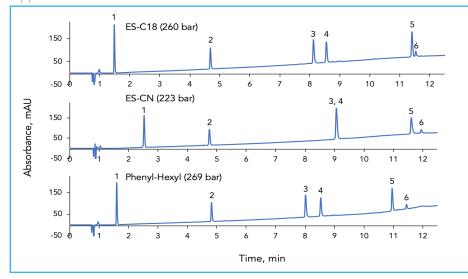
HALO

BIOPHARMACEUTICALS

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

Application Note 159-PE



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

TEST CONDITIONS:

Columns:

1) HALO 160 Å ES-C18, 2.7 μm, 2.1 x 150 mm Part Number: 92122-702 2) HALO 160 Å ES-CN, 2.7 µm, 2.1 x 150 mm Part Number: 92122-704 3) HALO 160 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 150 mm 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 Gradient: 10-60% B in 15 min Flow Rate: 0.4 mL/min Temperature: 60 °C Detection: UV 220 nm, PDA Injection Volume: 2.0 µL Sample Solvent: Water, 0.1% TFA Response Time: 0.24 sec Data Rate: 12.5 Hz Flow Cell: 1.0 µL LC System: Shimadzu Nexera