

Enhanced Selectivity With the HALO® 160Å Phenyl-Hexyl Column

A NEW LIGHT



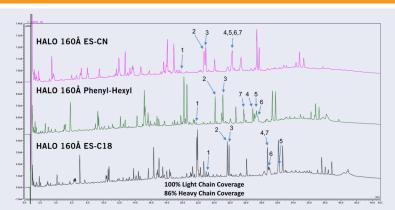


# INTRODUCING HALO® 160Å PHENYL-HEXYL

HALO® 160Å Phenyl-Hexyl columns are specifically designed to offer alternate selectivity to HALO 160Å ES-C18 and HALO 160Å ES-CN for separations of peptides and tryptic digests. The Fused-Core® particle design with a total particle size of 2.7 µm and 0.5 µm shell with 160Å pores enables high resolution at elevated flow rates. Additionally, HALO 160Å Phenyl-Hexyl columns have similar efficiency to sub-2-micron columns without the inconvenience of higher pressure.

## **UNIQUE SELECTIVITY**

Figure 1. The unique selectivity of HALO 160Å Phenyl-Hexyl enables different resolutions for tryptic digest fragments compared to HALO 160Å ES-C18 and HALO 160Å ES-CN.



#### **PEAK IDENTITIES:**

- 1. FTISADTSKNTAYLQMNSLR
- 2. LScAASGFNIKDTYIHWVR
- 3. GFYPSDIAVEWESNGQPENNYK 4. LLIYSASFLYSGVPSR
- 5. SGTASVVcLLNNFYPR
- 6. ScDKTHTcPPcPAPELLGGPSVFLFPPKPK
- 7. VVSVLTVLHQDWLNGKEYK

## **TEST CONDITIONS:**

**Column:** 2.1 x 100 mm

Top: HALO 160Å ES-CN, 2.7 µm

Middle: HALO 160Å Phenyl-Hexyl, 2.7 μm Bottom: HALO 160Å ES-C18, 2.7 µm

Part Numbers: **Top:** 92122-604

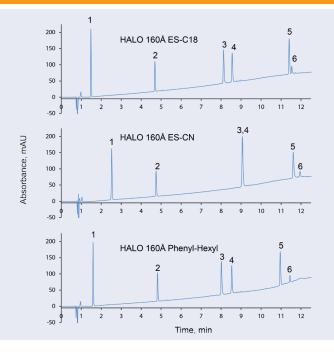
> Middle: 92112-606 Bottom: 92122-602

Mobile Phase A: Water/10 mM difluoroacetic acid Mobile Phase B: ACN/10 mM difluoroacetic acid

Gradient: 2-50% B in 60 min Flow Rate: 0.3 mL/min Temperature: 60 °C Detection: 220 nm Injection: 5 µL (0.2 mg/mL)

Sample: Trastuzumab Tryptic Digest

Figure 2. This figure demonstrates the utility of the unique selectivity of the 160Å Phenyl-Hexyl Peptide phase. The initial separation using HALO 160Å ES-C18 shows inadequate resolution of peaks five and six. The same separation was attempted on a HALO 160Å ES-CN column, which improved the resolution of peaks five and six, but resulted in coelution of peaks three and four. On the contrary, the HALO 160Å Phenyl-Hexyl column



### **TEST CONDITIONS:**

**Column:** 2.1 x 150 mm

**Top:** HALO 160Å ES-C18, 2.7 μm Middle: HALO 160Å ES-CN, 2.7 µm Bottom: HALO 160Å Phenyl-Hexyl, 2.7 µm

Part Numbers:

Top: 92122-702 Middle: 92122-704 Bottom: 92112-706

Mobile Phase A: 0.1% formic acid in water/10 mM ammonium formate

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

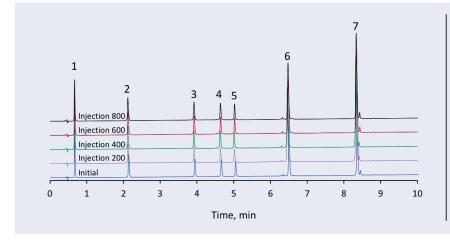
Gradient: 10-60% B in 15 min Flow Rate: 0.4 mL/min Temperature: 60 °C Detection: 220 nm Injection: 2 uL

Sample: (1) tyr-tyr-tyr, (2) angiotensin II, (3) angiotensin 1-12, (4) melittin, (5) sauvagine

and (6) B-endorphin

# **RUGGED STABILITY**

**Figure 3.** The rugged column stability under low pH and high temperature mobile phase conditions while using a sample containing peptides and small proteins is illustrated in Figure 3. HALO 160Å Phenyl-Hexyl columns can be run at a maximum temperature of 60 °C and with the low pH mobile phase conditions that are typically used for tryptic digests and polypeptides.



#### **TEST CONDITIONS:**

Column: 2.1 x 100 mm

HALO 160Å Phenyl-Hexyl, 2.7 µm

**Part Number:** 92112-606

Mobile Phase A: Water/0.1% TFA

Mobile Phase B: 70/30 ACN/water/0.1% TFA

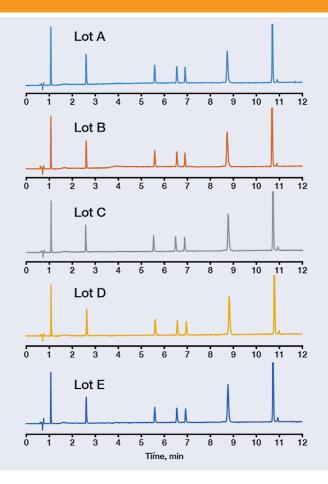
Gradient: 9-95% B in 10 min Flow Rate: 0.5 mL/min Temperature: 60 °C Detection: 220 nm Injection: 2 µL

Sample: (1) gly-tyr, (2) val-tyr-val, (3) methionine enkephalin, (4) angiotensin II, (5) leucine enkephalin,

(6) bovine RNase A and (7) bovine insulin

# **LOT-TO-LOT REPRODUCIBILITY**

**Figure 4.** The manufacturing process for HALO 160Å Phenyl-Hexyl bonded phase is tightly controlled to yield repeatable batches. Each lot is quality tested with a mix of peptides and small proteins to ensure lot-to-lot reproducibility. A lot-to-lot comparison is shown below in Figure 4.



#### **TEST CONDITIONS:**

**Column:** 4.6 x 100 mm

HALO 160Å Phenyl-Hexyl, 2.7 µm

Part Number: 92114-606

Mobile Phase A: 10/90 ACN/water/0.1% TFA Mobile Phase B: 70/30 ACN/water/0.1% TFA

Gradient: 0-50% B in 15 min Flow Rate: 1.5 mL/min Temperature: 30 °C Detection: 220 nm Injection: 5 µL

Sample: (1) gly-tyr, (2) val-tyr-val,

(3) methionine enkephalin, (4) angiotensin II, (5) leucine enkephalin, (6) bovine RNase A and

(7) bovine insulin

# **ACT NOW**

Contact your local distributor to be among the first to experience these new HALO® columns!

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# HALO 160Å Phenyl-Hexyl

| Dimension -<br>ID x length (mm) | Part No.  | Dimension -<br>ID x length (mm) | Part No.  |  |
|---------------------------------|-----------|---------------------------------|-----------|--|
| 2.1 x 20                        | 92112-206 | 3.0 x 100                       | 92113-606 |  |
| 2.1 x 30                        | 92112-306 | 3.0 x 150                       | 92113-706 |  |
| 2.1 x 50                        | 92112-406 | 3.0 x 250                       | 92113-906 |  |
| 2.1 x 75                        | 92112-506 | 4.6 x 20                        | 92114-206 |  |
| 2.1 x 100                       | 92112-606 | 4.6 x 30                        | 92114-306 |  |
| 2.1 x 150                       | 92112-706 | 4.6 x 50                        | 92114-406 |  |
| 2.1 x 250                       | 92112-906 | 4.6 x 75                        | 92114-506 |  |
| 3.0 x 20                        | 92113-206 | 4.6 x 100                       | 92114-606 |  |
| 3.0 x 30                        | 92113-306 | 4.6 x 150                       | 92114-706 |  |
| 3.0 x 50                        | 92113-406 | 4.6 x 250                       | 92114-906 |  |
| 3.0 x 75                        | 92113-506 |                                 |           |  |
|                                 |           |                                 |           |  |

# HALO 160Å Phenyl-Hexyl Guard Columns, 3/Pack

| Dimension -<br>ID x length (mm) | Part No.  |
|---------------------------------|-----------|
| 2.1 x 5                         | 92112-106 |
| 3.0 x 5                         | 92113-106 |
| 4.6 x 5                         | 92114-106 |
|                                 |           |
|                                 |           |
|                                 |           |
|                                 |           |
|                                 |           |
|                                 |           |
| Guard Column<br>Holder (1)      | 94900-001 |



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