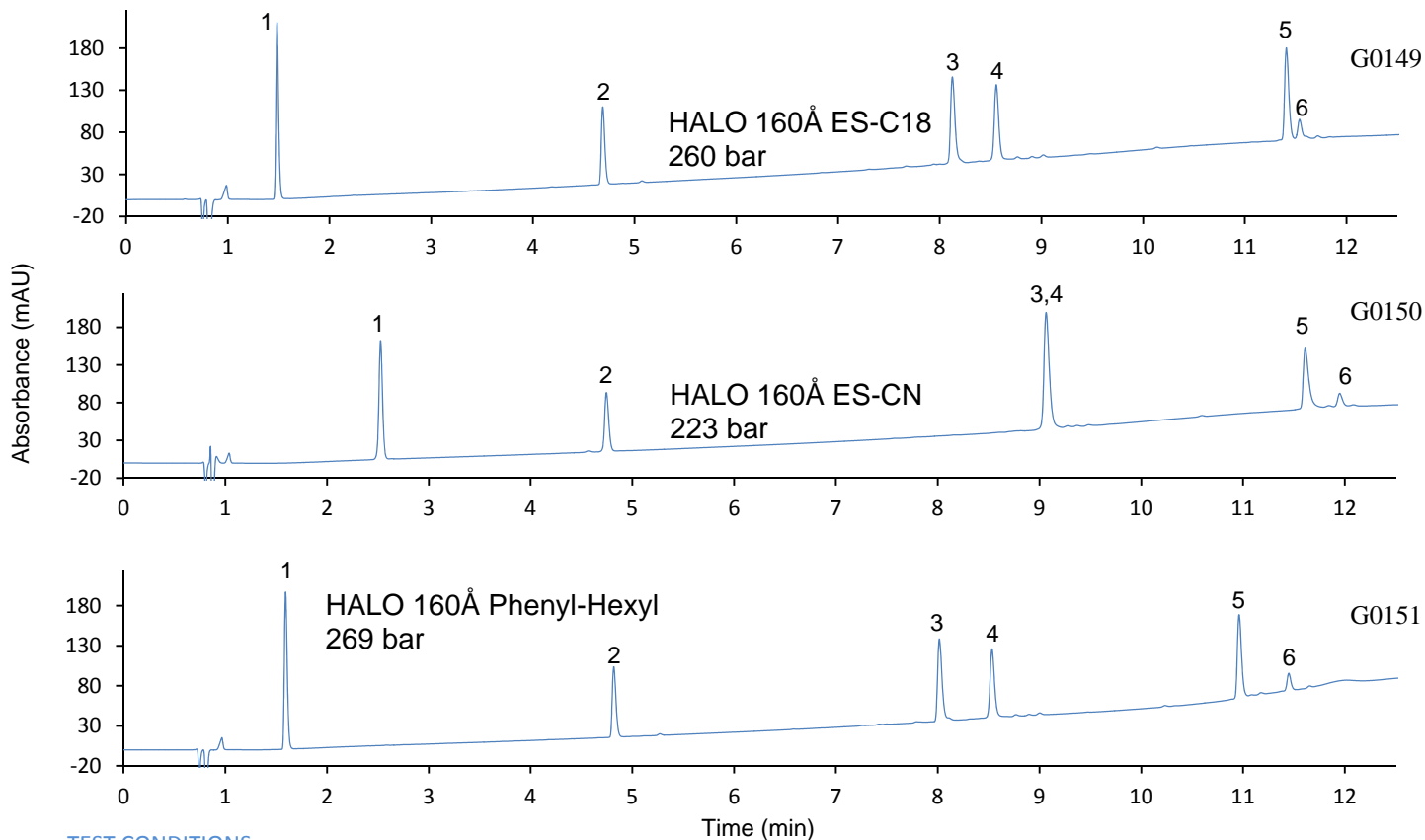


Application Note: 159-PE

Enhanced Selectivity for the Separation of Peptides Comparing HALO 160Å with Three Different Bonded Phases



TEST CONDITIONS:

Columns: HALO 160Å ES-C18, 2.7 μm , 2.1 x 150mm
Part Number: 92122-702
HALO 160Å ES-CN, 2.7 μm , 2.1 x 150mm
Part Number: 92122-704
HALO 160Å Phenyl-Hexyl, 2.7 μm , 2.1 x 150mm
Part Number: 92112-706

Mobile Phase:

A = 0.1% formic acid in water + 10mM ammonium formate
B = 50/50 n-propanol/water + 0.1% formic acid + 10mM ammonium formate (pH: 3.45)

Flow Rate: 0.4 mL/min

Gradient: 10-60%B in 15 min

Temperature: 60 °C

Detection: UV 220 nm, PDA

Injection Volume: 2 μL

Sample Solvent: water, 0.1% TFA

Response Time: 0.24 sec

Data Rate: 12.5 Hz

LC System: Shimadzu Nexera

Flow Cell: 1 μL

PEAK IDENTITIES:

1. Tyr-Tyr-Tyr
2. Angiotensin II
3. Angiotensin 1-12
4. Melittin
5. Sauvagine
6. β -Endorphin

The initial separation using a HALO 160Å ES-C18 column showed inadequate resolution of peaks 5 and 6. The same separation was attempted on a 160Å ES-CN column which provided improved resolution of peaks 5 and 6, but resulted in coelution of peaks 3 and 4. The HALO 160Å Phenyl-Hexyl column delivered excellent resolution between both peak pairs.