

HALO<sup>®</sup>

Discover the Advantages of HALO and HALO BioClass Fused-Core<sup>®</sup> Columns



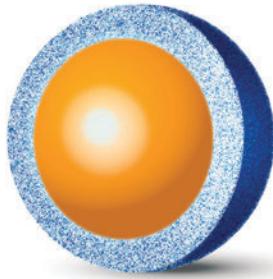
## SMALL MOLECULE



90 Å 2.0 micron particle



90 Å 2.7 micron particle



90 Å 4.6 micron particle

## BIOCLASS



160 Å 2.0 micron particle



160 Å 2.7 micron particle



160 Å 4.6 micron particle

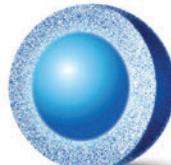
### PEPTIDE



1000 Å 2.7 micron particle



400 Å 3.4 micron particle



90 Å 2.7 micron particle

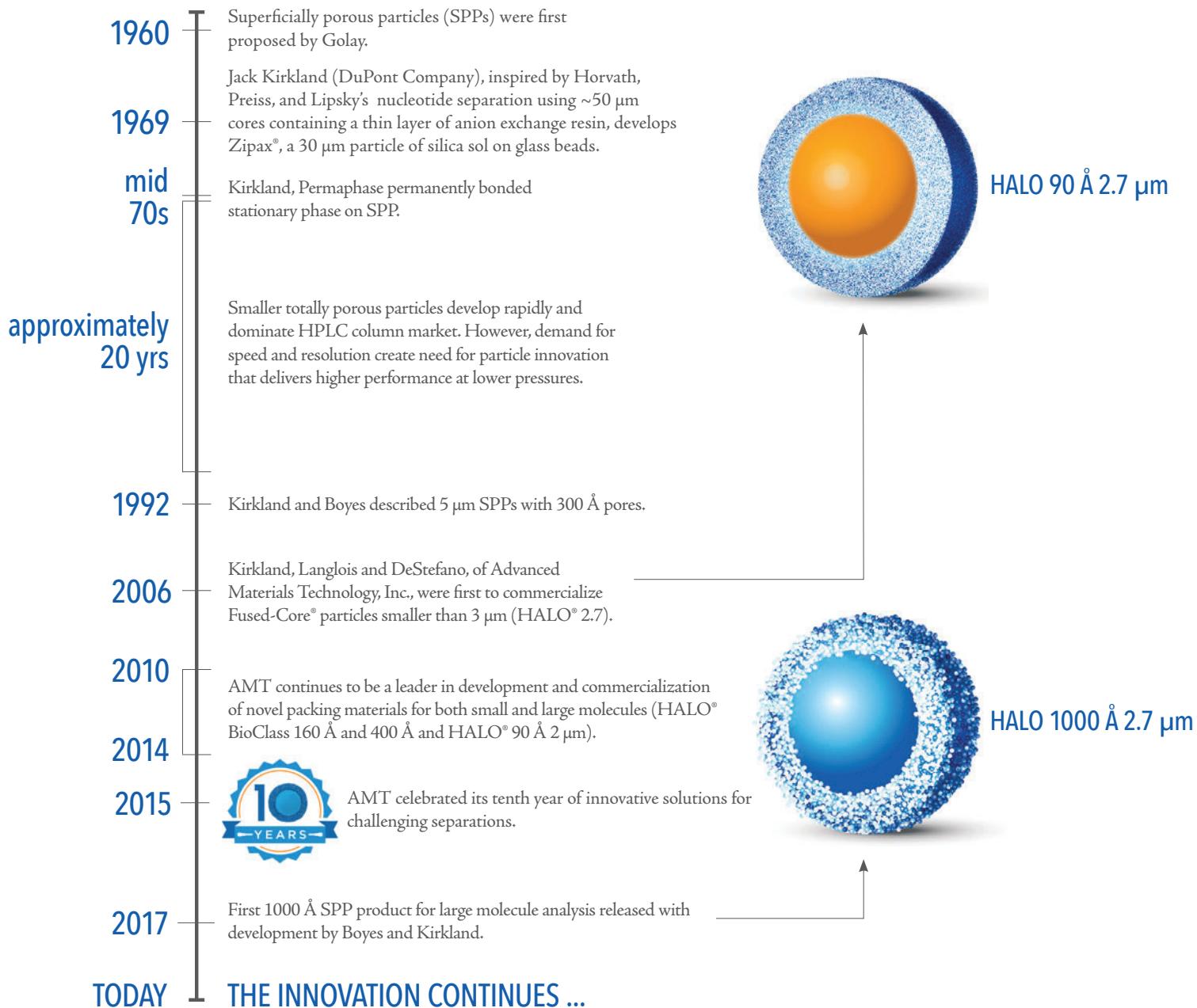
### PROTEIN

### GLYCAN

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# MILESTONES IN THE DEVELOPMENT OF FUSED-CORE PARTICLES



## SUMMARY:

• Dr. Joseph (Jack) Kirkland was involved in the development of HPLC packings, including porous and Fused-Core (SPP), throughout his distinguished career.

- Columns packed with these 2.7 µm particles created a revolution in HPLC technology.
  - Performance is comparable to the performance of sub-2 µm non-core particles, but with half the back pressure.
  - Analysts can obtain very high efficiencies and faster separations using their existing HPLC instruments, which may be limited to 400–600 bar.

# SUPERIOR PERFORMANCE OF HALO FUSED-CORE COLUMNS:

## HALO FUSED-CORE COLUMNS

HALO 2  $\mu\text{m}$  columns will deliver reliable high speed and high resolution separations at pressures lower than non-core sub-2  $\mu\text{m}$  columns.

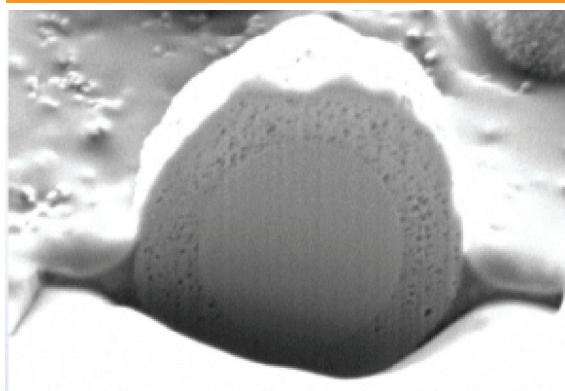
HALO 2.7  $\mu\text{m}$  columns can meet or exceed the performance of most non-core sub-2  $\mu\text{m}$  columns at pressures one-third to one-half the back pressure under the same conditions.

HALO 5  $\mu\text{m}$  columns match the performance of totally porous 3  $\mu\text{m}$  columns at roughly half the back pressure under the same conditions.

### Early Explanations for Superior Performance

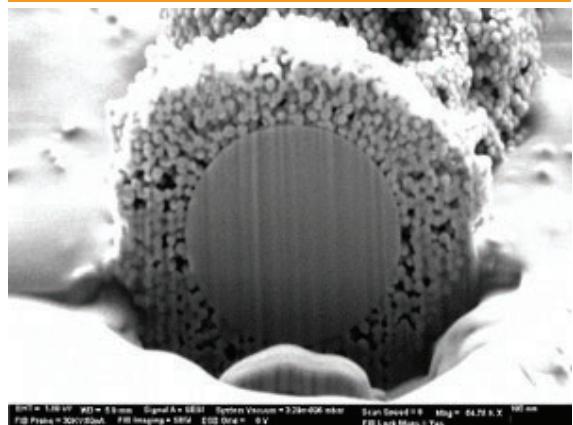
- *Faster Mass Transfer* due to a thin porous bonded-phase layer exterior to particle's solid silica core
- *More Uniform and Stable Column beds* due to very narrow particle size distribution (~4–6% RSD vs. ~20% RSD for non-core particles)

**Figure A.** FIB - SEM image of first commercial HALO particle with 2.7  $\mu\text{m}$  total size consisting of a 1.7  $\mu\text{m}$  solid silica core and a 0.5  $\mu\text{m}$  shell.



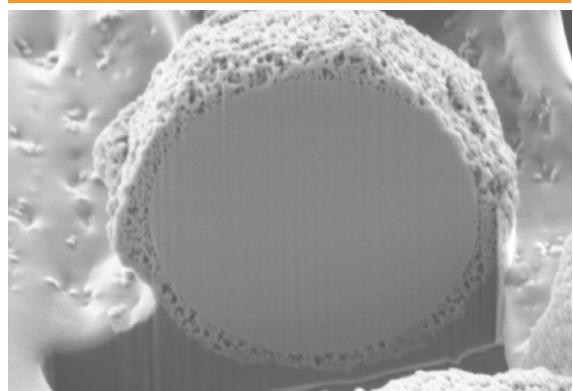
Beam = 1.00 kV WD = 8.0 mm Signal A = SEI System Vacuum = 0.000000 mbar Scan Speed = 0 Angl = 47.06 X, 200 nm  
FIB Probe = 50kV/50pA FIB Imaging = SEM ESB Grid = 0.09 V FIB Lock Mag = Yes

**Figure B.** SEM image of a focused-ion beam cleaved HALO 1000 Å 2.7  $\mu\text{m}$  silica particle. This "cut-away" view shows the solid core and shell with large pores allowing unrestricted access of macromolecules to the bonded phase.



Beam = 1.00 kV WD = 5.0 mm Signal A = SEI System Vacuum = 0.000000 mbar Scan Speed = 0 Angl = 64.79 X, 200 nm  
FIB Probe = 50kV/50pA FIB Imaging = SEM ESB Grid = 0.09 V FIB Lock Mag = Yes

**Figure C.** SEM image of a focused-ion-beam-cleaved HALO Protein 3.4  $\mu\text{m}$  silica particle. This "cut-away" view shows the solid core with its very thin 0.2  $\mu\text{m}$  outer porous layer.



Beam = 1.00 kV WD = 4.0 mm Signal A = SEI System Vacuum = 2.01e-004 Pa Scan Speed = 0 Angl = 23.62 X, 200 nm  
FIB Probe = 50kV/50pA FIB Imaging = SEM ESB Grid = 0.09 V FIB Lock Mag = Yes

## Understanding SPP Performance (Figure D)

The superior performance of Fused-Core SPP columns is now believed to be due to:

- Reduction in eddy diffusion
  - 40% smaller van Deemter "A term" due to more uniform analyte flow paths through the column bed
- Much lower longitudinal broadening, flat van Deemter plot and higher optimum linear velocity (flow rate)
  - Due to the presence of the particle's solid core (25–30% smaller van Deemter "B term")
- Much smaller reduced plate heights and high efficiencies for SPP columns due to smaller van Deemter A and B terms for SPP particles

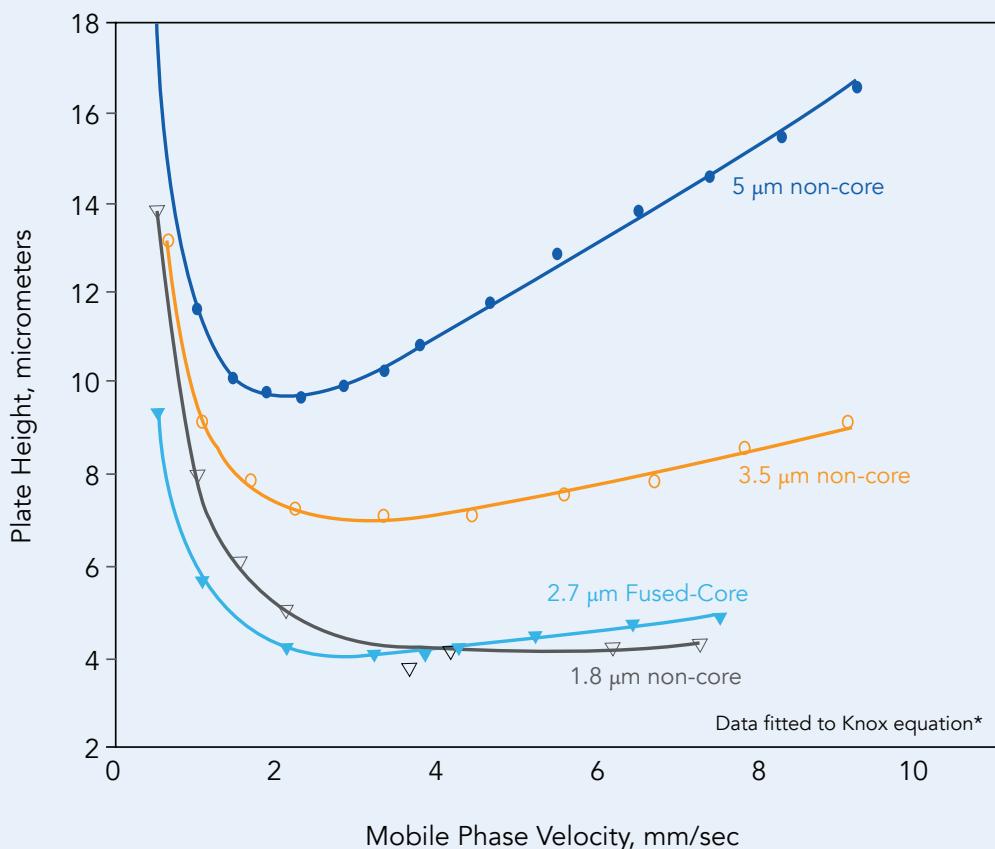
**Figure D. van Deemter Plot of Plate Height vs. Linear Velocity (flow rate)**

### Effect of Particle Size and Type

Column Dimensions: 4.6 x 50 mm, Non-core C18, 5  $\mu\text{m}$ ; Non-core C18, 3.5  $\mu\text{m}$ ;

Non-core C18, 1.8  $\mu\text{m}$ ; HALO C18, 2.7  $\mu\text{m}$

Solute: naphthalene; mobile phase: 60% ACN/40% water, 24 °C



$$H = A + \frac{B}{\mu} + C\mu$$

H = height equivalent to theoretical plate  
 A = eddy diffusion term  
 B = longitudinal diffusion term

C = resistance to mass transfer term  
 $\mu$  = mobile phase linear velocity ( $L/t_0$ )

van Deemter Equation

\*G.J. Kennedy, J.H. Knox, J. Chromatogr. Sci 10 (1972) 549.

# KEY ADVANTAGES OF HALO FUSED-CORE COLUMNS

## HALO FUSED-CORE PERFORMANCE

### High Speed Separations (Figures F and G)

- Smaller reduced plate heights lead to high efficiencies; narrower and taller peaks, for improved resolution and lower detection limits (LODs and LOQs)

- Flat van Deemter plot and higher linear velocity optimum (Figure D, page 3) allow higher flow rates with minimal column efficiency loss

### High Resolution Separations (Figures E and H)

- High efficiency with longer geometries (100, 150, 250 mm) provides greater resolving power for challenging applications
- Lower back pressure permits columns to be used in series for the most demanding UHPLC and HPLC separations

### Excellent Ruggedness and Reproducibility

- Less plugging, longer usable column lifetime and greater uptime due to larger porosity frits (vs. sub-2 µm totally porous (non-core) columns)
  - 2 µm frits for HALO 2.7 µm and 5 µm columns
  - 1 µm frits for HALO 2 µm columns vs. 0.2–0.5 µm frits for sub-2 µm non-core columns

- Excellent column-to-column and lot-to-lot reproducibility thanks to tight manufacturing controls

- Robust pores in multiple sizes for a tailored application solution (90 Å, 160 Å, 400 Å and 1000 Å)

## HALO BIOCLASS

### Solutions for Proteins, Peptides and Glycans

- Application specific columns for bioseparations that outperform non-core columns

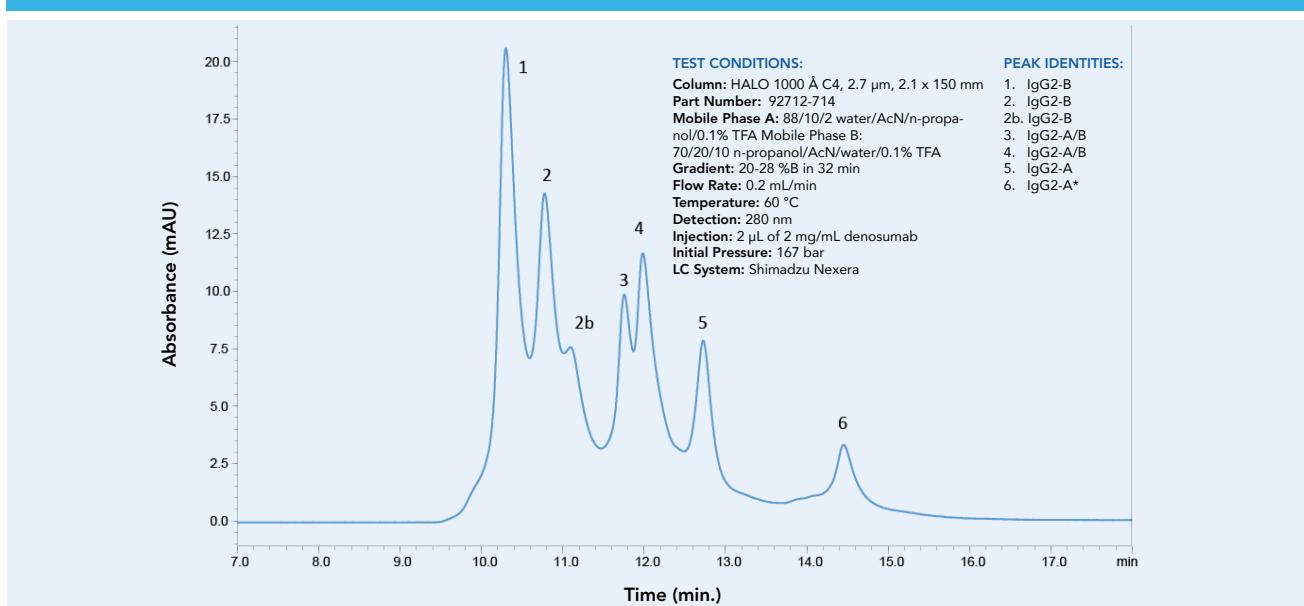
- Up to 1/2 the back pressure

- Offer better peak shape and peak capacity

- Breakthrough 1000 Å pore particles for large molecule enablement

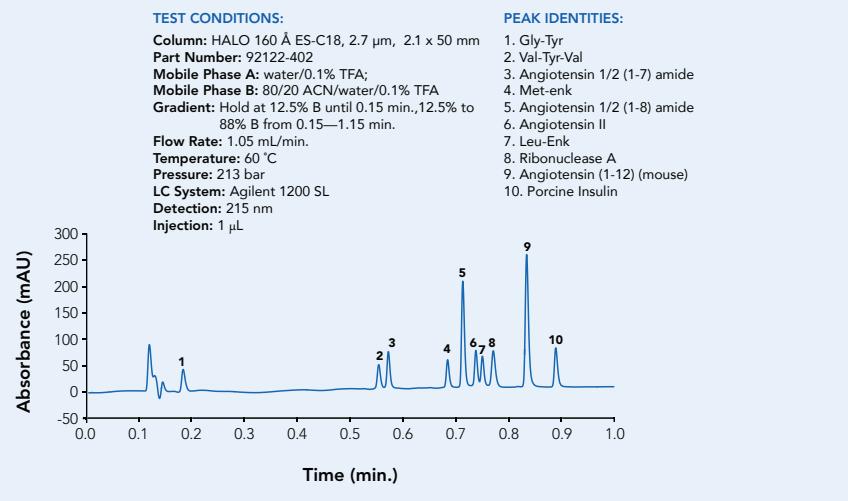
**Figure E.** High Resolution of IgG2 with HALO 1000 Å C4

Very high resolution separations are achieved with HALO 1000 Å C4 for a complex IgG2 such as denosumab. The assignments are based on non-reduced Lys-C digestion mapping.



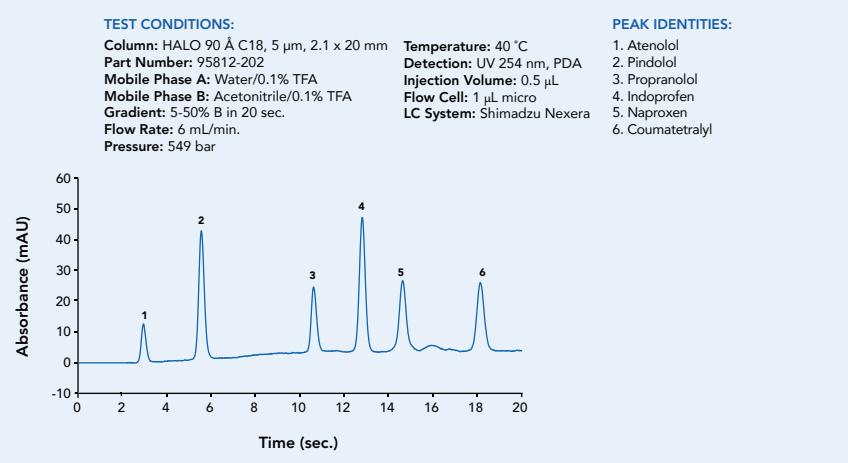
## ULTRAFAST PEPTIDE SEPARATION

**Figure F.** Separation of a 10 peptide mixture is accomplished in less than one minute using a HALO Peptide ES-C18 column on a delay-volume minimized and optimized Agilent 1200 system.



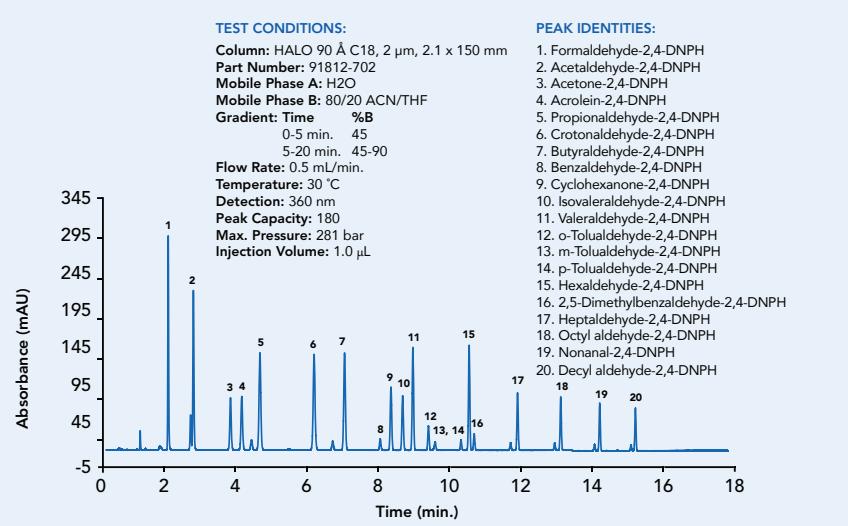
## ULTRAFAST BALLISTIC GRADIENT USING HALO 5 µm

**Figure G.**  
 Many researchers have found HALO 5 µm columns in 2.1 mm ID to be very useful for high-throughput, ballistic separations by LC and LC-MS.



## CARBONYL-DNPH HIGH RESOLUTION SEPARATION

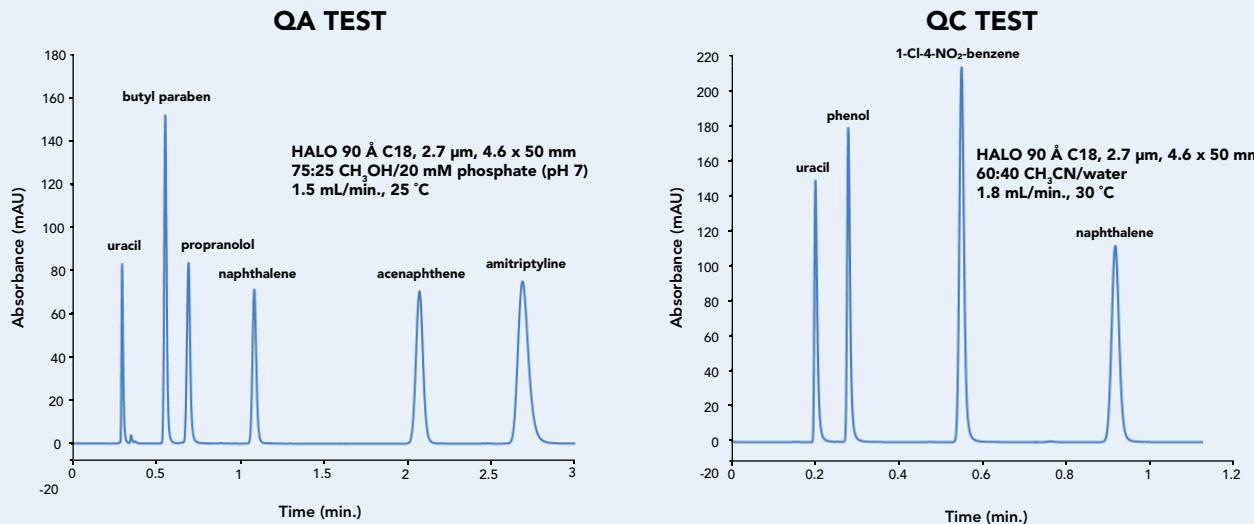
**Figure H.** Environmental samples can be quite complex as demonstrated by this gradient separation of dinitrophenylhydrazone (DNPH) carbonyl compound derivatives using a HALO 90 Å C18, 2 µm, 2.1 x 150 mm column.



# HALO QUALITY PROMISE: PERFORMANCE AND REPRODUCIBILITY – EVERY TIME

As the originators of Fused-Core particles, Advanced Materials Technology incorporates the most knowledge in the industry to bring high-quality innovative products to our customers. Our principal scientists have over 150 years of combined experience in liquid chromatography, particle synthesis and column manufacturing.

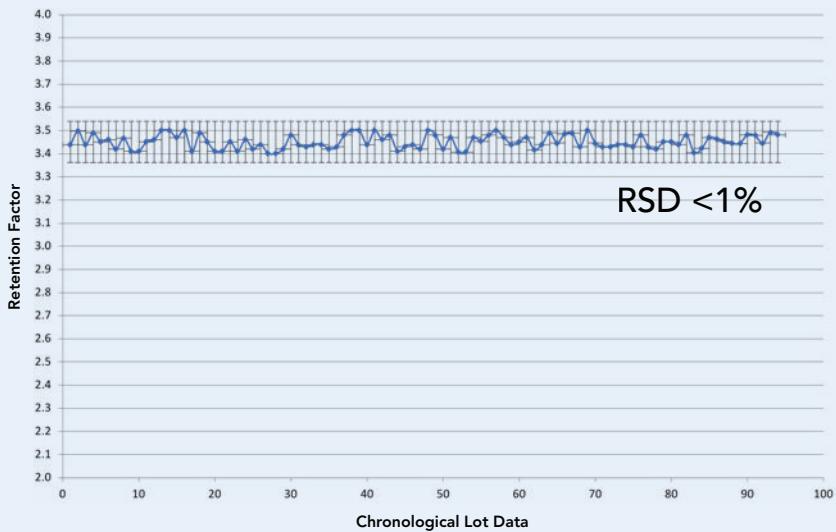
**Figure I.** Consistent reproducible performance from column to column and lot to lot is ensured because of well-designed processes and practices in the manufacture of HALO Fused-Core particles, HALO phases and HALO columns. Representative chromatograms of QA and QC tests are shown below, along with a historical plot of selectivity between a neutral and basic analyte.



## REPRODUCIBLE PERFORMANCE OVER TIME

**Figure J.** Advanced Materials Technology (AMT) is one of only a few HPLC column manufacturers that completes the entire column manufacturing process in-house. The scientists and engineers at AMT have expertise in every aspect of the column development process. Every step that comprises the creation of a HALO column is monitored and controlled. From the solid silica cores to the bonded Fused-Core particles to the final loaded and QC-tested column, customers can be confident that the HALO products they receive are reliable and reproducible. The graph demonstrates the superior reproducibility of the retention of HALO 90 Å C18, 2.7 µm columns over a 10-year period.

## HALO Reproducibility Data for 10 Years (QA Retention Factor)



# SELECTING THE APPROPRIATE PORE SIZE

AMT tailors pore sizes to your challenging separations. So how do you choose the correct one?

- Match the column pore size according to your molecule size and the range of molecular weights (MWs) of the analytes in your sample (Table A)
- Small molecules (< 5000 Da) are usually analyzed using HALO 90 Ångstrom columns
  - Packing materials with smaller pores have greater surface area, which allows improved retention and loading capacity for lower MW analytes
  - When an analyte is too large for the pores, restricted diffusion can occur, which can lead to peak broadening and reduced retention
- For macrocyclic antibiotics and biomolecules such as peptides and proteins, use larger pore sizes such as HALO 160 Å Peptide and HALO 400 Å Protein BioClass columns
- For mAbs and intact proteins of molecular sizes > 50 kDa, consider the HALO 1000 Å products

Table A. Guidance for Pore Size Selection

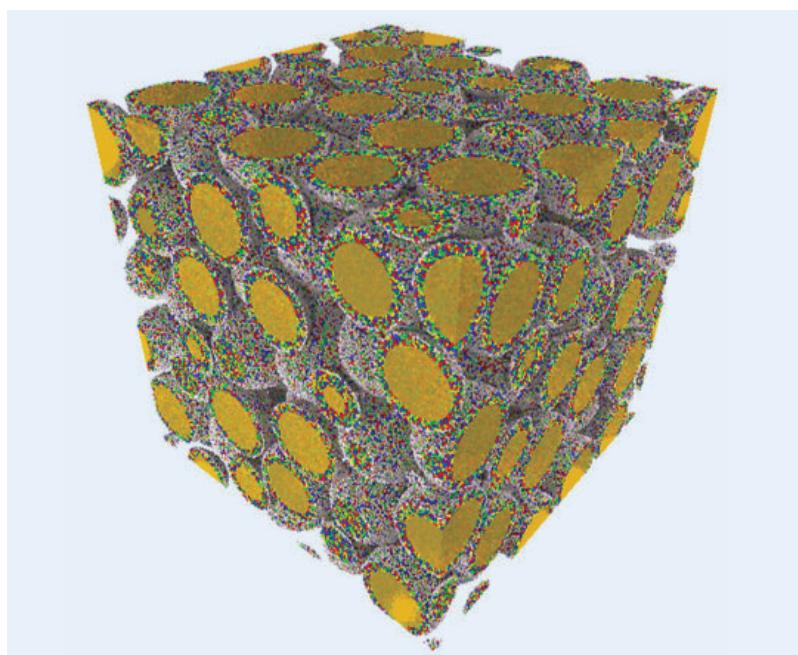
Molecule Size	Pore Size (Å)	Application	Particle Sizes (µm)	Column Family
SMALL (<5000 Da)	90	Small Molecules	2, 2.7, 5.0	HALO BIOCLASS
SMALL (< 20 kDa*)	90	Glycan	2.7	
MEDIUM (100 Da < MW < 15 kDa)	160	Peptide	2, 2.7, 5.0	
LARGE (2 kDa < MW < 500 kDa)	400	Protein	3.4	
LARGE (> 50 kDa)	1000		2.7	

\* for glycans, glycopeptides and glycoproteins

## QUALITY BY DESIGN

**Figure K.** HALO particles are manufactured with quality by design in mind. AMT tightly controls the manufacturing process through the use of control charts and in-process monitoring. The particles are designed with target core sizes, shell thicknesses and pore sizes that have been determined to be the best compromise of each of these variables. The narrow particle size distribution of HALO Fused-Core particles is one of the features that sets the columns apart from columns of fully porous particles. This image shows a simulation of a packed bed of HALO wide pore particles. Notice the solid silica cores in yellow and the porous shell in multicolors.

M. R. Schure, R. S. Maier, T. J. Shields, C. M. Wunder, B. M. Wagner Intraparticle and interstitial flow in wide-pore superficially porous and fully porous particles, Chemical Engineering Science 174 (2017) 445–458.



# HALO COLUMNS FOR SMALL MOLECULE ANALYSES

Of the three variables in the general resolution equation, including efficiency ( $N$ ) and retention ( $k$ ), **selectivity ( $\alpha$ )** is the most powerful parameter for adjusting and improving resolution between peaks in a chromatographic separation.

EFFICIENCY

SELECTIVITY

RETENTION

$$R_s = \left( \frac{\sqrt{N}}{4} \right) x \left[ \frac{(\alpha - 1)}{\alpha} \right] x \left[ \frac{k_2}{(1 + k)} \right]$$

where

$$\bar{k} = \frac{(k_1 + k_2)}{2}, \quad \alpha = \frac{k_2}{k_1} \quad \text{and} \quad N = \frac{L}{H} = \frac{L}{h \times d_p}$$

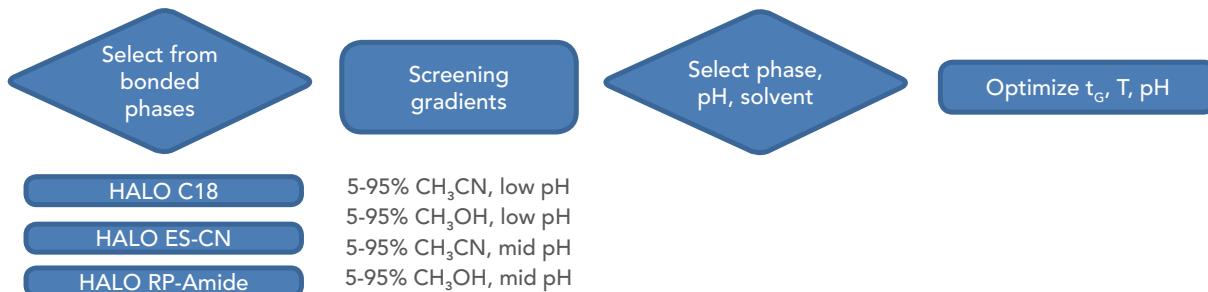
Moreover, column phase selectivity is one of the four most powerful and useful parameters for adjusting HPLC separation selectivity (see Table B). For ionizable analytes, mobile phase pH is, by far, the most effective parameter. However, column stationary phase is comparable to organic modifier choice (acetonitrile vs. methanol) and percent organic modifier/gradient steepness in its ability to change relative retention for UHPLC and HPLC separations. When developing a method, there are multiple ways to achieve a separation that meets specific resolution and retention requirements. One way is to take a systematic approach and screen multiple phases. HALO columns are available in several different stationary phases for various types of analyses. The HALO phases that are available for reversed-phase separations of small molecules are shown in Table C, and the phases are listed according to their differences in selectivity compared to HALO C18 at both pH 2.8 and pH 7. For example, if you were looking for a column with a different selectivity to a HALO C18 column at low pH, you might consider Table C and select a HALO PFP

column as one most likely to be orthogonal to C18. However, the other available HALO phases (Phenyl-Hexyl, ES-CN, Biphenyl, RP-Amide) also retain and separate analytes via retention mechanisms different from HALO C18, HALO C8 and HALO AQ-C18, so it might be prudent to consider one or more of the former phases as part of a comprehensive column screening or method development strategy (Figure L). Another approach to method development is to use trial and error with columns that have similar bonded phases, such as HALO C18 and HALO AQ-C18. According to Table C, these phases are not very orthogonal to each other, but the polar aspects of HALO AQ-C18 may be needed for retention of polar analytes.

Table B. Parameters That Affect HPLC Selectivity in Order of Increasing Effectiveness (Refs. 1 and 2)

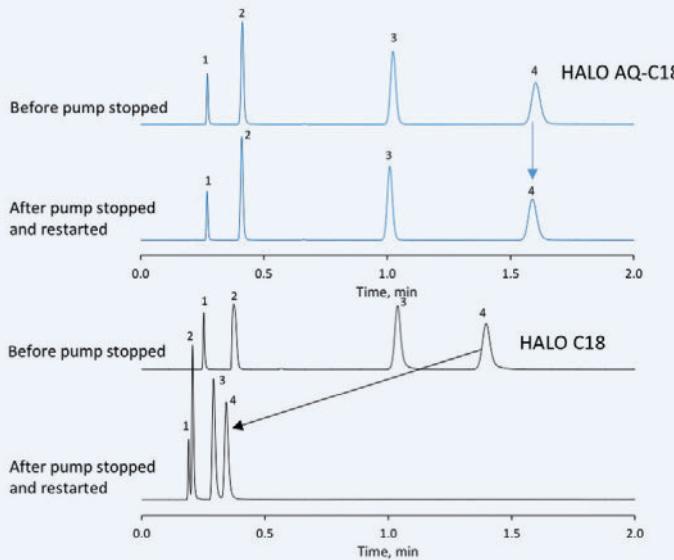
HPLC Parameter	Effectiveness for Changing Selectivity
Mobile phase pH (ionizable analytes only)	Most Effective
Organic modifier choice	
Percent organic modifier or gradient steepness	
Column stationary phase	
Column temperature	
Buffer choice	
Buffer concentration	Least Effective

Figure L. Example Strategy for Comprehensive Method Development Using Multiple HALO Stationary Phases and Column/Condition Screening, Followed by Optimization of Gradient Time, Temperature and pH



## RESISTANCE TO DEWETTING

**Figure M.** The unique polar modified bonded phase of HALO AQ-C18 enables it to be run in 100% aqueous mobile phase without experiencing loss in retention due to dewetting when pressure is relieved. The retention is nearly 100% maintained compared to the HALO C18 after the pump is stopped and restarted.



### TEST CONDITIONS:

**Column:** 4.6 x 50 mm

**Top:** HALO 90Å AQ-C18, 2.7 µm

**Bottom:** HALO 90Å C18, 2.7 µm

### Part Numbers:

**Top:** 92814-422

**Bottom:** 92814-402

**Mobile Phase:** 100% 20 mM Potassium Phosphate buffer, pH 7

**Flow Rate:** 2 mL/min

**Temperature:** 30 °C

**Detection:** 254 nm

**Injection:** 0.5 µL

**Sample:** (1) thiourea, (2) 5-fluorocytosine, (3) adenine and (4) thymine

Another item that must be considered during method development is phase dewetting. Dewetting occurs when the stationary phase is highly hydrophobic and the mobile phase is changed from one with a high amount of organic solvent component (> 40% ACN or MeOH) to one that is entirely aqueous or mostly aqueous. When the column is under pressure, the aqueous mobile phase is forced into the porous structure where most of the retention occurs. When the pump is stopped, the aqueous mobile phase is no longer forced into the packing pores and is expelled from the interior of the particles. Restarting the pump will not force the aqueous mobile phase

back into the pores since the phase is hydrophobic. The retention of the sample components drastically decreases and resolution is lost. Figure M demonstrates what happens to a separation when dewetting occurs with HALO C18. In contrast, HALO AQ-C18 phase has an added amount of polar characteristic that prevents it from dewetting as shown in Figure M. Even when the pump is stopped and restarted, the retention and resolution are both maintained with the HALO AQ-C18 column. All of the HALO phases except HALO C18 may be used under 100% aqueous conditions without dewetting.

Table C. Orthogonality of HALO Phases

	pH 2.8	pH 7
Most Similar	HALO C18	HALO C18
	HALO C8	HALO C8
	HALO AQ-C18	HALO AQ-C18
	HALO Phenyl-Hexyl	HALO PFP
	HALO ES-CN	HALO Phenyl-Hexyl
	HALO Biphenyl	HALO Biphenyl
	HALO RP-Amide	HALO ES-CN
Most Orthogonal	HALO PFP	HALO RP-Amide

# HALO COLUMNS FOR SMALL MOLECULE SEPARATIONS

Table D. HALO Small Molecule Column Specifications

Bonded Phase	USP Designation	Particle Size(s) ( $\mu\text{m}$ )	Carbon Load (%)	Surface Area ( $\text{m}^2/\text{g}$ )	Low pH/T Limit	High pH/T Limit	Endcapped
C18	L1	2	7.2	120			
		2.7	7.7	135	2/60 °C	9/40 °C	
		5	6.4	90			Yes
AQ-C18	L1	2	6.5	120			
		2.7	6.7	135	2/60 °C	9/40 °C	
		5	5.6	90			Yes
C8	L7	2	4.8	120			
		2.7	5.4	135	2/60 °C	9/40 °C	
		5	3.7	90			Yes
Phenyl-Hexyl	L11	2	6.3	120			
		2.7	7.1	135	2/60 °C	9/40 °C	
		5	5.2	90			Yes
Biphenyl	L11	2.7	7.0	135	2/60 °C	9/40 °C	Yes
PFP	L43	2	5.3	120			
		2.7	5.5	135	2/60 °C	8/40 °C	
		5	3.9	90			Yes
ES-CN	L10	2	3.4	120			
		2.7	3.5	135	1/80 °C	8/40 °C	
		5	2.5	90			Yes
RP-Amide	L60	2	7.3	120			
		2.7	8.2	135	2/60 °C	9/40 °C	
		5	5.1	90			Yes
HILIC	L3	2		120			
		2.7	Unbonded	135	1/60 °C	8/40 °C	N.A.
		5		90			
Penta-HILIC	L95	2	2.8	120			
		2.7	3.2	135	2/60 °C	9/40 °C	No
		5	2.1	90			

# HALO COLUMNS FOR SMALL MOLECULE SEPARATIONS

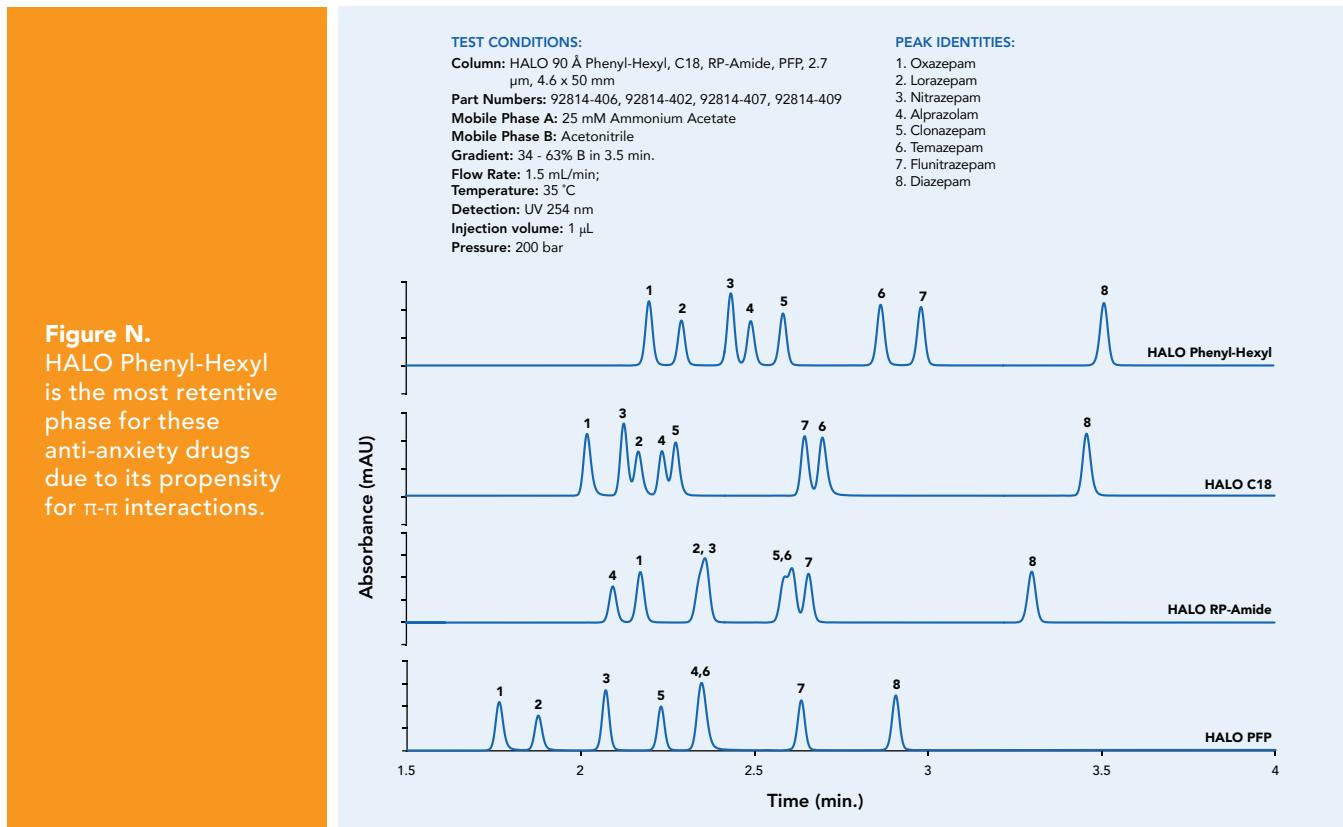
Table E. HALO Phases: Features and Benefits, Target Analytes and Best Applications

Bonded Phase	Features and Benefits	Target Analytes	Best Applications
C18 (dimethyloctadecylsilane)	+ Excellent performance for broad range of analyte polarities	Diverse analytes ranging from polar to non-polar	+ Pharmaceutical + Environmental + Cannabinoid + General purpose
AQ-C18 (polar modified)	+ Resistant to dewetting, making it 100% aqueous mobile phase compatible + Enhanced retention for polar molecules	Acids, bases, polar analytes	+ Pesticides + Nucleobases + Neurotransmitters + Polar acids
C8 (dimethyloctylsilane)	+ Excellent performance for broad range of analyte polarities	Diverse analytes ranging from polar to non-polar	+ Pharmaceutical + Environmental + Higher hydrophobic compounds
Phenyl-Hexyl (dimethylphenyl-hexylsilane)	+ Complementary selectivity to alkyl phases + Enhanced selectivity for stereoisomers	Electron-poor molecules, aromatic or unsaturated compounds (ketones, nitriles, alkenes)	+ Benzodiazepines + Aromatics + Drugs of abuse
Biphenyl (dimethylbiphenyl)	+ Complementary selectivity to alkyl phases + Enhanced selectivity for aromatic compounds	Electron-poor molecules, aromatic or unsaturated compounds (ketones, nitriles, alkenes)	+ Aromatic + Heterocycles + Drugs of abuse + Pain management drugs + Highly aqueous conditions
PFP (pentafluorophenylpropylsilane)	+ Complementary selectivity to alkyl phases + Enhanced selectivity for stereoisomers + Can be used in RPLC and HILIC modes	Electron-rich compounds, aromatics, unsaturated compounds with double and/or triple bonds	+ Steroids + Isomeric compounds + Substituted aromatics
ES-CN (diisopropylcyanopropylsilane)	+ Complementary selectivity to alkyl phases + More retention for polar analytes and much less retention for non-polar analytes	Polar and very polar bases, acids and neutrals	+ Explosives + Aromatics + Polar compounds
RP-Amide (C16 amide)	+ Complementary selectivity to alkyl phases + Enhanced stability for minimum bleed and long life	Alcohols, acids, phenols and catechins	+ Phenols + Alcohols + Catechins
HILIC (bare silica)	+ Can be used in HILIC and normal-phase modes	Polar and very polar bases, acids and neutrals, especially with $\log P < 0.5$	+ Polar compounds
Penta-HILIC (proprietary penta-hydroxy ligand)	+ Ideal for separation of highly polar compounds that are poorly retained in RPLC	Polar analytes with Log P values near or less than 0	+ Polar basic compounds

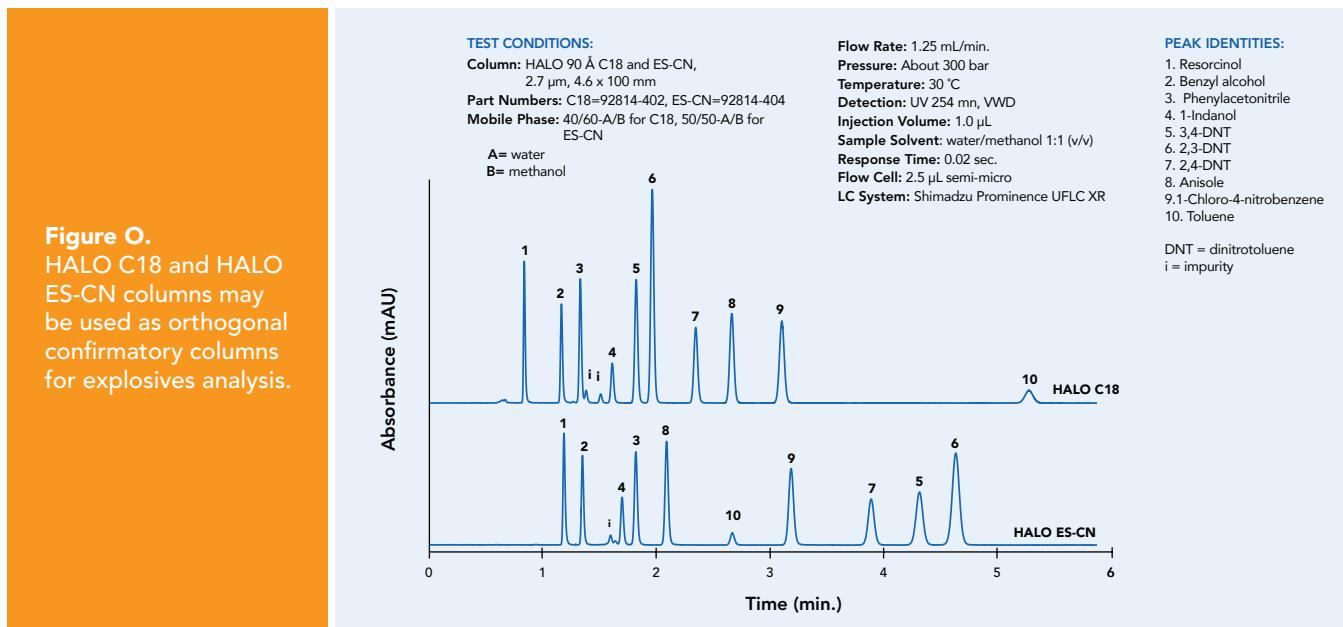
# REVERSED-PHASE SEPARATIONS WITH HALO

To illustrate the selectivity differences among the various HALO RPLC phases, the following examples are provided.

## BENZODIAZEPINES ON HALO FUSED-CORE BONDED PHASES

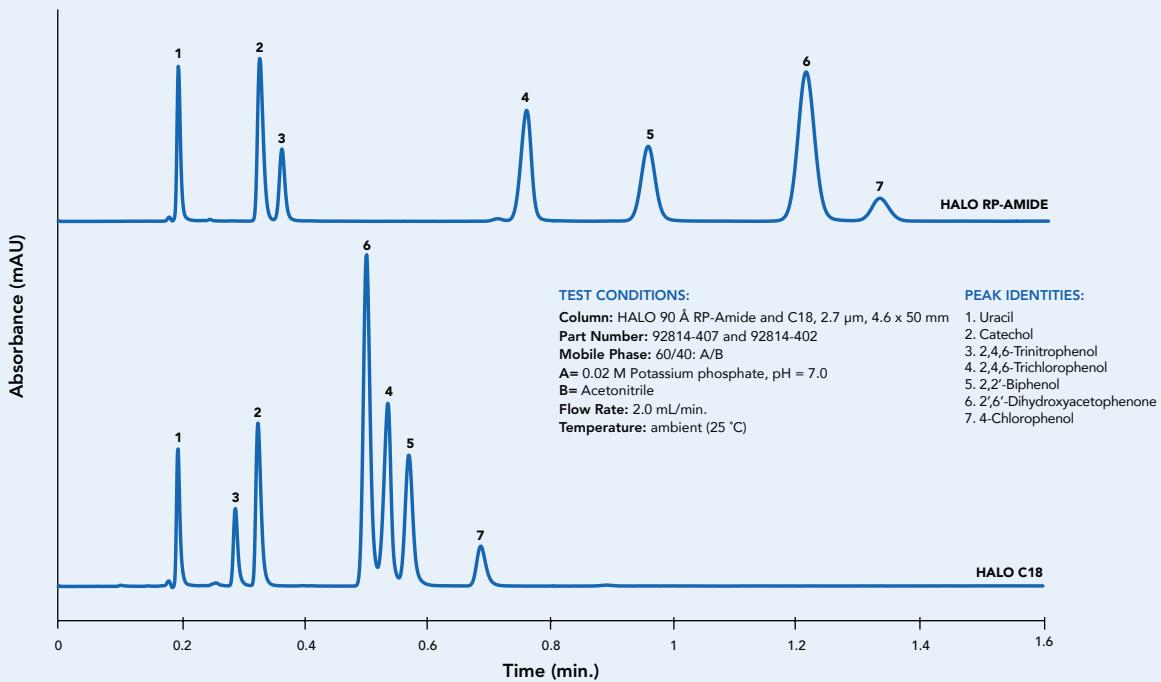


## AROMATIC AND NITROAROMATIC COMPOUNDS



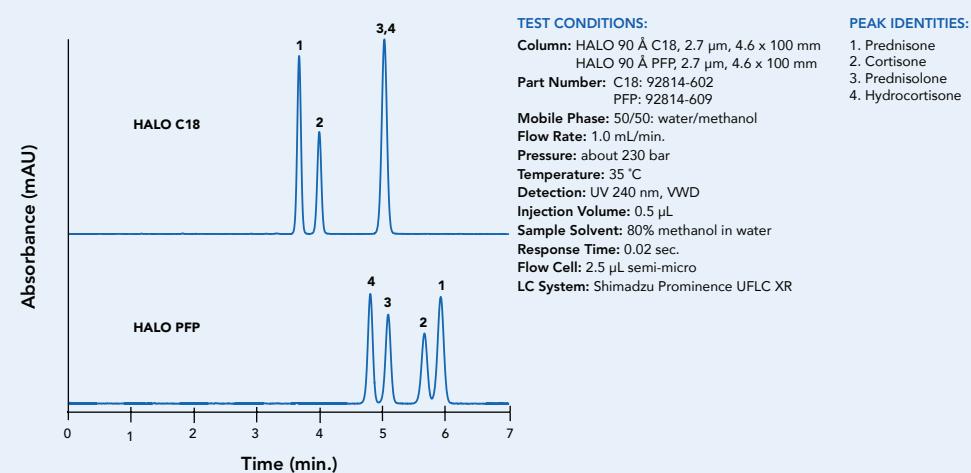
## HALO C18 VS. RP-AMIDE FOR PHENOLICS

**Figure P.** HALO RP-Amide provides greater retention and resolution compared to HALO C18 for this phenol mixture.



## SEPARATION OF STRUCTURALLY SIMILAR STEROIDS ON HALO C18 AND PFP

**Figure Q.**  
HALO PFP delivers improved resolution and different elution order compared to HALO C18 for this mixture of steroids.



# HILIC SEPARATIONS WITH HALO

Hydrophilic interaction liquid chromatography (HILIC) is a useful UHPLC and HPLC mode for the following situations:

- Polar analytes that are poorly or not retained in RPLC
- Basic analytes that have poor peak shape (overloading) and/or poor retention at low pH in RPLC
- Analytes that have log P values near or less than zero
- When conditions orthogonal to RPLC mode are needed (elution order change)

HALO columns are currently available in two different phases for HILIC separations:

- HALO HILIC
- HALO Penta-HILIC

**HALO HILIC** is a Fused-Core silica phase that can be used either in HILIC mode or in normal-phase mode with water-immiscible solvents (NPLC).

**HALO Penta-HILIC** is a bonded silica phase, which has a highly polar ligand with 5 hydroxyl groups tethered via novel proprietary linkage chemistry to Fused-Core silica particles.

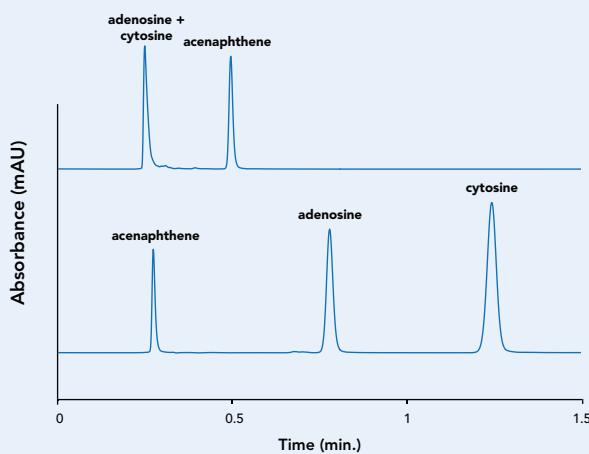
## Some Typical Analytes for HILIC Separations

- Basic pharmaceuticals
- Peptides
- Polar organic acids
- Catecholamines and other neurotransmitters
- Nucleosides and nucleobases
- Drug glycoside and glucuronide metabolites
- Mono-, di-, tri- and other oligosaccharides
- Opiates
- Glycosylceramides
- Polar triazines and pyrimidines
- Analytes from metabolomic profiling

For more information on HILIC separations, please see references 7-10 on page 31.

## RETENTION ORDER REVERSAL AND IMPROVED RETENTION WITH HILIC

**Figure R.**  
You can often obtain a complete reversal in elution order and different selectivity using HILIC mode compared to reversed-phase mode under the same or appropriate conditions.



### TEST CONDITIONS:

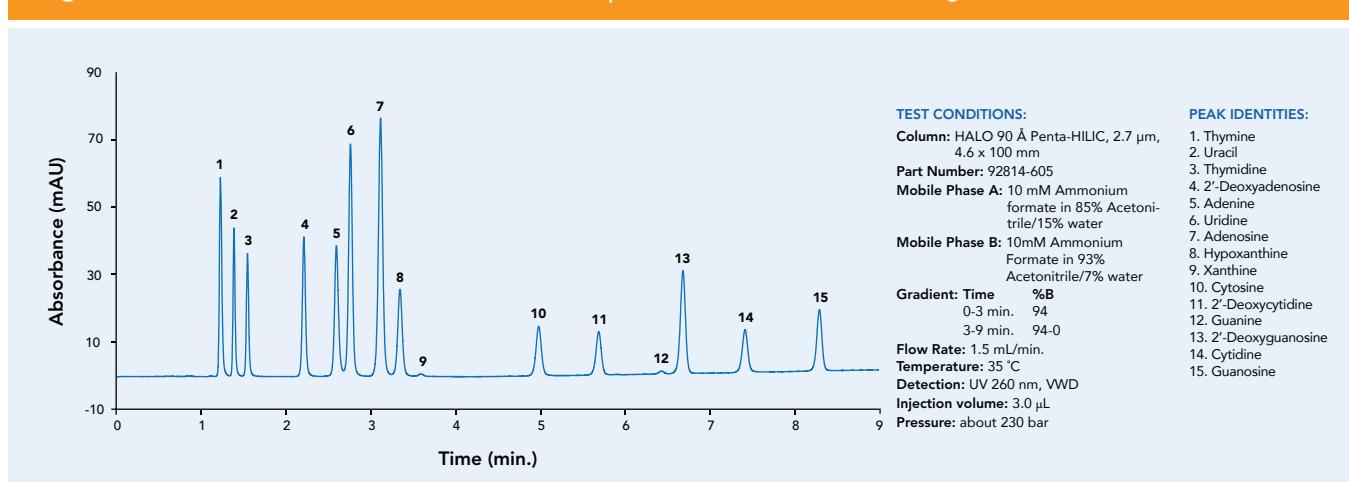
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm  
Part Number: 92814-402  
Mobile Phase A: 90/10 ACN/0.1 M Ammonium Formate  
Flow Rate: 1.8 mL/min.  
pH: 3.0

### TEST CONDITIONS:

Column: HALO 90 Å HILIC, 2.7 µm, 4.6 x 50 mm  
Part Number: 92814-401  
Mobile Phase A: 90/10 ACN/0.1 M Ammonium Formate  
Flow Rate: 1.8 mL/min.  
pH: 3.0

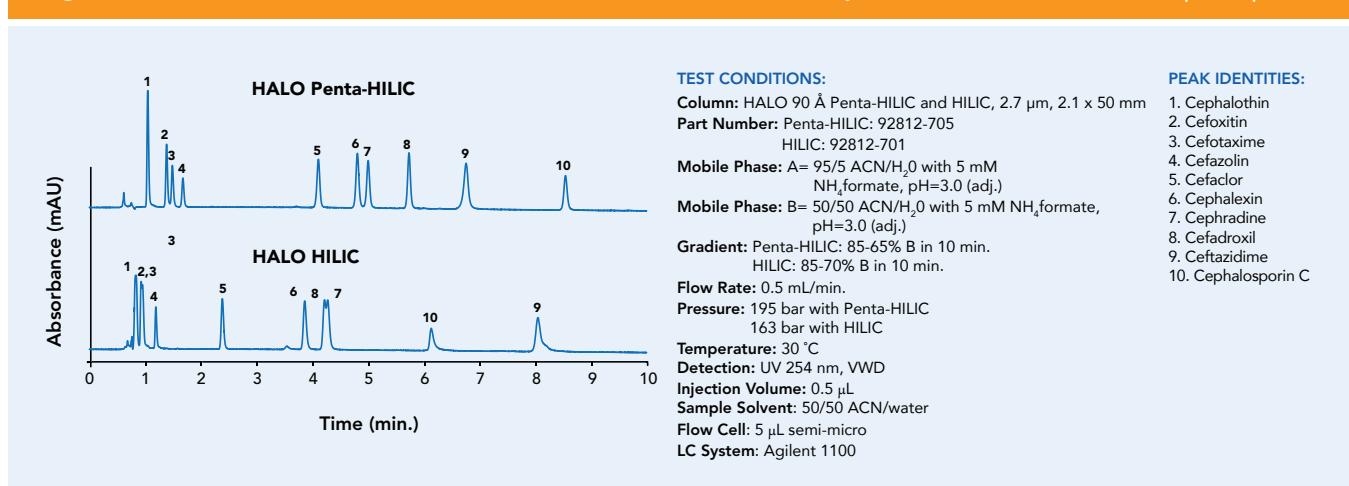
## NUCLEOSIDES AND NUCLEOBASES ON HALO PENTA-HILIC

**Figure S.** These 15 nucleosides and nucleobases are separated in under 10 minutes using a HALO Penta-HILIC column.



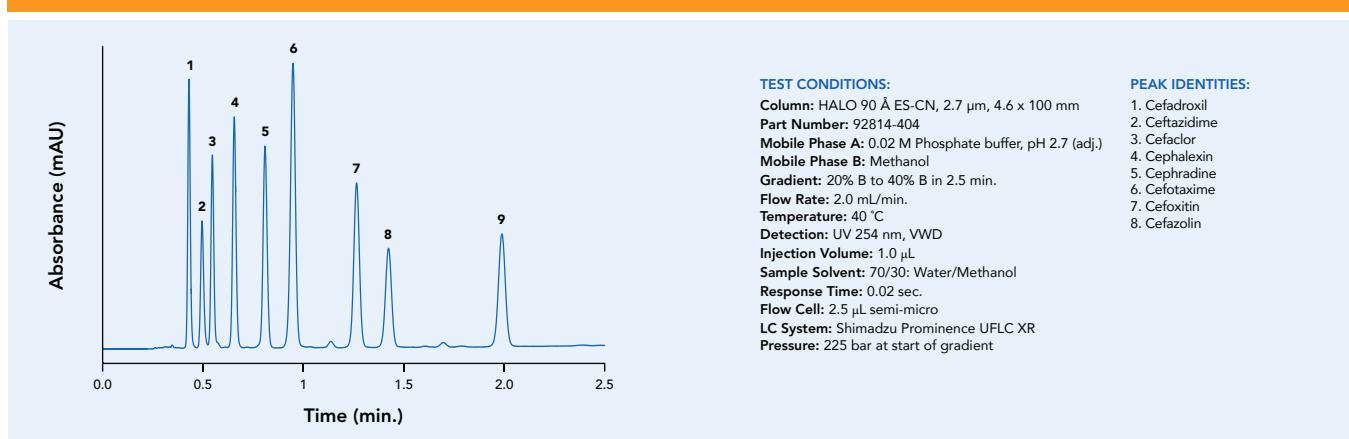
## CEPHALOSPORINS ON HALO PENTA-HILIC AND HALO HILIC

**Figure T.** HALO Penta-HILIC shows increased retention and different selectivity vs. HALO HILIC for these 10 cephalosporins.



## REVERSED-PHASE SEPARATION OF CEPHALOSPORINS USING HALO ES-CN

**Figure U.** HALO HILIC and Penta-HILIC columns often offer an orthogonal separation relative to reversed-phase separations, as shown here for HALO ES-CN for a subset of the same cephalosporins shown in Figure T.





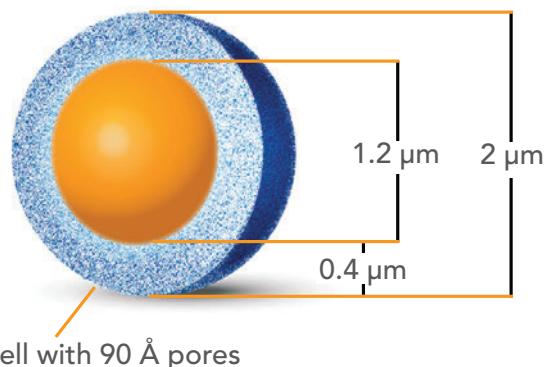
## HALO 90 Å 2 µm (UHPLC)

Highest UHPLC performance possible without the disadvantages of sub-2 µm columns

- Use when the highest efficiency is needed
- Excellent for fast method development and column/condition screening
- Best performance obtained with instrumentation having extracolumn volume (IBW < 10 µL)
- Ruggedness for R&D
- 1 µm inlet frit
- Pressure limit, 1000 bar/14,500 psi

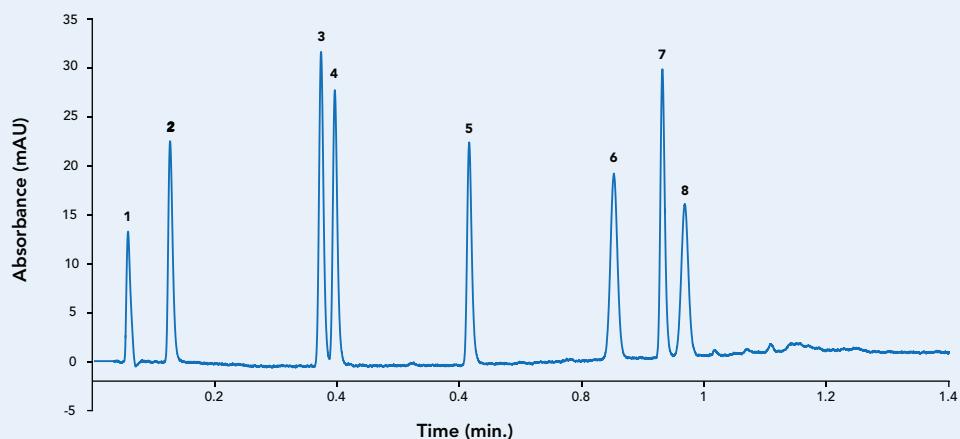
Extremely high efficiency columns such as the HALO 90 Å 2 µm columns require minimal band dispersion to see the greatest benefit.

## HALO 2 µm



## ULTRA-FAST SEPARATION OF ANTICOAGULANTS USING HALO 90 Å C18, 2 µm

**Figure V.** This separation of anticoagulants is completed in one minute using a short 2.1 x 30 mm HALO C18 column using a Shimadzu Nexera UHPLC system.



### TEST CONDITIONS:

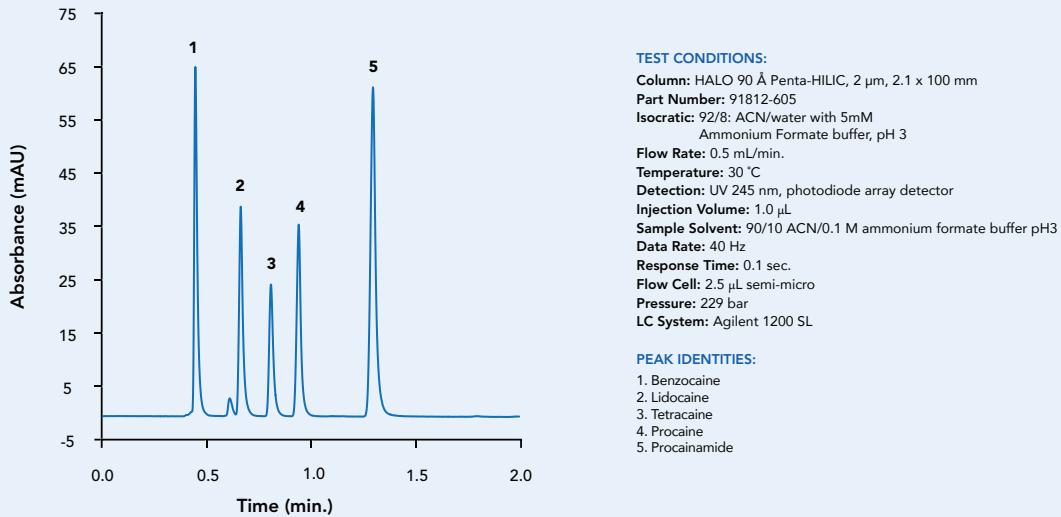
Column: HALO 90 Å C18, 2 µm, 2.1 x 30 mm  
Part Number: 91812-302  
Mobile Phase A: 20 mM Formic acid  
Mobile Phase B: 50/50 Acetonitrile/Methanol  
Gradient: Time %B  
0-0.06 20  
0.06-1.06 20-75  
Flow Rate: 1.1 mL/min.  
Temperature: 45 °C  
Detection: 254 nm  
Injection Volume: 0.2 µL  
Maximum Pressure: 430 bar

### PEAK IDENTITIES:

1. Uracil ( $t_b$ )
2. 6,7-Dihydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 6-Chloro-4-hydroxycoumarin
6. Warfarin
7. Coumatetralyl
8. Coumachlor

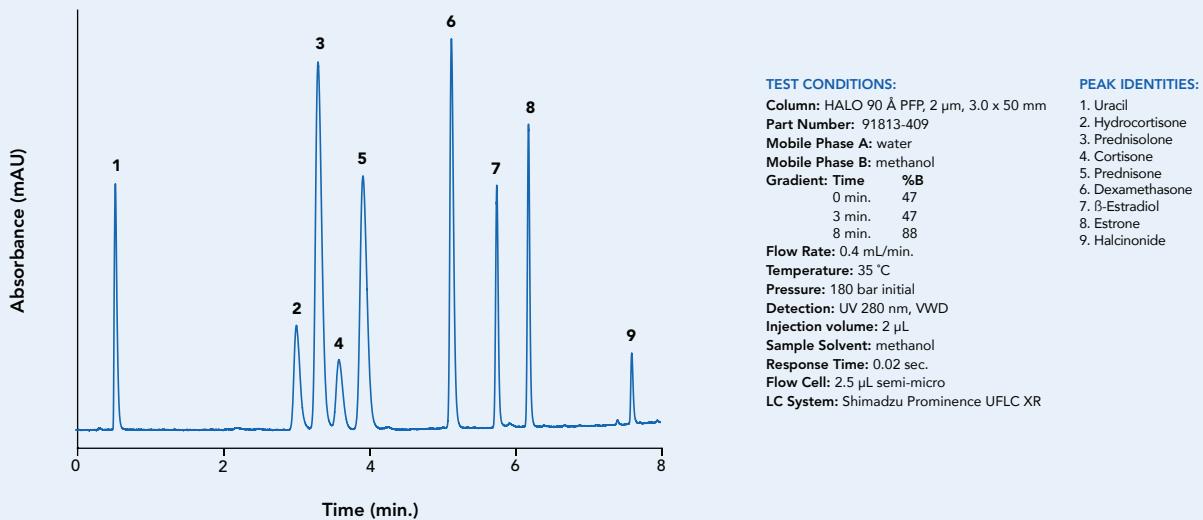
## FAST LOCAL ANESTHETIC SEPARATION USING HALO 2 $\mu$ m PENTA-HILIC

**Figure W.** This mixture of five local anesthetics is resolved isocratically in 1.5 minutes using a HALO 2  $\mu$ m Penta-HILIC column.



## STEROID SEPARATION USING HALO 2 $\mu$ m PFP

**Figure X.** HALO PFP columns often show excellent selectivity for steroids. HALO 2  $\mu$ m PFP is able to readily separate a mixture of 9 steroids in less than 8 minutes in gradient mode.



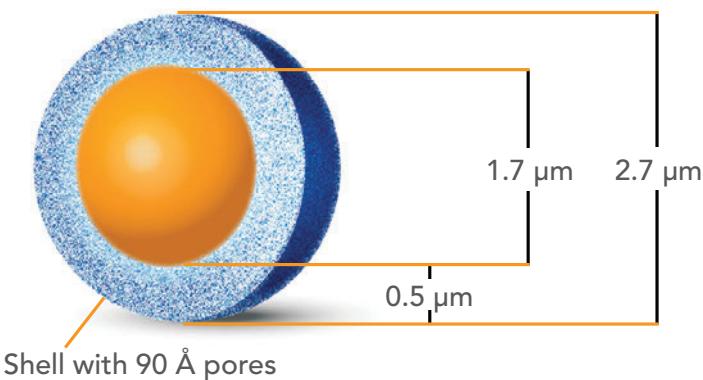


## HALO 90 Å 2.7 µm (UHPLC AND HPLC)

Reliable, efficient performance with lower back pressure compared to all sub-2 µm columns

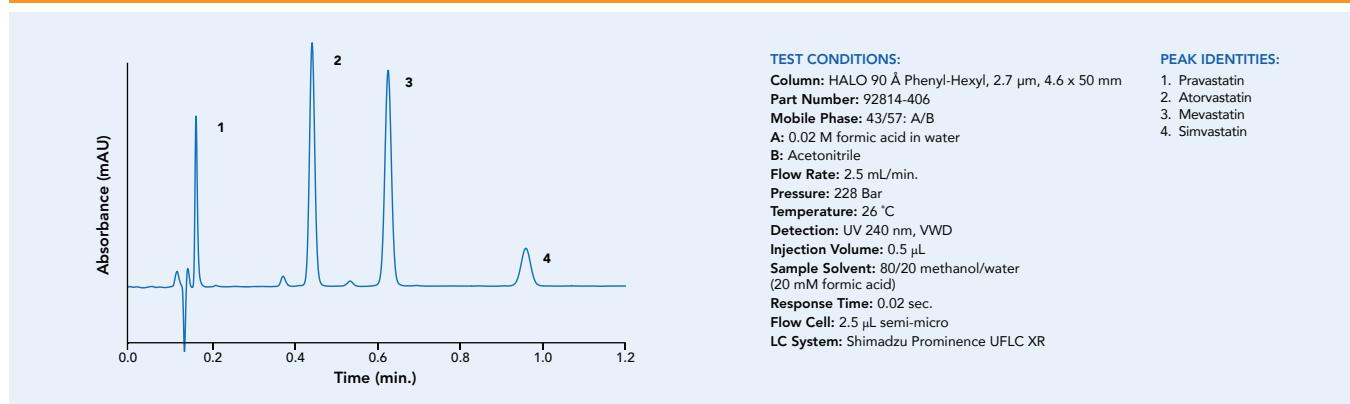
- Use for high speed or high resolution with UHPLC or HPLC applications
- Excellent for R&D and routine analyses
- 2 µm inlet frit
- Pressure limit, 600 bar/9000 psi

## HALO 2.7 µm



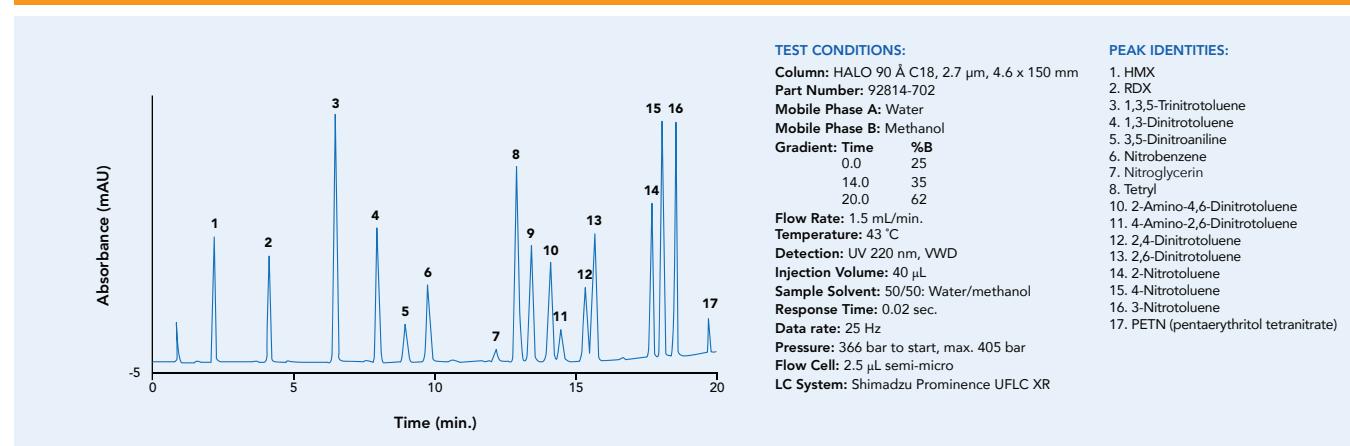
## ULTRAFAST SEPARATION OF STATIN DRUGS

**Figure Y.** These common statin drugs are separated in 1 minute using a 4.6 x 50 mm HALO Phenyl-Hexyl column.



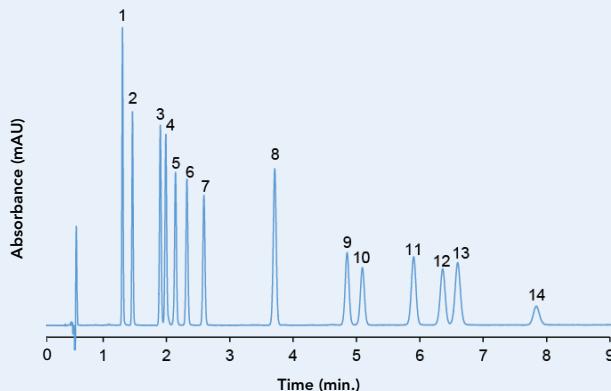
## HIGH RESOLUTION SEPARATION OF EXPLOSIVES

**Figure Z.** In this example, a 4.6 x 150 mm HALO C18 column is used to resolve 17 explosives in 20 minutes. This separation is quite sensitive to temperature, and was optimized using gradient time x temperature ( $t_g \times T$ ) computer modeling and simulation using DryLab® software.



## EFFICIENT CANNABINOID SEPARATION ON HALO 90 Å C18

**Figure AA.** Fourteen cannabinoids are resolved in less than eight minutes using a HALO 90 Å C18 column.



### TEST CONDITIONS:

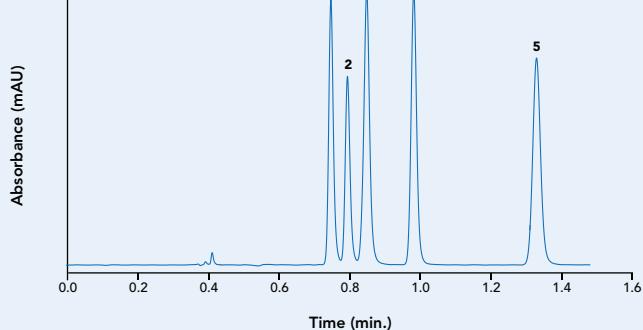
Column: HALO 90 Å C18, 2.7  $\mu$ m, 3.0 x 150 mm  
Part Number: 92813-702  
Mobile Phase: 25/75 A/B  
A: Water/0.1% formic acid  
B: Acetonitrile/0.085% formic acid  
Flow Rate: 1.0 mL/min  
Pressure: 350 bar  
Temperature: 30 °C  
Detection: UV 220 nm, PDA  
Injection: 0.6  $\mu$ L  
Sample Solvent: 75/25 methanol/ water  
Response Time: 0.025 sec.  
Data Rate: 100 Hz  
Flow Cell: 1  $\mu$ L  
LC System: Shimadzu Nexera X2

### PEAK IDENTITIES:

1. Cannabidivaricin acid (CBDVA)
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Cannabinol (CBN)
9. delta-9-Tetrahydrocannabinol ( $\Delta^9$ -THC)
10. delta-8-Tetrahydrocannabinol ( $\Delta^8$ -THC)
11. Cannabicyclol (CBL)
12. Cannabichromene (CBC)
13. delta-9-Tetrahydrocannabinolic acid A (THCA)
14. Cannabichromenic acid (CBCA)

## ULTRAFAST SEPARATION OF TRICYCLIC ANTIDEPRESSANTS

**Figure BB.** These basic tricyclic antidepressants are separated in less than two minutes, with excellent peak shape, using a HALO Penta-HILIC column.



### TEST CONDITIONS:

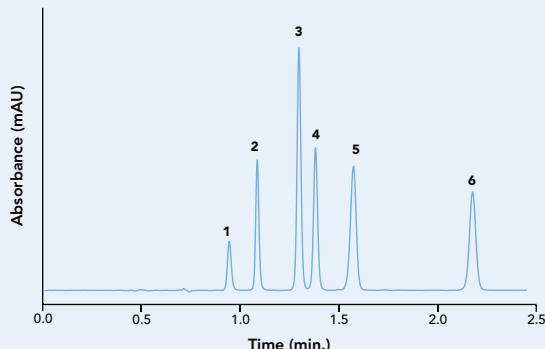
Column: HALO 90 Å Penta HILIC, 2.7  $\mu$ m, 4.6 x 100 mm  
Part Number: 92814-605  
Mobile Phase: 7/93: A/B  
A: 0.1 M Ammonium formate, pH=3.5 (adj.)  
B: Acetonitrile  
Flow Rate: 2.5 mL/min.  
Temperature: 30 °C  
Detection: UV 254 nm, VWD  
Injection Volume: 0.5  $\mu$ L  
Sample Solvent: 10/90: Water/acetonitrile  
Response Time: 0.02 sec.  
Maximum Pressure: 165 Bar  
Flow Cell: 2.5  $\mu$ L semi-micro  
LC System: Shimadzu Prominence UFC XR

### PEAK IDENTITIES:

1. Trimipramine
2. Amitriptyline
3. Doxepin
4. Nortriptyline
5. Amoxapine

## HIGH RESOLUTION OF NEONICOTINOIDS ON HALO 2.7 $\mu$ m ES-CN

**Figure CC.** Six neonicotinoids are separated using a HALO 2.7  $\mu$ m ES-CN column. The sub-3  $\mu$ m Fused-Core silica-based packing allows rapid separations at modest pressures.



### TEST CONDITIONS:

Column: HALO 90 Å ES-CN, 2.7  $\mu$ m, 4.6 x 100 mm  
Part Number: 92814-604  
Mobile Phase: 70/30: A/B  
A: 0.1% Formic acid in water  
B: Acetonitrile  
Flow Rate: 1.5 mL/min.  
Pressure: 205 Bar  
Temperature: 35 °C  
Detection: UV 254 nm, VWD  
Injection Volume: 0.5  $\mu$ L  
Sample Solvent: Acetonitrile  
Response Time: 0.02 sec.  
Flow Cell: 2.5  $\mu$ L semi-micro  
LC System: Shimadzu Prominence UFC XR

### PEAK IDENTITIES:

1. Nitopenpyram
2. Thiamethoxam
3. Clothianidin
4. Imidacloprid
5. Acetamiprid
6. Thiocloprid



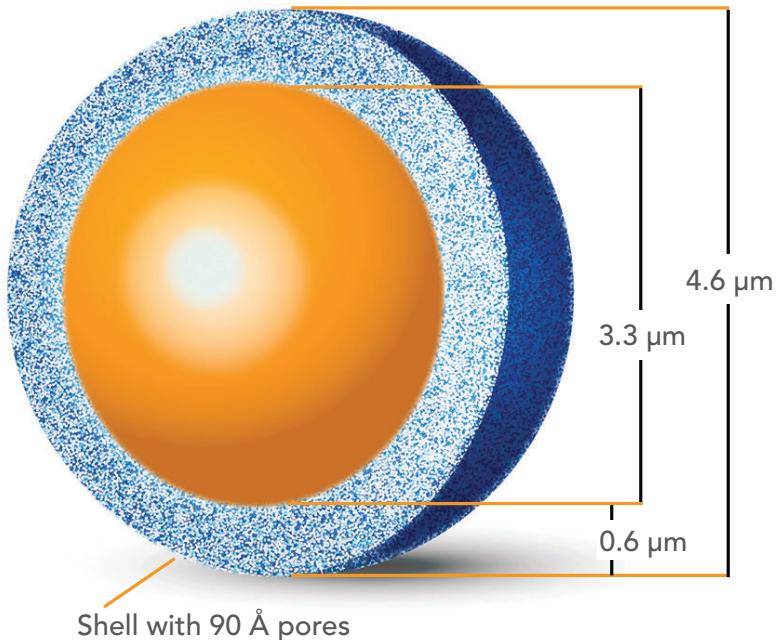
### HALO 90 Å 5 µm (HPLC)

Performance of 3 µm non-core column  
at 5 µm column pressures

Ideal for:

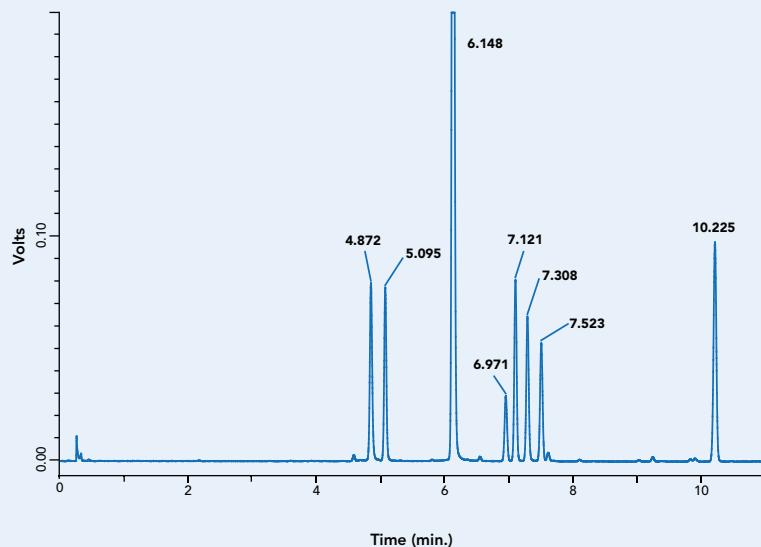
- QC laboratories
- Dirty samples
- High throughput, ballistic gradient and isocratic applications
- High resolution at HPLC back pressures (using columns in series)
- 2 µm inlet frit
- Pressure limit, 600 bar/9000 psi

### HALO 5 µm



### FAST, HIGH RESOLUTION GRADIENT FLAVONOID SEPARATION

**Figure DD.**  
This mixture of 8 flavonoids is baseline resolved in less than 11 minutes using a 2.1 x 150 mm HALO 5 µm C18 column with a fast 1.0-mL/min. flow rate with an LC-MS-compatible mobile phase.

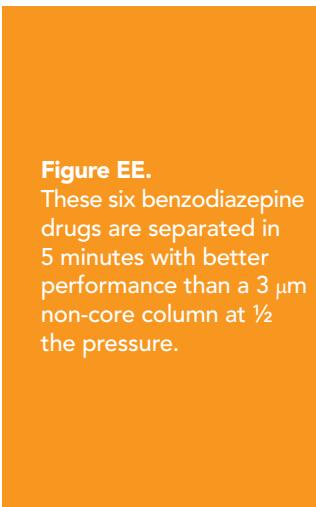


**SAMPLE:**  
Mixture of 8 flavonoids, 1 µL in MeOH

**TEST CONDITIONS:**  
Column: HALO 90 Å C18, 5 µm, 2.1 x 150 mm  
Part Number: 95812-702  
Flow Rate: 1.0 mL/min.  
Temperature: 40 °C  
Gradient: 5% CH<sub>3</sub>CN for 0.5 min.  
5-60% CH<sub>3</sub>CN/10 mM NH<sub>4</sub>COO (0.1% HCOOH) in 15 min.  
Max. Pressure: 280 bar

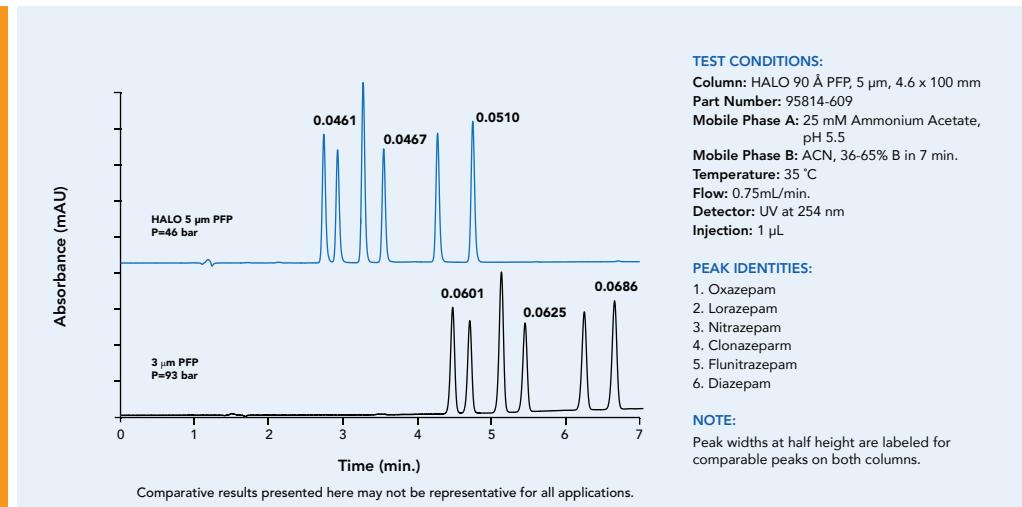
**ANALYTES:**  
1. Hesperidin  
2. Myricetin  
3. Quercetin  
4. Naringenin  
5. Apigenin  
6. Hesperetin  
7. Kaempferol  
8. Biochanin

## BENZODIAZEPINE SEPARATION USING HALO 5 $\mu$ m PFP

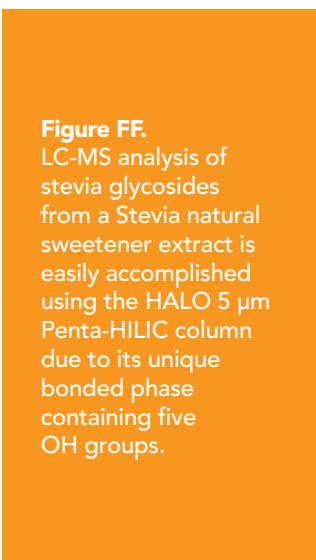


**Figure EE.**

These six benzodiazepine drugs are separated in 5 minutes with better performance than a 3  $\mu$ m non-core column at  $\frac{1}{2}$  the pressure.

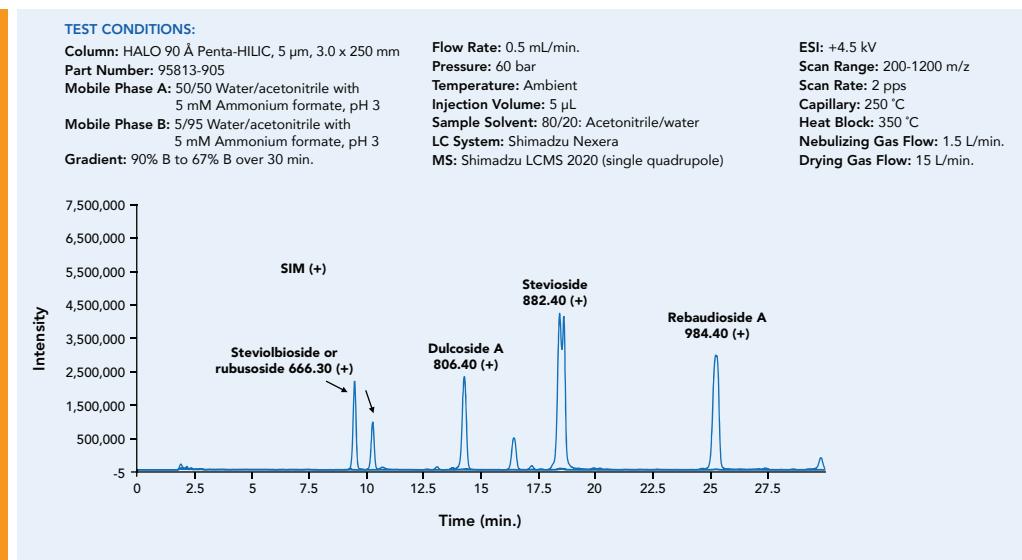


## LC-MS ANALYSIS OF STEVIA GLYCOSIDES USING HALO PENTA-HILIC

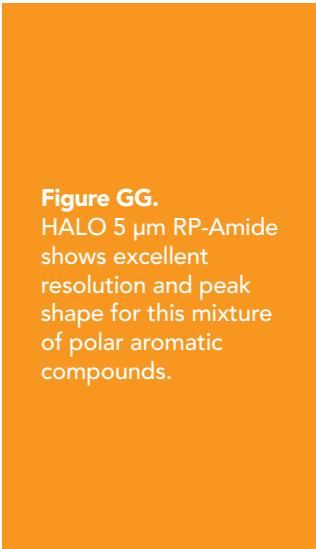


**Figure FF.**

LC-MS analysis of stevia glycosides from a Stevia natural sweetener extract is easily accomplished using the HALO 5  $\mu$ m Penta-HILIC column due to its unique bonded phase containing five OH groups.

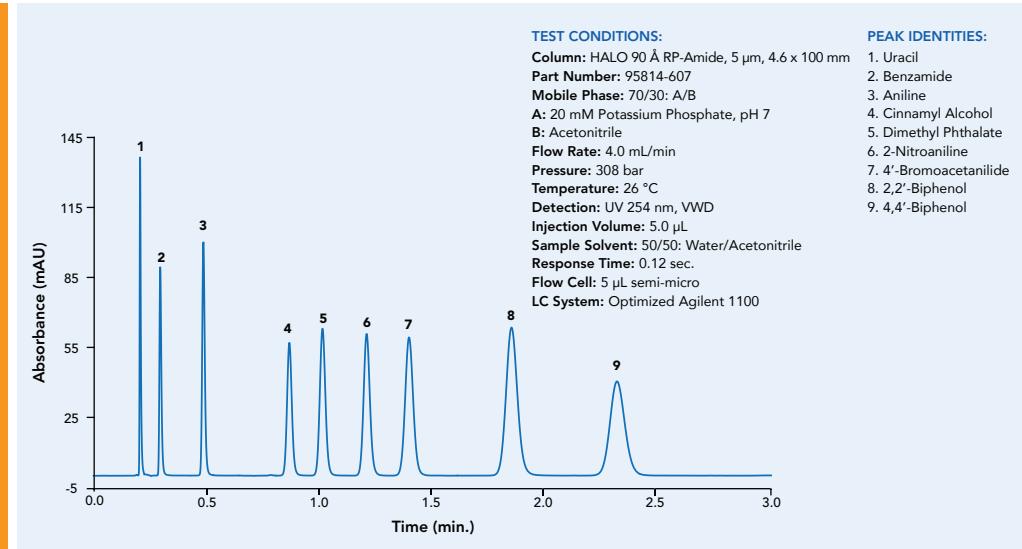


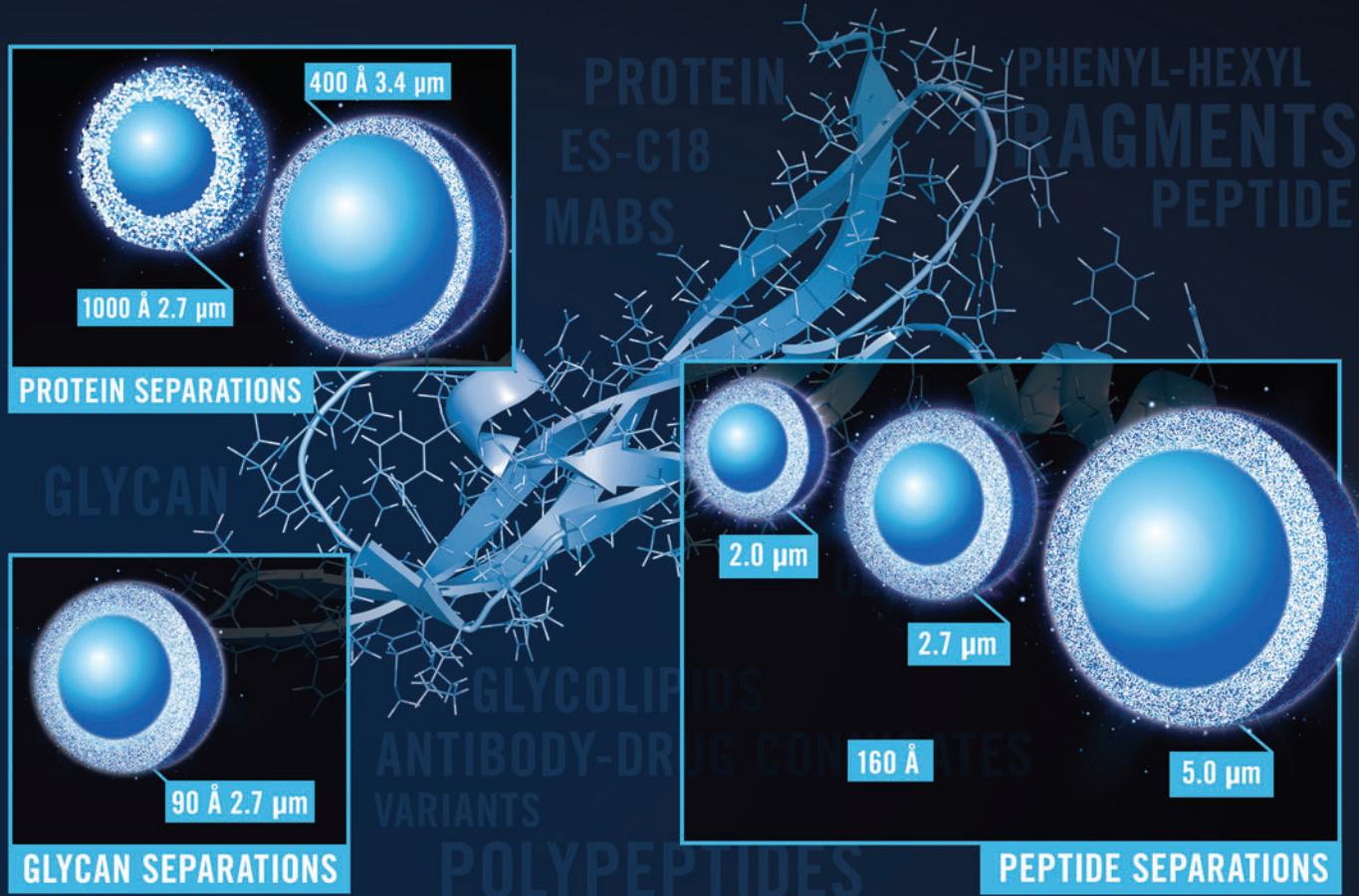
## POLAR AROMATIC COMPOUNDS ON HALO 5 $\mu$ m RP-AMIDE



**Figure GG.**

HALO 5  $\mu$ m RP-Amide shows excellent resolution and peak shape for this mixture of polar aromatic compounds.





### HALO ENABLED LARGE MOLECULE ANALYSIS

Today, researchers are keenly interested in both fast and high-resolution separations of numerous biomolecules. The HALO Fused-Core technology supports the development of novel therapeutic proteins and peptides in pharmaceutical drug development to advance understanding in modern university laboratories, enabling researchers to characterize protein post-translational modifications and fully assess subtle differences in biosimilars and other products of bioengineering and manufacture. HALO BioClass columns have been specifically designed to accomplish these bioseparation goals with a simplified and more effective solution.

With both tailored particle and pore size options, HALO BioClass offers application specific solutions for:

- Intact proteins, monoclonal antibodies (mAbs), biosimilars, and other large biomolecules such as pegylated proteins, antibody drug conjugates (ADCs), etc.
- Peptide mapping (analysis of enzyme digests) for characterization and monitoring of synthetic protein drugs
- Analysis of therapeutic peptides and peptide biomarkers (protein surrogates)
- High resolution separations of complex mixtures of glycans released from N- and O-linked glycoproteins

**Table F. HALO BioClass Column Specifications**

Bonded Phase	USP Designation	Particle Sizes (s) ( $\mu\text{m}$ )	Pore Size ( $\text{\AA}$ )	Carbon Load (%)	Surface Area ( $\text{m}^2/\text{g}$ )	Low pH/T Limit	High pH/T Limit	Endcapped
Protein	C4	L26	2.7	1000	0.6	22	2/90 °C	9/40 °C Yes
	ES-C18	L1	2.7	1000	1.4	22	1/90 °C	8/40 °C Yes
	C4	L26	3.4	400	0.4	15	2/90 °C	9/40 °C Yes
	ES-C18	L1	3.4	400	1.0	15	1/90 °C	8/40 °C Yes
Peptide	ES-C18	L1	2 2.7 5	160	4.0 4.6 4.0	65 90 60	1/90 °C	8/40 °C No
	ES-CN	L10	2.7 5	160	2.2 1.5	90 60	1/90 °C	8/40 °C Yes
	Phenyl-Hexyl	L11	2.7	160	4.7	90	2/90 °C	9/40 °C Yes
Glycan	Proprietary Ligand	L95	2.7	90	3.2	135	2/65 °C	9/40 °C No

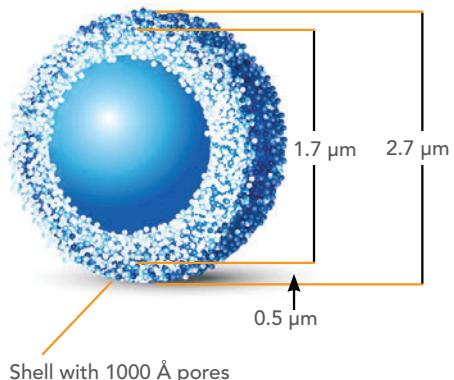
**Table G. HALO BioClass Features & Benefits**

	Bonded Phase	Features and Benefits	Target Analytes	Best Applications
1000 $\text{\AA}$ Protein	C4 (dimethylbutylsilane)	<ul style="list-style-type: none"> <li>Outstanding high temperature stability at low pH</li> <li>Unrestricted access to bonded phase</li> <li>Exceptional mass transfer kinetics</li> <li>Compatible with UHPLC and HPLC</li> <li>Low LC-MS bleed</li> </ul>	Monoclonal antibodies, antibody-drug conjugates, antibody fragments and large proteins with MWs $\leq 500 \text{ kDa}$	High resolution separations of monoclonal antibodies and their variants and antibody-drug conjugates
	ES-C18 (diisobutyloctadecylsilane)	<ul style="list-style-type: none"> <li>Even better stability up to 90 °C</li> <li>Can elute very large proteins with good peak shape and recovery</li> <li>Compatible with UHPLC and HPLC</li> <li>Very low LC-MS bleed</li> </ul>	Monoclonal antibodies, antibody-drug conjugates, antibody fragments and large proteins with MWs $\leq 500 \text{ kDa}$	High resolution separations of monoclonal antibodies and their variants and antibody-drug conjugates
400 $\text{\AA}$ Protein	C4 (dimethylbutylsilane)	<ul style="list-style-type: none"> <li>Stability up to 90°C</li> <li>Can elute very large proteins with good peak shape and recovery</li> <li>Compatible with UHPLC and HPLC</li> <li>Low LC-MS bleed</li> </ul>	Monoclonal antibodies, proteins and polypeptides $< 500 \text{ kDa}$	Monoclonal antibodies and mid-to-high molecular weight proteins and polypeptides
	ES-C18 (diisobutyloctadecylsilane)	<ul style="list-style-type: none"> <li>Even better stability up to 90 °C</li> <li>Can elute very large proteins with good peak shape and recovery</li> <li>Compatible with UHPLC and HPLC</li> <li>Very low LC-MS bleed</li> </ul>	Proteins and polypeptides $< 500 \text{ kDa}$	Mid-to-high molecular weight proteins and polypeptides
160 $\text{\AA}$ Peptide	ES-C18 (diisobutyloctadecylsilane)	<ul style="list-style-type: none"> <li>Fast separations</li> <li>High peak capacity</li> <li>Rugged, reliable performance</li> <li>Use with either UHPLC or HPLC</li> </ul>	Peptides and polypeptides $< 20 \text{ kDa}$	Intermediate molecular weight proteins and polypeptides
	ES-CN (diisopropylcyanopropylsilane)	<ul style="list-style-type: none"> <li>Alternative selectivity to ES-C18 and Phenyl-Hexyl for peptide mapping and proteomic applications</li> </ul>	Peptides and polypeptides $< 20 \text{ kDa}$	Intermediate molecular weight proteins and polypeptides
	Phenyl-Hexyl (dimethylphenyl-hexylsilane)	<ul style="list-style-type: none"> <li>Alternative selectivity to ES-C18 and ES-CN for peptide mapping and proteomic applications</li> </ul>	Peptides and polypeptides $< 20 \text{ kDa}$	Intermediate molecular weight proteins and polypeptides
Glycan	Proprietary hydrophilic ligand	<ul style="list-style-type: none"> <li>Improved retention of acids and zwitterions</li> <li>Very low sensitivity to buffer concentration</li> <li>Able to separate isobaric oligosaccharides with different linkages</li> </ul>	Glycans ( $< 20 \text{ kDa}$ ), glycopeptides and polar peptides	Provides orthogonal HILIC selectivity to HALO Peptide ES-C18

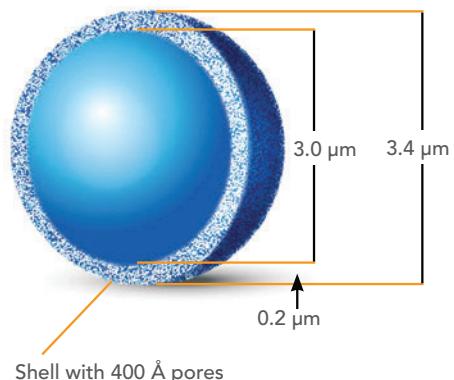
## PROTEIN SOLUTIONS

- As the first manufacturer of the 1000 Å fused-core particle, AMT recognizes the benefit of unrestricted pore access and offers both 400 Å and 1000 Å products to tailor the perfect large molecule solution.
- Benefits of HALO protein solutions include:
  - Provides narrower peaks and better recoveries for large biomolecules (vs. smaller pore sizes and non-core particles)
  - Allows HALO Protein columns to be used with both UHPLC and HPLC instrumentation for fast bioseparations at moderate back pressures
- C4 and sterically-protected ES-C18 phases
- Excellent high temperature stability (up to 90 °C) for improved peak shape and recovery
- 2 µm inlet frit
- Pressure limit, 600 bar/9000 psi

HALO 1000 Å 2.7 µm

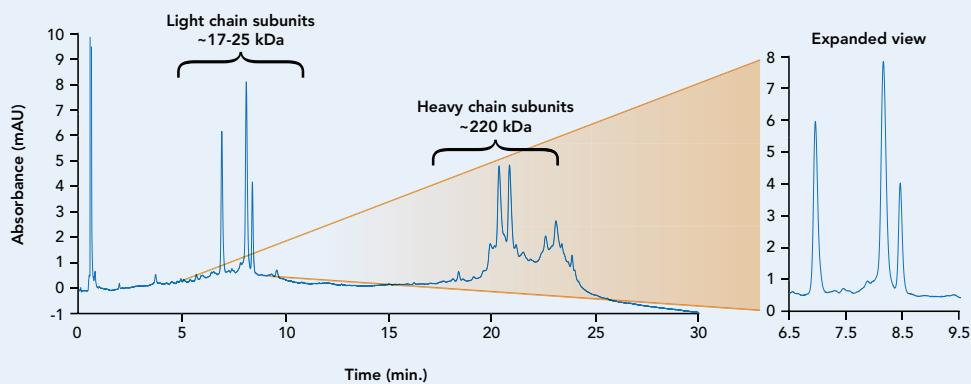


HALO 400 Å 3.4 µm



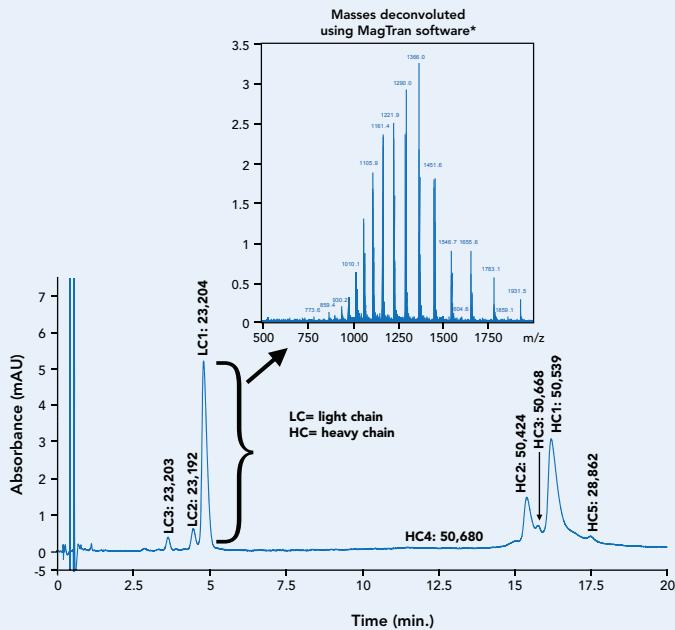
## LARGE PROTEIN SEPARATION USING HALO PROTEIN C4 FUSED-CORE COLUMN

**Figure HH.** High resolution separation of light and heavy chains of a denatured contractile protein (whole myosin from purified rabbit skeletal muscle) using HALO 400 Å Protein C4 at 80 °C.



## HIGH RESOLUTION OF LIGHT AND HEAVY CHAIN VARIANTS OF IgG1

**Figure II.**  
Very high resolution is obtained between variants of light and heavy chains of a reduced and alkylated monoclonal antibody (IgG1) sample using a HALO 400 Å Protein C4 column.

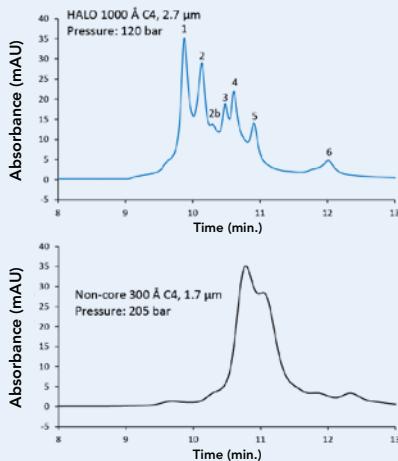


### TEST CONDITIONS:

Column: HALO 400 Å C4, 3.4 µm, 2.1 x 100 mm  
Part Number: 93412-614  
Mobile Phase A: 0.5% formic acid with 20 mM Ammonium Formate  
Mobile Phase B: 45% ACN/45% IPA/10% A solvent gradient: 29–32% B in 20 min.  
Temperature: 80 °C  
Detection: 280 nm and MS using 2pps scan rate from 500 to 2000 m/z  
Injection Volume: 2 µL of 2 µg/µL reduced and alkylated IgG1  
Sample Solvent: 0.25% (v/v) formic acid in water  
MS Parameters: Positive ion mode, ESI at +4.5 kV, 400 °C heatblock, 225 °C capillary  
LC-MS System: Shimadzu Nexera and LCMS-2020 (single quadrupole MS)

## INCREASED RESOLUTION OF IgG2 OVER TOTALLY POROUS COLUMN

**Figure JJ.**  
The larger pores of the HALO 1000 Å C4 column allow improved access to the stationary phase and increased resolution for IgG2 isoforms compared to the smaller 300 Å pores of the non-core C4 column.



### TEST CONDITIONS:

Column: HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm  
Part Number: 92712-714  
Mobile Phase A: 88/10/2 water/ACN/n-propanol/0.1% DFA  
Mobile Phase B: 70/20/10 n-propanol/ACN/water/0.1% DFA  
Gradient: 14–24 %B in 20 min  
Flow Rate: 0.2 mL/min  
Temperature: 80 °C  
Detection: UV 280 nm, PDA  
Injection: 2 µL of 2 mg/mL denosumab in water/0.1% DFA  
LC System: Shimadzu Nexera

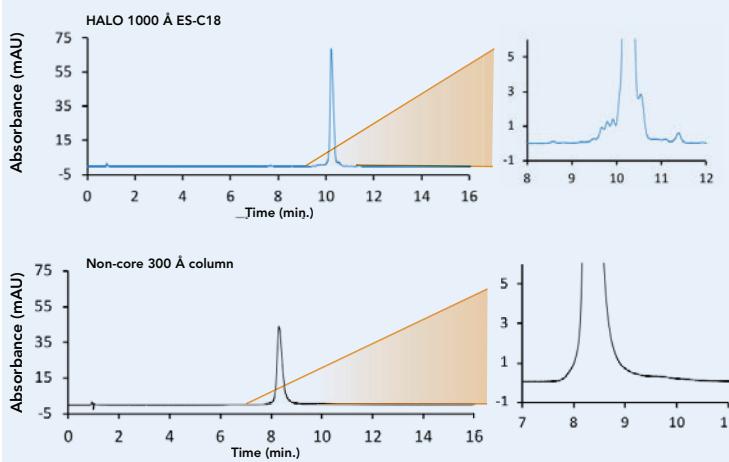
### PEAK IDENTITIES

1. IgG2-B
2. IgG2-B
- 2b. IgG2-B
3. IgG2-A/B
4. IgG2-A/B
5. IgG2-A
6. IgG2-A\*

Comparative results presented here may not be representative for all applications.

## NARROWER PEAK AND MORE RESOLUTION THAN TOTALLY POROUS COLUMN

**Figure KK.**  
HALO 1000 Å ES-C18 outperforms a non-core column with 300 Å pores. The zoomed-in region of the base of the NISTmAb peak shows more resolution with HALO 1000 Å ES-C18, as well.



### TEST CONDITIONS:

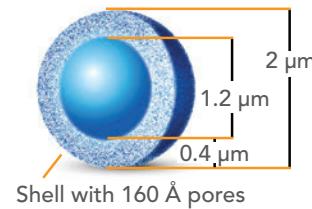
Column: HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm  
Part Number: 92712-702  
Mobile Phase A: Water/0.1% TFA  
Mobile Phase B: Acetonitrile/0.1% TFA  
Gradient: 36–44 %B in 16 min  
Flow Rate: 0.4 mL/min  
Temperature: 60 °C  
Detection: UV 280 nm, PDA  
Injection: 2 µL of 2 mg/mL NISTmAb  
Flow Cell: 1 µL  
LC System: Shimadzu Nexera

Comparative results presented here may not be representative for all applications.

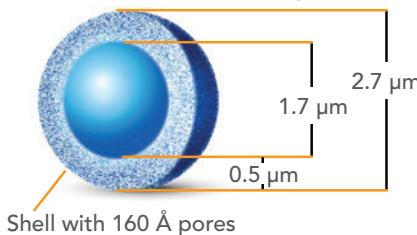
## PEPTIDE SOLUTIONS

- Extremely stable at high temperatures and low pH
- Ideal for both ultrafast and ultrahigh resolution separations of peptides and polypeptides up to 20 kDa
- Outperforms non-core 3 µm, 300 Angstrom columns in terms of peak width, peak capacity and peak height (Figure MM)
- Offers comparable peak capacity to sub-2 µm non-core columns at 40–50% back pressure (2.7 µm)
- ~ 20% higher peak capacity than sub-2 µm non-core columns at comparable back pressure (2 µm)
- Columns (Peptide 2.7 and 5 µm) can be used in series to increase peak capacity for UHPLC and HPLC analyses of complex tryptic digest samples (Figure NN)
- HALO Peptide ES-CN (2.7 and 5 µm) offers different selectivity and improved retention for polar peptides (Figure OO)
- 2 µm inlet frit (2.7 and 5 µm); 1 µm inlet frit (2 µm) provides extra protection from plugging

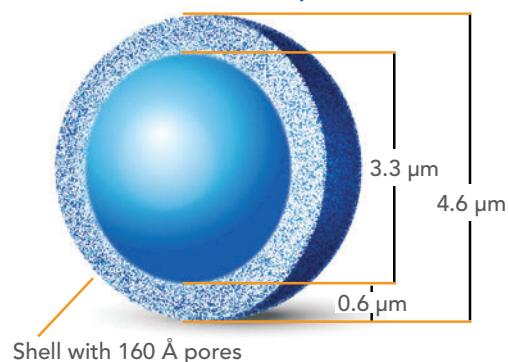
### HALO 2 µm Peptide



### HALO 2.7 µm Peptide

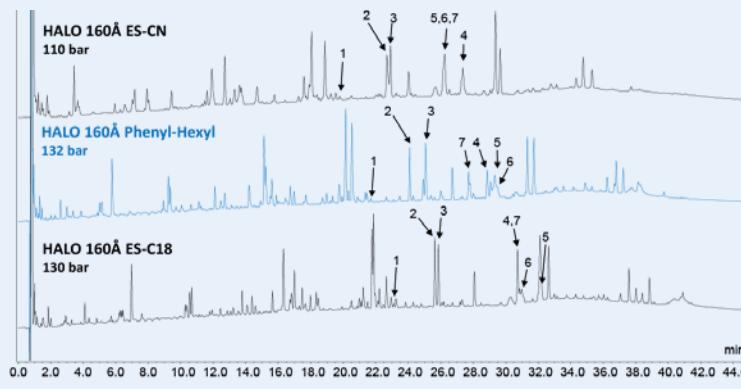


### HALO 5 µm Peptide



## ENHANCED SELECTIVITY WITH HALO 160 Å PHENYL-HEXYL FOR A TRYPTIC DIGEST

**Figure LL.**  
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.



#### TEST CONDITIONS:

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

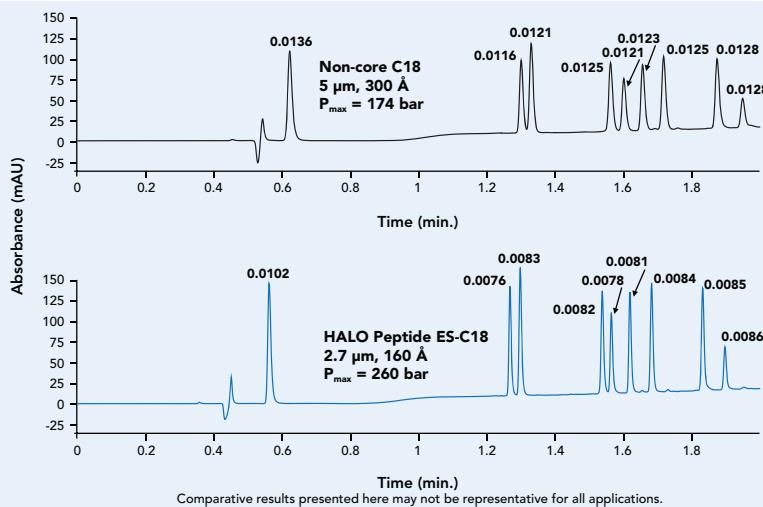
Mobile Phase:  
A = water + 10 mM difluoroacetic acid (DFA)  
B = ACN + 10 mM difluoroacetic acid  
Flow Rate: 0.3 mL/min  
Gradient: 2–50 %B in 60 min  
Temperature: 60 °C  
Detection: UV 220 nm, VWD  
Injection Volume: 5 µL of 0.2 mg/mL digest  
Sample Solvent: 50 mM Tris-HCl/1.5 M Guanidine-HCl with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

#### PEAK IDENTITIES:

- FTISADTTSKNTAYLQMNLSR (754 m/z)
- LScAAASGFNIKDTYIHWR (747 m/z)
- GFPSPDIADVWESNOOPENNYK (849 m/z)
- LLYASFLYSGVPSR (592 m/z)
- SGTASVVcLLNNFVPR (849 m/z)
- SdCKTHTcPcPAPELLGPGSFLFPPPKPK (834 m/z)
- WVSvLTVLHQDWLNKEYK (1115 m/z)

## COMPARISON OF FUSED-CORE TO NON-CORE COLUMNS FOR PEPTIDE SEPARATIONS

**Figure MM.** HALO Peptide 160 Å 2.7 µm column produces significantly taller peaks and higher peak capacity than a non-core 3 µm column.



Comparative results presented here may not be representative for all applications.

### TEST CONDITIONS:

Columns: HALO 160 Å ES-C18, 2.7 µm, 4.6 x 100 mm and non-core C18, 3 µm, 4.6 x 100 mm  
Part Number: 92124-602  
Mobile Phase A: 90% water/10% ACN/0.1% TFA  
Mobile Phase B: 30% water/70% ACN/0.1% TFA  
Gradient: 0-87.5% B in 2 min.  
Flow Rate: 2.5 mL/min.  
Temperature: 60 °C  
Injection Volume: 5 µL  
LC System: Agilent 1100

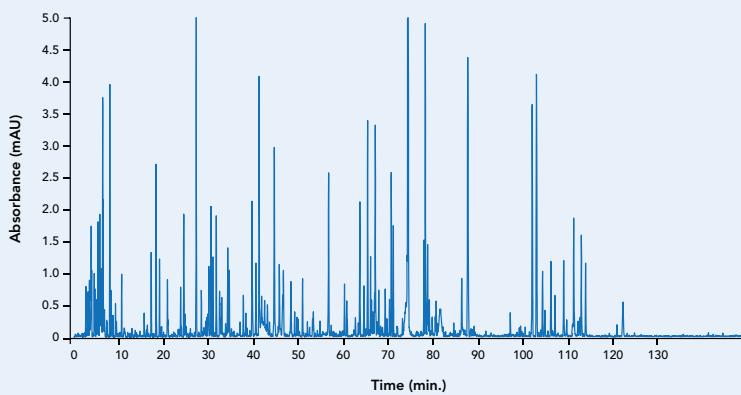
### PEAK IDENTITIES:

1. Gly-Tyr
2. Angiotensin 1/2 (1-7) amide
3. Val-Tyr-Val
4. Met-Enk
5. Angiotensin 1/2 (1-8) amide
6. Angiotensin II
7. Leu-Enk
8. Angiotensin (1-12) human
9. Angiotensin (1-12) mouse

Peak widths at half height are shown above respective peaks.

## COUPLED HALO PEPTIDE COLUMNS FOR MAXIMUM PEAK CAPACITY

**Figure NN.** Three HALO Peptide 160 Å ES-C18, 2.7 µm 150-mm columns (450 mm total length) were connected in series to achieve a peak capacity of 560 for this mixture of tryptic digests of  $\alpha$ -1-glycoprotein and apotransferrin.

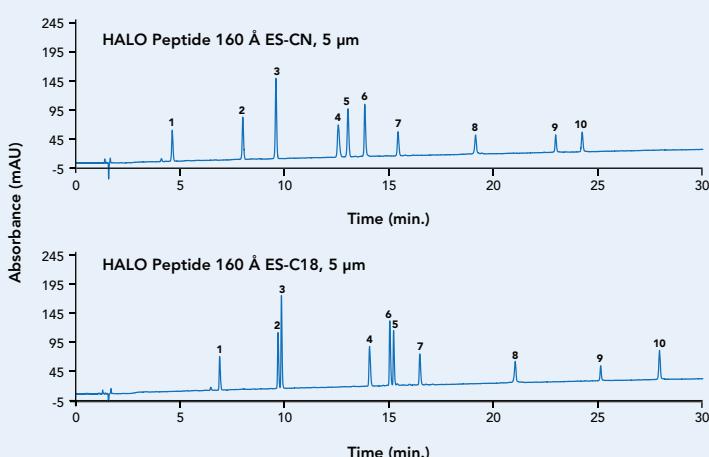


### TEST CONDITIONS:

Columns: HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm (3)  
Part Number: 3 of 92122-702  
Mobile Phase A: water/0.1% formic acid/20 mM ammonium formate  
Mobile Phase B: A with 80% acetonitrile  
Gradient: 5-55% B in 150 min.  
Flow Rate: 0.5 mL/min.  
Temperature: 70 °C  
Detection: 220 nm  
Injection Volume: 50 µL [25 µg each] of  $\alpha$ -1-glycoprotein tryptic digest and apotransferrin tryptic digest

## ALTERNATE SELECTIVITY USING HALO 160 Å 5 µm PEPTIDE ES-CN

**Figure OO.** HALO Peptide 160 Å ES-CN, 5 µm offers alternative selectivity to HALO Peptide 160 Å ES-C18, 5 µm for this mixture of 10 peptides and polypeptides.



### TEST CONDITIONS:

Column: HALO 160 Å ES-CN, 5 µm  
HALO 160 Å ES-C18, 5 µm  
Part Numbers: ES-CN: 95124-704  
ES-C18: 95124-702  
Instrument: Optimized Agilent 1100  
Injection Volume: 10 µL  
Detection: 215 nm  
Temperature: 40 °C  
Flow Rate: 1.0 mL/min  
Mobile Phase A: water/0.1% TFA  
Mobile Phase B: ACN/0.1% TFA  
Gradient: 5-50% B in 30 min.

### PEAK IDENTITIES:

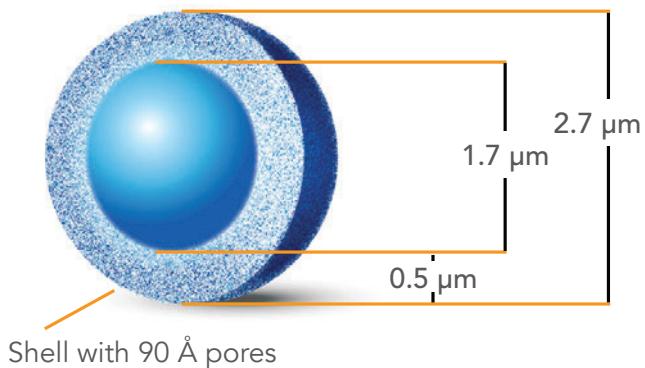
1. Asp-Phe
2. Angiotensin (1-7) amide
3. Tyr-Tyr-Tyr
4. Bradykinin
5. Leu-Enk
6. Angiotensin II
7. Neuropeptides
8.  $\beta$ -endorphin
9. Sauvagine
10. Mellitin



## GLYCAN SOLUTIONS

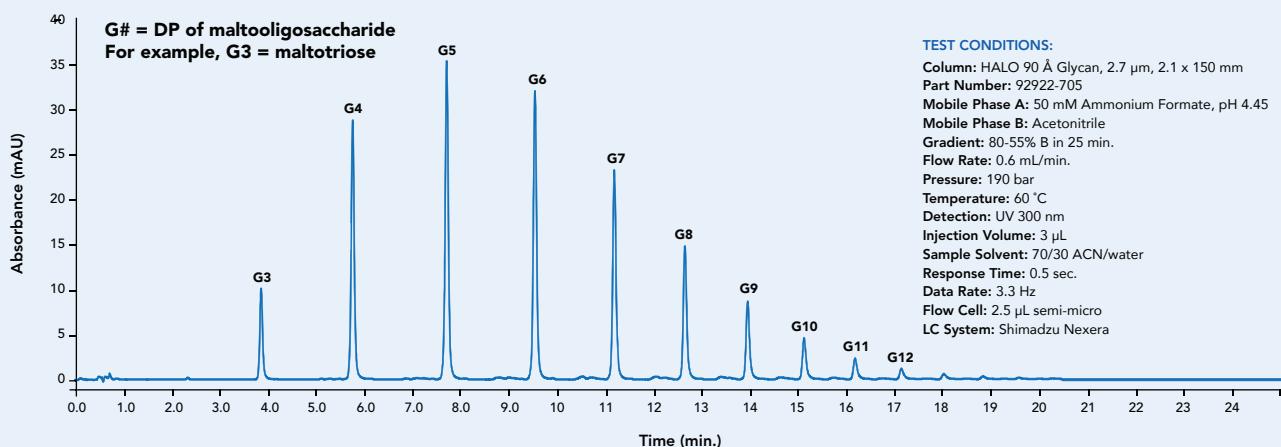
- 90 Ångstrom pore size
- Incorporates a highly polar ligand that contains 5 hydroxyl groups tethered to 2.7 µm Fused-Core silica particles via novel, proprietary linkage chemistry
- Ideal for hydrophilic interaction liquid chromatography (HILIC) separations of oligosaccharides, and particularly, of released and labeled glycans from glycoproteins and proteoglycans
- Mobile phases typically consist of acetonitrile and aqueous ammonium formate buffer (50 mM, pH 4.4) used to form a gradient of increasing water content during elution
- Each lot of HALO Glycan material is tested for quality assurance (Figure PP) by separation of a procainamide-reducing-end-labeled glycan ladder of oligosaccharides having 2–25 glucose units (GU).
  - Peaks for oligosaccharides composed of 5 and 10 GU must meet tight specifications for retention and peak width before lot is approved for glycan analysis
- 2 µm inlet frit
- Pressure limit, 600 bar/9000 psi

## HALO Glycan



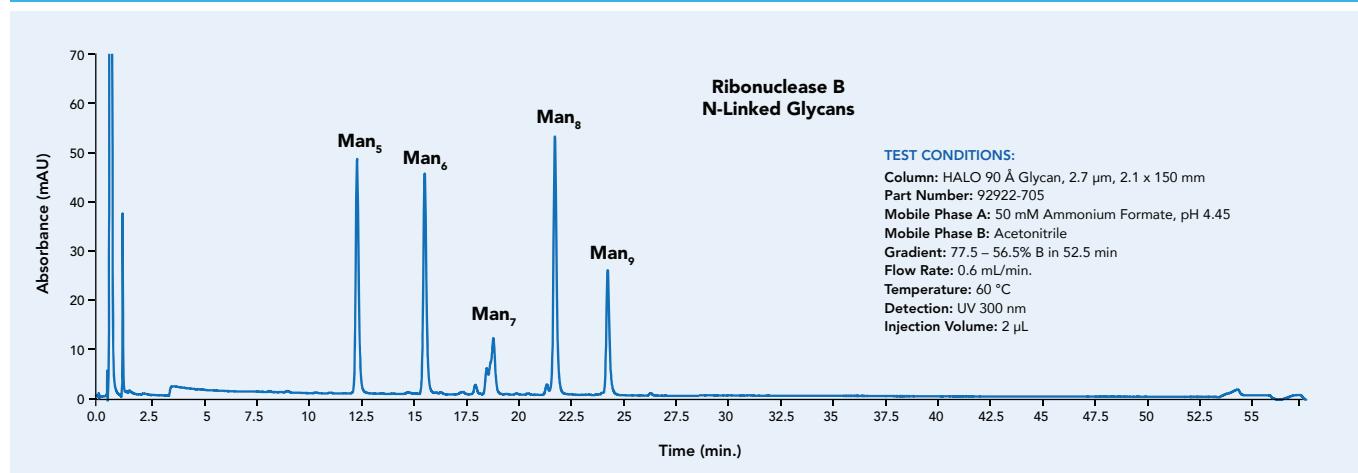
## QA ANALYSIS OF HALO GLYCAN

**Figure PP.** Example QA Chromatogram for HALO Glycan column. Each HALO Glycan packing lot is tested using this glycan ladder mixture to assess and ensure lot-to-lot reproducibility.



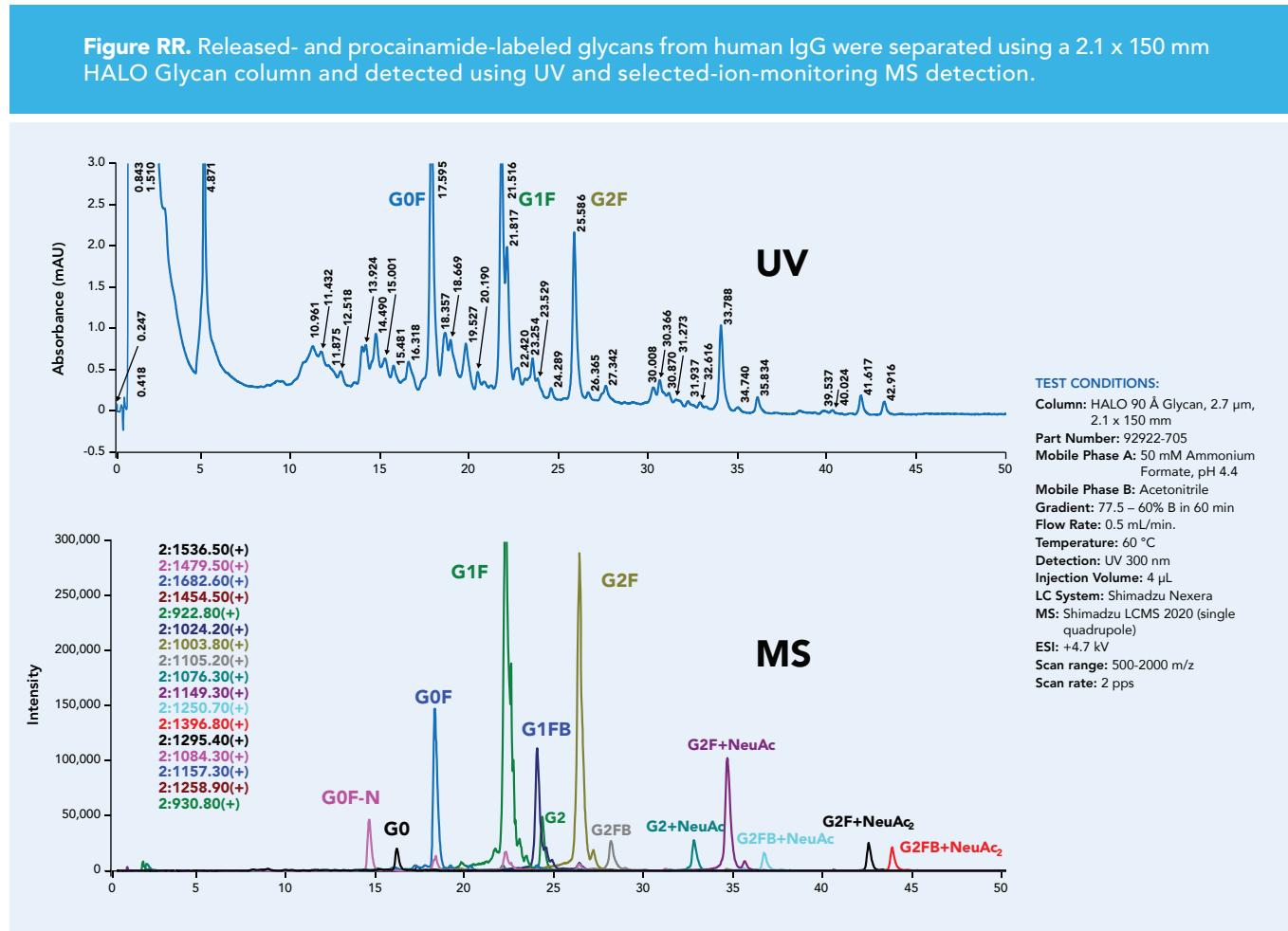
## SEPARATION OF N-LINKED GLYCANS FROM RIBONUCLEASE B

**Figure QQ.** Gradient HILIC-MS separation of N-linked glycans, which had been released using PNGase from ribonuclease B, using the HALO Glycan column.



## SEPARATION OF N-LINKED GLYCANS FROM HUMAN IgG

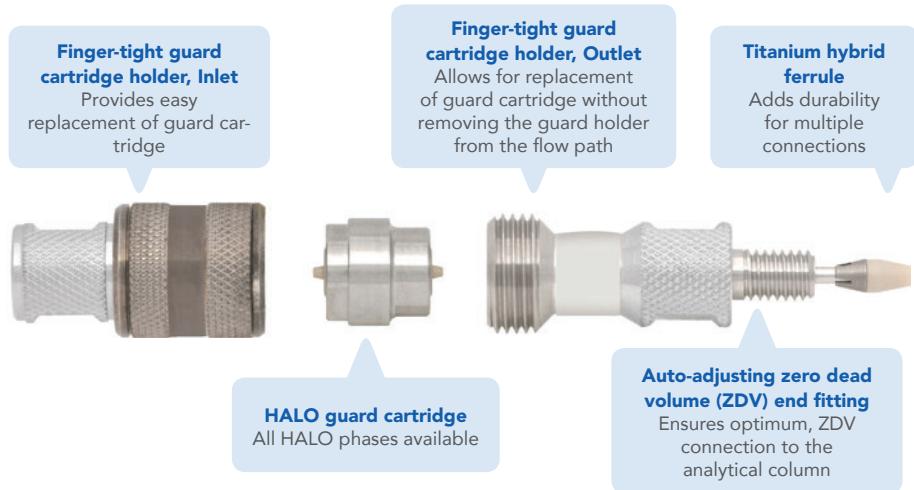
**Figure RR.** Released- and procainamide-labeled glycans from human IgG were separated using a 2.1 x 150 mm HALO Glycan column and detected using UV and selected-ion-monitoring MS detection.



# HALO UHPLC AND HPLC GUARD COLUMNS

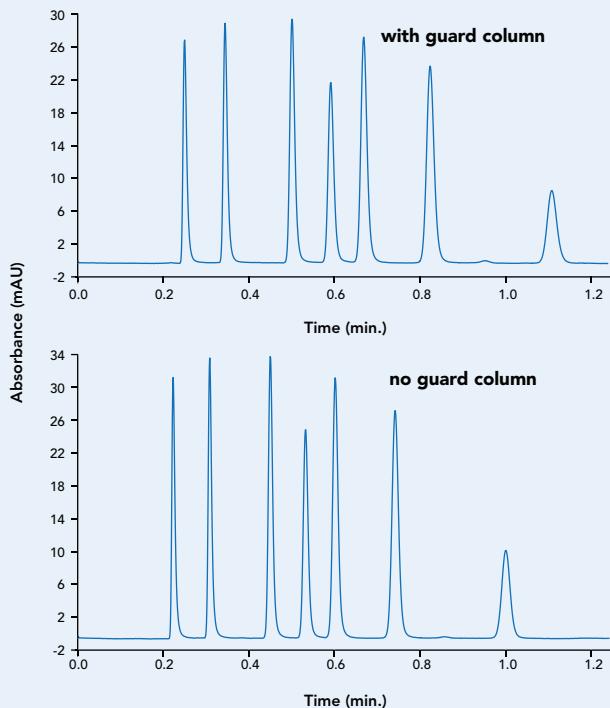
- Collect strongly retained compounds from the sample and minimizes column fouling
- Ultra-low dispersion, easy to use, operate at pressures up to 1000 bar
- Finger-tight, direct-connect units that auto-adjust to any column with a 10–32 inlet port
- Easily replace guard cartridge without removing guard holder from the flow path
- Available for all HALO analytical geometries (2.1, 3.0 and 4.6 mm ID) and phases

See below for an exploded view of the HALO guard cartridge and guard holder.  
Please see pages 32–36 for ordering information.



## HALO GUARD COLUMNS: PROTECTION + PERFORMANCE

**Figure S5.**  
HALO guard columns provide optimum protection for your HALO HPLC and UHPLC column without sacrificing column efficiency.



**TEST CONDITIONS:**  
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm  
Mobile Phase: 60/40 ACN/water  
Flow Rate: 1.8 mL/min.  
Temperature: 30 °C  
Detection: 254 nm  
Injection Volume: 1 µL  
Pressure: 158 bar with guard column  
146 bar without guard column  
Instrument: Optimized Agilent 1100 bypassed semi-micro flow cell  
0.05" ID tubing  
14 Hz data rate

The Optimize Technologies EXP® Direct Connect Holder: U.S. Patent No. 8,201,854 & 8,696,902 and Foreign Patents Pending.

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2. "Orthogonal" separations for reversed-phase liquid chromatography; J. Pellett, P. Lukulay, Y. Mao, W. Bowen, R. Reed, M. Ma, R.C. Munger, J.W. Dolan, L. Wrisley, K. Medwid, N.P. Toltl, C.C. Chan, M. Skibic, K. Biswas, K. A. Wells, and L.R. Snyder; *Journal of Chromatography A*, 1101 (2006) 122–135.
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8. A.J. Alpert. *Anal. Chem.* 80, 62–76 (2008).
9. D.V. McCalley, *J. Chromatogr. A* 1171, 46–56 (2007).
10. A.J. Alpert et al., *Anal. Chem.* 82, 5253–5259 (2010).



# HALO 90 Å 2 µm COLUMNS

The part numbers for HALO 90 Å 2 µm columns are presented below and available in 2.1 and 3.0 mm internal diameters. Guard columns are also available for these IDs for UHPLC to provide additional protection when necessary.

<i>Dimensions ID x Length (in mm)</i>	C18	AQ-C18	C8	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 20	91812-202	91812-222	91812-208	91812-206	91812-207	91812-209	91812-204	91812-205	91812-201
2.1 x 30	91812-302	91812-322	91812-308	91812-306	91812-307	91812-309	91812-304	91812-305	91812-301
2.1 x 50	91812-402	91812-422	91812-408	91812-406	91812-407	91812-409	91812-404	91812-405	91812-401
2.1 x 75	91812-502	91812-522	91812-508	91812-506	91812-507	91812-509	91812-504	91812-505	91812-501
2.1 x 100	91812-602	91812-622	91812-608	91812-606	91812-607	91812-609	91812-604	91812-605	91812-601
2.1 x 150	91812-702	91812-722	91812-708	91812-706	91812-707	91812-709	91812-704	91812-705	91812-701
3.0 x 20	91813-202	91813-222	91813-208	91813-206	91813-207	91813-209	91813-204	91813-205	91813-201
3.0 x 30	91813-302	91813-322	91813-308	91813-306	91813-307	91813-309	91813-304	91813-305	91813-301
3.0 x 50	91813-402	91813-422	91813-408	91813-406	91813-407	91813-409	91813-404	91813-405	91813-401
3.0 x 75	91813-502	91813-522	91813-508	91813-506	91813-507	91813-509	91813-504	91813-505	91813-501
3.0 x 100	91813-602	91813-622	91813-608	91813-606	91813-607	91813-609	91813-604	91813-605	91813-601
3.0 x 150	91813-702	91813-722	91813-708	91813-706	91813-707	91813-709	91813-704	91813-705	91813-701
<b>2 µm, 90 Å Guard Columns, 3-Pack</b>									
<i>Dimensions ID x Length (in mm)</i>	C18	AQ-C18	C8	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 5	91812-102	91812-122	91812-108	91812-106	91812-107	91812-109	91812-104	91812-105	91812-101
3.0 x 5	91813-102	91813-122	91813-108	91813-106	91813-107	91813-109	91813-104	91813-105	91813-101
<b>Guard Column Holder</b>	94900-001								





# HALO 1000 Å AND 400 Å PROTEIN COLUMNS

Part numbers for nano, capillary, analytical and semi-preparative HALO 1000 and 400 Å in 2.7 and 3.4 µm phases are provided below. Guard columns are available in 2.1, 3.0 and 4.6 mm IDs for UHPLC and HPLC applications to provide additional column protection when desired.

Dimensions ID x Length (in mm)	400 Å, 3.4 µm		1000 Å, 2.7 µm	
	C4	ES-C18	C4	ES-C18
0.075 x 50	94319-414	94319-402	97219-414	97219-402
0.075 x 100	94319-614	94319-602	97219-614	97219-602
0.075 x 150	94319-714	94319-702	97219-714	97219-702
0.1 x 50	94318-414	94318-402	97218-414	97218-402
0.1 x 100	94318-614	94318-602	97218-614	97218-602
0.1 x 150	94318-714	94318-702	97218-714	97218-702
0.2 x 50	94317-414	94317-402	97217-414	97217-402
0.2 x 100	94317-614	94317-602	97217-614	97217-602
0.2 x 150	94317-714	94317-702	97217-714	97217-702
0.3 x 50	94316-414	94316-402	97216-414	97216-402
0.3 x 100	94316-614	94316-602	97216-614	97216-602
0.3 x 150	94316-714	94316-702	97216-714	97216-702
0.5 x 50	94315-414	94315-402	97215-414	97215-402
0.5 x 100	94315-614	94315-602	97215-614	97215-602
0.5 x 150	94315-714	94315-702	97215-714	97215-702
1.0 x 30	93411-314	93411-302	92711-314	92711-302
1.0 x 50	93411-414	93411-402	92711-414	92711-402
1.0 x 75	93411-514	93411-502	92711-514	92711-502
1.0 x 100	93411-614	93411-602	92711-614	92711-602
1.0 x 150	93411-714	93411-702	92711-714	92711-702
2.1 x 20	93412-214	93412-202	92712-214	92712-202
2.1 x 30	93412-314	93412-302	92712-314	92712-302
2.1 x 50	93412-414	93412-402	92712-414	92712-402
2.1 x 75	93412-514	93412-502	92712-514	92712-502
2.1 x 100	93412-614	93412-602	92712-614	92712-602
2.1 x 150	93412-714	93412-702	92712-714	92712-702
2.1 x 250	93412-914	93412-902	92712-914	92712-902
3.0 x 20	93413-214	93413-202	92713-214	92713-202
3.0 x 30	93413-314	93413-302	92713-314	92713-302
3.0 x 50	93413-414	93413-402	92713-414	92713-402
3.0 x 75	93413-514	93413-502	92713-514	92713-502
3.0 x 100	93413-614	93413-602	92713-614	92713-602
3.0 x 150	93413-714	93413-702	92713-714	92713-702
3.0 x 250	93413-914	93413-902	92713-914	92713-902
4.6 x 20	93414-214	93414-202	92714-214	92714-202
4.6 x 30	93414-314	93414-302	92714-314	92714-302
4.6 x 50	93414-414	93414-402	92714-414	92714-402
4.6 x 75	93414-514	93414-502	92714-514	92714-502
4.6 x 100	93414-614	93414-602	92714-614	92714-602
4.6 x 150	93414-714	93414-702	92714-714	92714-702
4.6 x 250	93414-914	93414-902	92714-914	92714-902
10.0 x 50	93410-414	93410-402	92710-414	92710-402
10.0 x 75	93410-514	93410-502	92710-514	92710-502
10.0 x 100	93410-614	93410-602	92710-614	92710-602
10.0 x 150	93410-714	93410-702	92710-714	92710-702
<b>Guard Columns, 3-Pack</b>				
Dimensions ID x Length (in mm)	C4	ES-C18	C4	ES-C18
2.1 x 5	93412-114	93412-102	92712-114	92712-102
3.0 x 5	93413-114	93413-102	92713-114	92713-102
4.6 x 5	93414-114	93414-102	92714-114	92714-102
<b>Guard Column Holder</b> 94900-001				

# HALO 90 Å GLYCAN COLUMNS

HALO Glycan columns are available in 2.1 and 4.6 mm diameters in the following lengths as a 2.7 µm particle size. Guard columns are available for UHPLC and HPLC applications if additional protection is desired.

Dimensions ID x Length (in mm)	HALO Glycan
2.1 x 50	92922-405
2.1 x 100	92922-605
2.1 x 150	92922-705
4.6 x 50	92924-405
4.6 x 100	92924-605
4.6 x 150	92924-705
<b>Guard Columns, 3-Pack</b>	
Dimensions ID x Length (in mm)	HALO Glycan
2.1 x 5	92922-105
4.6 x 5	92924-105
<b>Guard Column Holder</b> 94900-001	

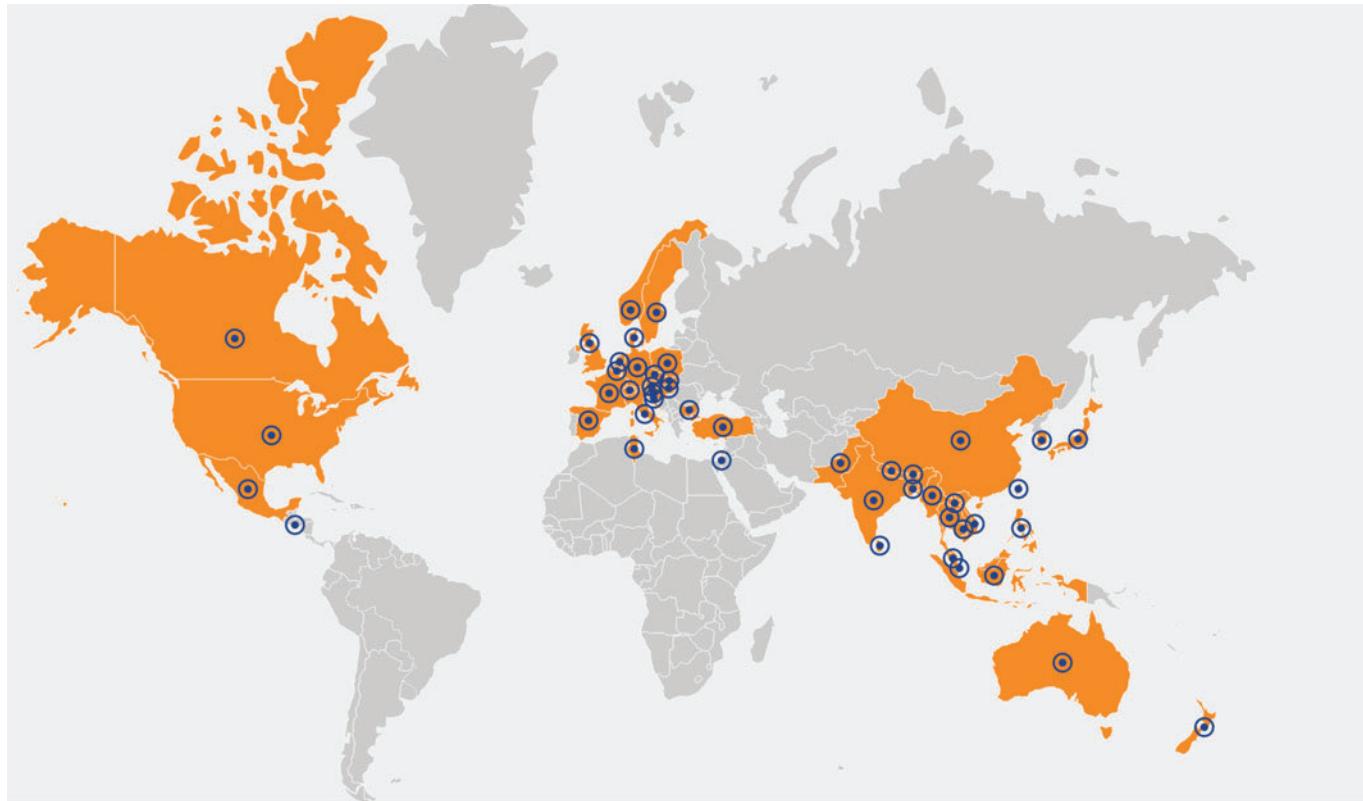


# HALO 160 Å PEPTIDE COLUMNS

The part numbers are provided below for the nano, capillary, analytical and semi-preparative HALO 160 Å 2, 2.7 and 5 µm phases. Guard columns are available for 2.1, 3.0 and 4.6 mm internal diameters for UHPLC and HPLC applications, if additional protection is desired.

	160 Å, 2 µm		160 Å, 2.7 µm			160 Å, 5 µm	
Dimensions ID x Length (in mm)	ES-C18	ES-C18	ES-CN	Phenyl-Hexyl	ES-C18	ES-CN	
0.075 x 50		91229-402	91229-404	91219-406	91529-402	91529-404	
0.075 x 100		91229-602	91229-604	91219-606	91529-602	91529-604	
0.075 x 150		91229-702	91229-704	91219-706	91529-702	91529-704	
0.1 x 50		91228-402	91228-404	91218-406	91528-402	91528-404	
0.1 x 100		91228-602	91228-604	91218-606	91528-602	91528-604	
0.1 x 150		91228-702	91228-704	91218-706	91528-702	91528-704	
0.2 x 50		91227-402	91227-404	91217-406	91527-402	91527-404	
0.2 x 100		91227-602	91227-604	91217-606	91527-602	91527-604	
0.2 x 150		91227-702	91227-704	91217-706	91527-702	91527-704	
0.3 x 50		91226-402	91226-404	91216-406	91526-402	91526-404	
0.3 x 100		91226-602	91226-604	91216-606	91526-602	91526-604	
0.3 x 150		91226-702	91226-704	91216-706	91526-702	91526-704	
0.5 x 50		91225-402	91225-404	91215-406	91525-402	91525-404	
0.5 x 100		91225-602	91225-604	91215-606	91525-602	91525-604	
0.5 x 150		91225-702	91225-704	91215-706	91525-702	91525-704	
1.0 x 30		92121-302	92121-304	92111-306	95121-302	95121-304	
1.0 x 50		92121-402	92121-404	92111-406	95121-402	95121-404	
1.0 x 75		92121-502	92121-504	92111-506	95121-502	95121-504	
1.0 x 100		92121-602	92121-604	92111-606	95121-602	95121-604	
1.0 x 150		92121-702	92121-704	92111-706	95121-702	95121-704	
2.1 x 20	91122-202	92122-202	92122-204	92112-206	95122-202	95122-204	
2.1 x 30	91122-302	92122-302	92122-304	92112-306	95122-302	95122-304	
2.1 x 50	91122-402	92122-402	92122-404	92112-406	95122-402	95122-404	
2.1 x 75	91122-502	92122-502	92122-504	92112-506	95122-502	95122-504	
2.1 x 100	91122-602	92122-602	92122-604	92112-606	95122-602	95122-604	
2.1 x 150	91122-702	92122-702	92122-704	92112-706	95122-702	95122-704	
2.1 x 250	91122-902	92122-902	92122-904	92112-906	95122-902	95122-904	
3.0 x 20	91123-202	92123-202	92123-204	92113-206	95123-202	95123-204	
3.0 x 30	91123-302	92123-302	92123-304	92113-306	95123-302	95123-304	
3.0 x 50	91123-402	92123-402	92123-404	92113-406	95123-402	95123-404	
3.0 x 75	91123-502	92123-502	92123-504	92113-506	95123-502	95123-504	
3.0 x 100	91123-602	92123-602	92123-604	92113-606	95123-602	95123-604	
3.0 x 150	91123-702	92123-702	92123-704	92113-706	95123-702	95123-704	
3.0 x 250	91123-902	92123-902	92123-904	92113-906	95123-902	95123-904	
4.6 x 20		92124-202	92124-204	92114-206	95124-202	95124-204	
4.6 x 30		92124-302	92124-304	92114-306	95124-302	95124-304	
4.6 x 50		92124-402	92124-404	92114-406	95124-402	95124-404	
4.6 x 75		92124-502	92124-504	92114-506	95124-502	95124-504	
4.6 x 100		92124-602	92124-604	92114-606	95124-602	95124-604	
4.6 x 150		92124-702	92124-704	92114-706	95124-702	95124-704	
4.6 x 250		92124-902	92124-904	92114-906	95124-902	95124-904	
10.0 x 50		92120-402	92120-404	92110-406	95120-402	95120-404	
10.0 x 75		92120-502	92120-504	92110-506	95120-502	95120-504	
10.0 x 100		92120-602	92120-604	92110-606	95120-602	95120-604	
10.0 x 150		92120-702	92120-704	92110-706	95120-702	95120-704	
10.0 x 250					95120-902	95120-904	
<b>Guard Columns, 3-pack</b>							
Dimensions ID x Length (in mm)	ES-C18	ES-C18	ES-CN	Phenyl-Hexyl	ES-C18	ES-CN	
2.1 x 5	91122-102	92122-102	92122-104	92112-106	95122-102	95122-104	
3.0 x 5	91123-102	92123-102	92123-104	92113-106	95123-102	95123-104	
4.6 x 5		92124-102	92124-104	92114-106	95124-102	95124-104	
<b>Guard Column Holder</b>							
94900-001							

# HALO GLOBAL DISTRIBUTION NETWORK



Visit our web site ([www.advanced-materials-tech.com](http://www.advanced-materials-tech.com)) for distributor contact information.



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[info@advanced-materials-tech.com](mailto:info@advanced-materials-tech.com)  
[www.advanced-materials-tech.com](http://www.advanced-materials-tech.com)

# HALO® 1000 Å DIPHENYL

## INNOVATION YOU CAN TRUST!

In alignment with Advanced Materials Technology's ongoing innovation of wide pore materials for bioanalysis, we are excited to introduce another 1000 Å phase to our repertoire.

Your protein separation challenges are unique so why should your column choices be limited?

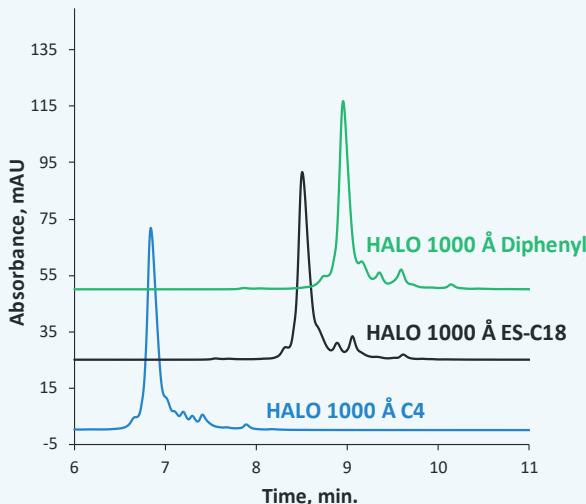


## ADVANTAGES OF THE NEW HALO DIPHENYL

- Extremely stable bonded phase using proven Fused-Core® technology
- Alternate selectivity for large biomolecules
- High resolution

### High Temperature Comparison of HALO 1000 Å Phases

**Figure 1.** Three bonded phases (C4, ES-C18, and Diphenyl) are now available on HALO 1000 Å superficially porous particles. Each phase offers unique selectivity for mAbs as demonstrated by this high temperature separation of trastuzumab.



#### TEST CONDITIONS

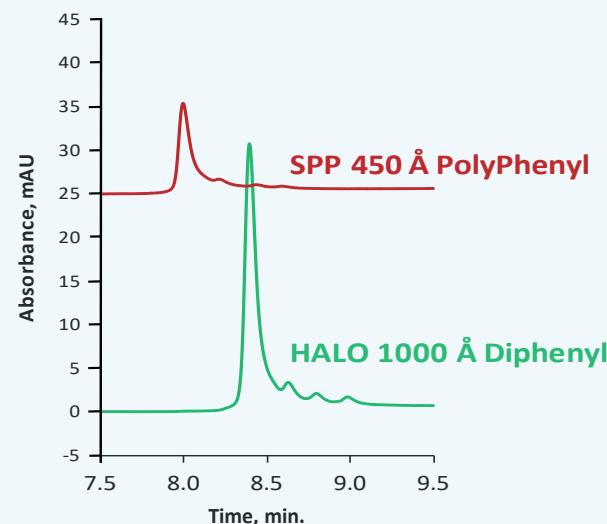
Column:  
HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm HALO  
1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm HALO  
1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm

Flow Rate: 0.4 mL/min  
Temperature: 80 °C  
Injection Volume: 2 µL of 2 mg/mL trastuzumab in water/0.1% TFA  
Instrument: Shimadzu Nexera  
Detection: PDA at 280 nm

Mobile Phase A: water/0.1% TFA  
Mobile Phase B: ACN/0.1% TFA  
Gradient:  
32-40% B in 16 min (C4 and ES-C18)  
32-38% B in 12 min (Diphenyl)

### Comparison of HALO 1000 Å Diphenyl to Competitor PolyPhenyl at 40 °C

**Figure 2.** HALO 1000 Å Diphenyl shows improved resolution, retention, and peak area compared to a competitor column of 450 Å SPPs with PolyPhenyl phase. The increased retention clearly demonstrates the benefit of unrestricted large pore access to the bonded phase. The HALO Diphenyl phase can also be operated at 40 °C without showing peak area loss.



#### TEST CONDITIONS

Column:  
HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm  
SPP 450 Å PolyPhenyl, 2.7 µm, 2.1 x 150 mm

Flow Rate: 0.4 mL/min  
Temperature: 40 °C  
Injection Volume: 2 µL of 2 mg/mL trastuzumab in water/0.1% TFA  
Instrument: Shimadzu Nexera  
Detection: PDA at 280 nm

Mobile Phase A: water/0.1% TFA  
Mobile Phase B: ACN/0.1% TFA  
Gradient:  
30-45% B in 15 min



## PART NUMBERS

### ANALYTICAL COLUMNS

1000 Å, 2.7 µm

Dimensions: ID x Length (in mm)	Diphenyl
2.1 x 20	92712-226
2.1 x 30	92712-326
2.1 x 50	92712-426
2.1 x 75	92712-526
2.1 x 100	92712-626
2.1 x 150	92712-726
2.1 x 250	92712-926
3.0 x 20	92713-226
3.0 x 30	92713-326
3.0 x 50	92713-426
3.0 x 75	92713-526
3.0 x 100	92713-626
3.0 x 150	92713-726
3.0 x 250	92713-926
4.6 x 20	92714-226
4.6 x 30	92714-326
4.6 x 50	92714-426
4.6 x 75	92714-526
4.6 x 100	92714-626
4.6 x 150	92714-726
4.6 x 250	92714-926
10.0 x 50	92710-426
10.0 x 75	92710-526
10.0 x 100	92710-626
10.0 x 150	92710-726

### CAPILLARY COLUMNS

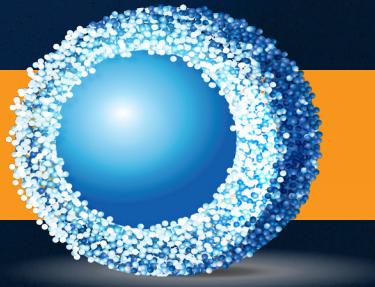
1000 Å, 2.7 µm

Dimensions: ID x Length (in mm)	Diphenyl
0.075 x 50	97219-426
0.075 x 100	97219-626
0.075 x 150	97219-726
0.1 x 50	97218-426
0.1 x 100	97218-626
0.1 x 150	97218-726
0.2 x 50	97217-426
0.2 x 100	97217-626
0.2 x 150	97217-726
0.3 x 50	97216-426
0.3 x 100	97216-626
0.3 x 150	97216-726
0.5 x 50	97215-426
0.5 x 100	97215-626
0.5 x 150	97215-726

### GUARD COLUMNS

2.7 µm, 1000 Å Guard columns, 3-pack

Dimensions: ID x Length (in mm)	Diphenyl
2.1 x 5	92712-126
3.0 x 5	92713-126
4.6 x 5	92714-126
Guard Column Holder	92814-111



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# HALO<sup>®</sup>

## BIPHENYL

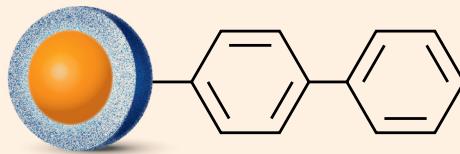
SAY HELLO TO ENHANCED  
RETENTION, SELECTIVITY AND EFFICIENCY.



# HALO 90 Å BIPHENYL

## SELECTIVITY, EFFICIENCY AND RETENTION packed into one powerful new phase

The HALO Biphenyl offers a new perspective on retention mechanisms for polar compounds. With a combination of hydrophobic, aromatic, and polar selectivities, the HALO Biphenyl, joined with the efficiency of robust Fused-Core® technology, unlocks powerful separation forces. Experience the difference one phase can make!

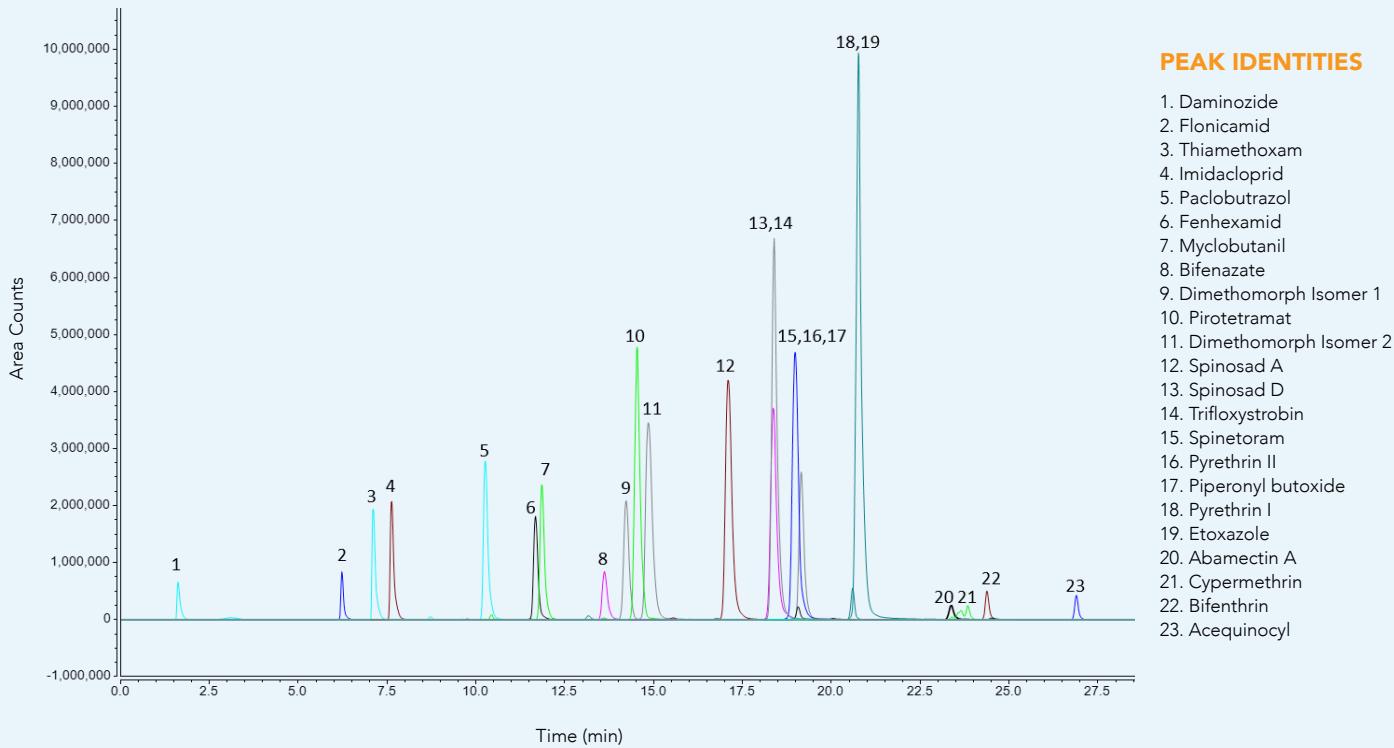


## ADVANTAGES OF THE NEW HALO BIPHENYL

- Enhanced retention, high sensitivity, and increased efficiencies without sacrifice to robustness or reproducibility
- Increased selectivity for challenging polar compounds
- High efficiency resulting in sharp resolved peaks with excellent peak shapes

## CHALLENGING SEPARATIONS REQUESTED

**Figure 1.** The HALO Biphenyl is ready for the challenge of complex samples. Both polar and non-polar pesticides are well resolved with this commonly required test for cannabis.



### TEST CONDITIONS

Column: HALO 90Å Biphenyl, 2.7 µm,  
2.1 x 100 mm  
Flow Rate: 0.2 mL/min  
Injection Volume: 1 µL  
Sample Solvent: Nevada Pesticide Mix  
Detection: MS-TOF, ESI+, XIC

Mobile Phase: A= Water/0.1% formic acid/4 mM ammonium formate  
B= Acetonitrile/0.1% formic acid/4 mM ammonium formate

Gradient:	Time (min.)	%B
	0.00	0
	1.01	15
	4.00	35
	5.00	62
	30.00	100
	34.00	100

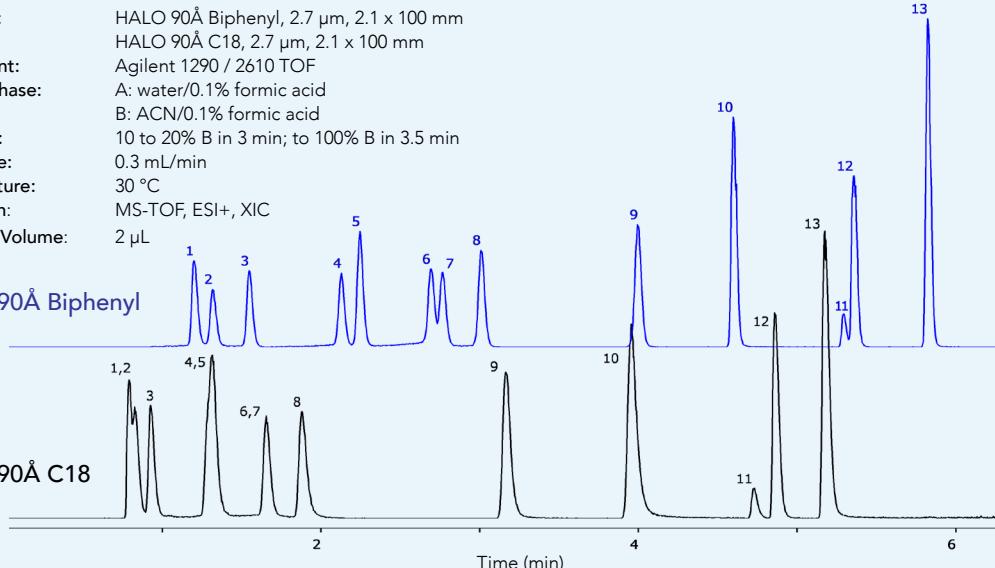
## ENHANCED RETENTION WITH ALTERNATE SELECTIVITY

**Figure 2.** Selectivity comparisons are demonstrated between the HALO Biphenyl and HALO C18 on this common 13 opiate pain panel screening used by clinical laboratories.

### TEST CONDITIONS

Columns: HALO 90Å Biphenyl, 2.7 µm, 2.1 x 100 mm  
 HALO 90Å C18, 2.7 µm, 2.1 x 100 mm  
 Instrument: Agilent 1290 / 2610 TOF  
 Mobile Phase:  
 A: water/0.1% formic acid  
 B: ACN/0.1% formic acid  
 Gradient: 10 to 20% B in 3 min; to 100% B in 3.5 min  
 Flow Rate: 0.3 mL/min  
 Temperature: 30 °C  
 Detection: MS-TOF, ESI+, XIC  
 Injection Volume: 2 µL

HALO 90Å Biphenyl



### PEAK IDENTITIES

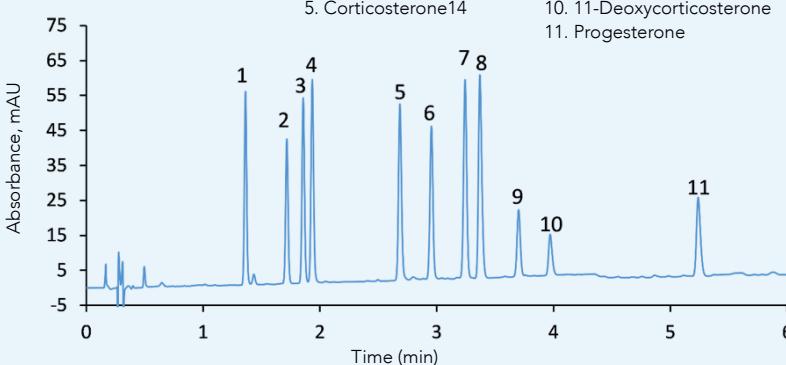
1. Morphine
2. Oxymorphone
3. Hydromorphone
4. Naloxone
5. Codeine
6. Naltrexone
7. Oxycodone
8. Hydrocodone
9. cis-Tramadol HCl
10. Meperidine
11. Fentanyl
12. Buprenorphine
13. (±)-Methadone

## HIGH RESOLUTION SEPARATIONS

**Figure 3.** The sharp, fully resolved peaks demonstrate excellent peak shape in this high efficiency steroid separation.

### PEAK IDENTITIES

1. Estriol
2. Hydrocortisone
3. Prednisone
4. Cortisone
5. Corticosterone
6. β-Estradiol
7. Cortisone Acetate
8. Testosterone
9. 17-α-Hydroxyprogesterone
10. 11-Deoxycorticosterone
11. Progesterone



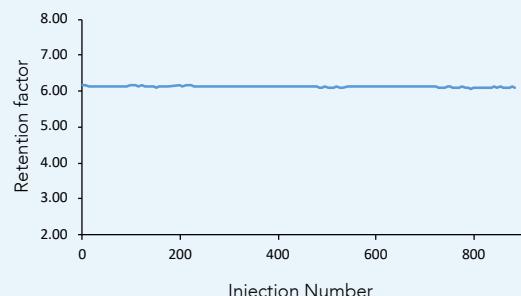
### TEST CONDITIONS

Column: HALO 90Å Biphenyl, 2.7 µm, 4.6 x 50mm  
 Gradient: 20-60% B in 6 minutes  
 Flow Rate: 1.85 mL/min  
 Temperature: 30°C  
 Detection: UV 215 nm, PDA  
 Injection Volume: 4 µL  
 Mobile Phase A: Water  
 Mobile Phase B: Acetonitrile

## QUALITY YOU CAN COUNT ON

**Figure 4.** Built to perform! The HALO Biphenyl provides robust column lifetimes.

Ruggedness after 900 injections as demonstrated with a 0.29% RSD



### TEST CONDITIONS

Column: HALO 90Å Biphenyl, 2.7 µm 2.1x100 mm  
 Mobile Phase: 60/40 Methanol/ H<sub>2</sub>O, 0.1% Formic Acid, 10mM Ammonium Formate  
 Temperature: 35°C  
 Flow Rate: 0.6 mL/min  
 Detection: 254 nm  
 Injection Volume: 0.5 µL  
 Sample: Naphthalene

# PART NUMBERS

## ANALYTICAL COLUMNS

**90 Å, 2.7 µm**

Dimensions: ID x Length (in mm)	Biphenyl
2.1 x 20	92812-211
2.1 x 30	92812-311
2.1 x 50	92812-411
2.1 x 75	92812-511
2.1 x 100	92812-611
2.1 x 150	92812-711
2.1 x 250	92812-911
3.0 x 20	92813-211
3.0 x 30	92813-311
3.0 x 50	92813-411
3.0 x 75	92813-511
3.0 x 100	92813-611
3.0 x 150	92813-711
3.0 x 250	92813-911
4.6 x 20	92814-211
4.6 x 30	92814-311
4.6 x 50	92814-411
4.6 x 75	92814-511
4.6 x 100	92814-611
4.6 x 150	92814-711
4.6 x 250	92814-911
10.0 x 50	92810-411
10.0 x 75	92810-511
10.0 x 100	92810-611
10.0 x 150	92810-711

## CAPILLARY COLUMNS

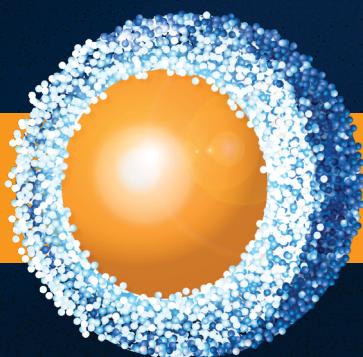
**90 Å, 2.7 µm**

Dimensions: ID x Length (in mm)	Biphenyl
0.075 x 50	98219-411
0.075 x 100	98219-611
0.075 x 150	98219-711
0.1 x 50	98218-411
0.1 x 100	98218-611
0.1 x 150	98218-711
0.2 x 50	98217-411
0.2 x 100	98217-611
0.2 x 150	98217-711
0.3 x 50	98216-411
0.3 x 100	98216-611
0.3 x 150	98216-711
0.5 x 50	98215-411
0.5 x 100	98215-611
0.5 x 150	98215-711
1.0 x 30	92811-311
1.0 x 50	92811-411
1.0 x 75	92811-511
1.0 x 100	92811-611
1.0 x 150	92811-711

## GUARD COLUMNS

**2.7 µm, 90 Å Guard columns, 3-pack**

Dimensions: ID x Length (in mm)	Biphenyl
2.1 x 5	92812-111
3.0 x 5	92813-111
4.6 x 5	92814-111
Guard Column Holder	92814-111



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# HALO® C30

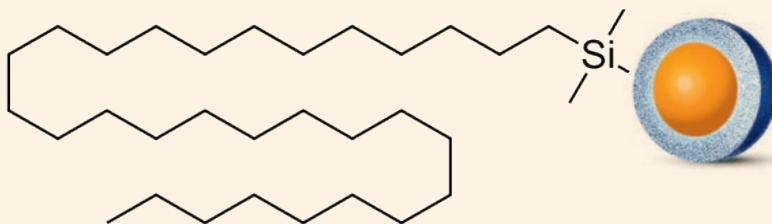
ISOMERS HAVE MET THEIR MATCH



# HALO® C30

## INTRODUCING THE NEW HALO® C30!

Built on proven Fused-Core® particle technology, the HALO® C30 is designed to deliver fast separations ideal for lipids and isomers compared to your C18.



## FEATURES OF HALO® C30

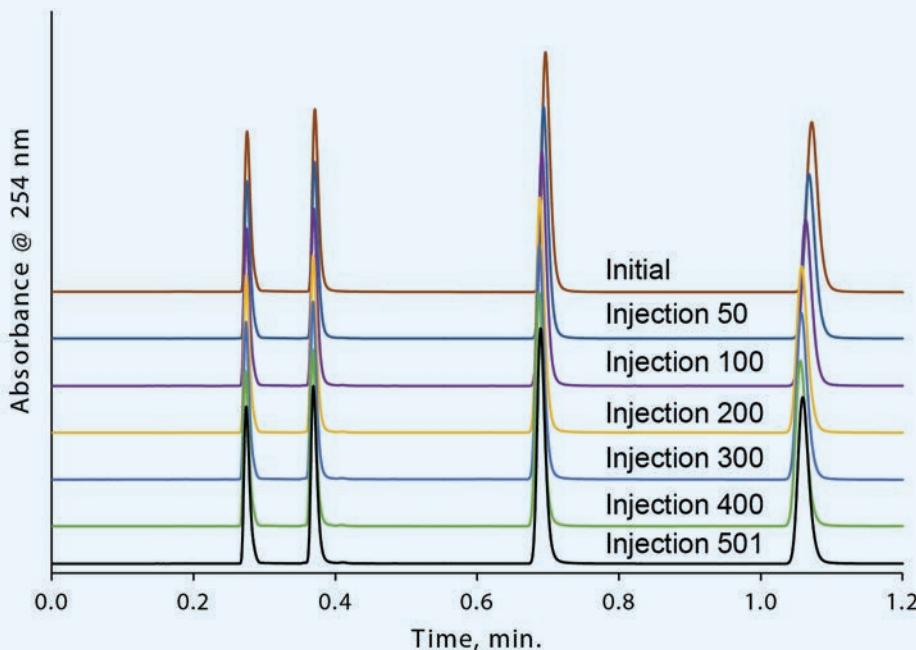
- High shape selectivity for hydrophobic, long-chain structurally related isomers
- Highly reproducible bonded phase coverage resulting in fast, highly efficient, rugged separations
- 100 % Aqueous Compatibility

### Best Applications:

- Fat/Water Soluble Vitamins
- Carotenoids
- Lipids
- Steroids

## QUALITY YOU CAN COUNT ON

The HALO® C30 exhibits reliable stability providing you assurance in your separations – injection to injection.

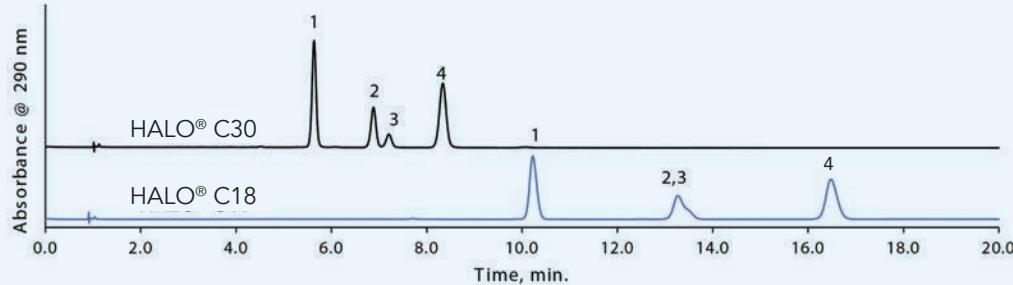


### TEST CONDITIONS

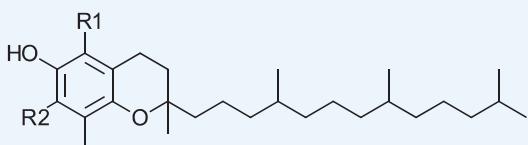
Column: HALO 160 Å C30, 2.7 $\mu$ m, 2.1 x 150mm  
Isocratic: 50-50 ACN/H<sub>2</sub>O  
Flow Rate: 1.1 mL/min.  
Back Pressure: 602 bar  
Temperature: 60°C  
Injection Volume: 1  $\mu$ l test mix containing uracil, phenol, 1-chloro-4-nitrobenzene, and naphthalene  
Instrument: Shimadzu Nexera  
Detection: UV 254 nm, PDA

## ISOMERS HAVE MET THEIR MATCH

Tocopherols, a form of vitamin E, known for their antioxidant properties contain many isomers which are easily baseline separated on the HALO® C30 compared to a C18 due to the shape selectivity property of the phase.



### STRUCTURE



Tocopherol	R1	R2
Alpha ( $\alpha$ )	CH <sub>3</sub>	CH <sub>3</sub>
Beta ( $\beta$ )	CH <sub>3</sub>	H
Gamma ( $\gamma$ )	H	CH <sub>3</sub>
Delta ( $\delta$ )	H	H

### PEAK IDENTITIES

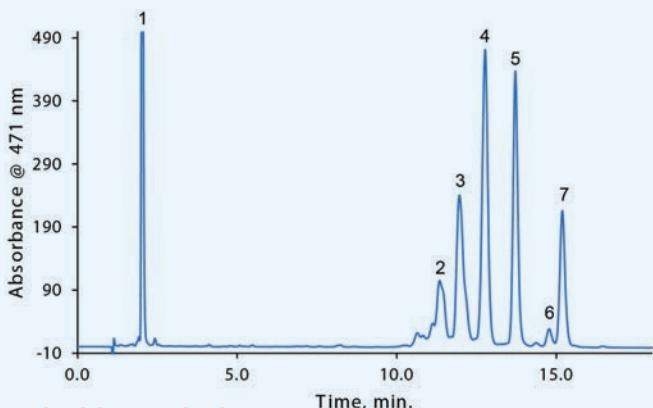
1.  $\delta$ -tocopherol
2.  $\gamma$ - tocopherol
3.  $\beta$ - tocopherol
4.  $\alpha$ - tocopherol

### TEST CONDITIONS

Columns: HALO 160 Å C30 and 90 Å C18, 2.7  $\mu$ m, 4.6 x 150mm  
Mobile Phase A: Water  
Mobile Phase B: Methanol  
Isocratic: 95% B  
Flow Rate: 1.5 mL/min  
Temperature: 10°C  
Injection Volume: 1.5  $\mu$ L  
Instrument: Agilent 1200 SL  
Detection: UV 290 nm, PDA

## CAROTENOID SEPARATION

These seven carotenoids and related isomers from a commercially available vitamin formulation are easily resolved under simple mobile phase conditions.



### TEST CONDITIONS

Column: HALO 160 Å C30, 2.7  $\mu$ m, 3.0 x 150 mm  
Mobile Phase A: Methanol  
Mobile Phase B: Ethanol  
Gradient: 100% A with gradient to 40% B at 20 min.  
Flow Rate: 0.65 mL/min  
Temperature: 38°C  
Data acquisition rate: 2.5 Hz  
Injection volume: 0.60  $\mu$ L  
Instrument: Agilent 1100  
Detection: UV 471 nm, PDA

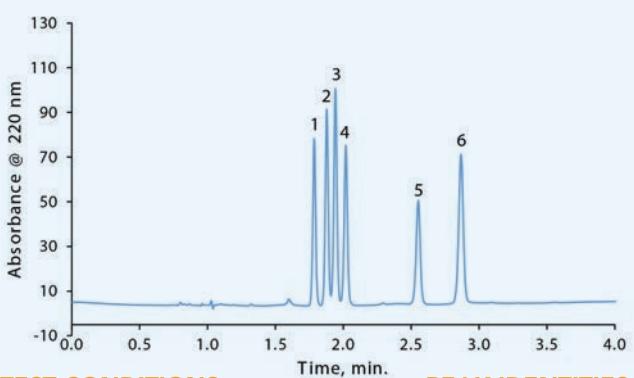
Data courtesy of Nature's Sunshine Products.

### PEAK IDENTITIES

1. Lutein
2. cis-carotenoid 1
3. cis-carotenoid 2
4.  $\alpha$ -carotene
5.  $\beta$ -carotene
6. cis-lycopene
7. Lycopene

## STEROID SEPARATIONS USING HALO® C30

Glucocorticoids are a powerful form of steroid hormone both naturally produced and prescribed as a means to control a number of conditions caused by inflammation. In the example below six glucocorticoids are separated within three minutes and demonstrate excellent peak shape.



### TEST CONDITIONS

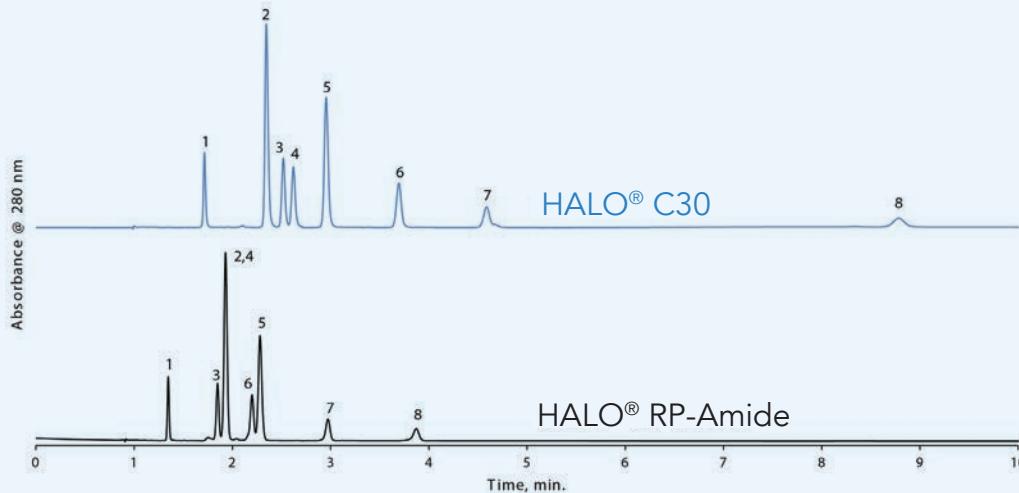
Column: HALO 160 Å C30, 2.7  $\mu$ m, 4.6 x 150 mm  
Mobile Phase A: Water  
Mobile Phase B: 50/50 Acetonitrile/ Methanol  
Isocratic: 50% B  
Flow Rate: 1.5 mL/min  
Back Pressure: 309 bar  
Temperature: 60°C  
Injection Volume: 0.5  $\mu$ L  
Instrument: Shimadzu Nexera X2  
Detection: UV 220 nm, PDA

### PEAK IDENTITIES

1. Prednisone
2. Cortisone
3. Prednisolone
4. Hydrocortisone
5. Dexamethasone
6. Corticosterone

## ENHANCED SELECTIVITY AND RESOLUTION FOR FAT SOLUBLE VITAMINS

For a panel of fat soluble vitamins, the HALO® C30 demonstrates superior resolution and selectivity in comparison with a commonly employed RP-Amide phase.



### PEAK IDENTITIES

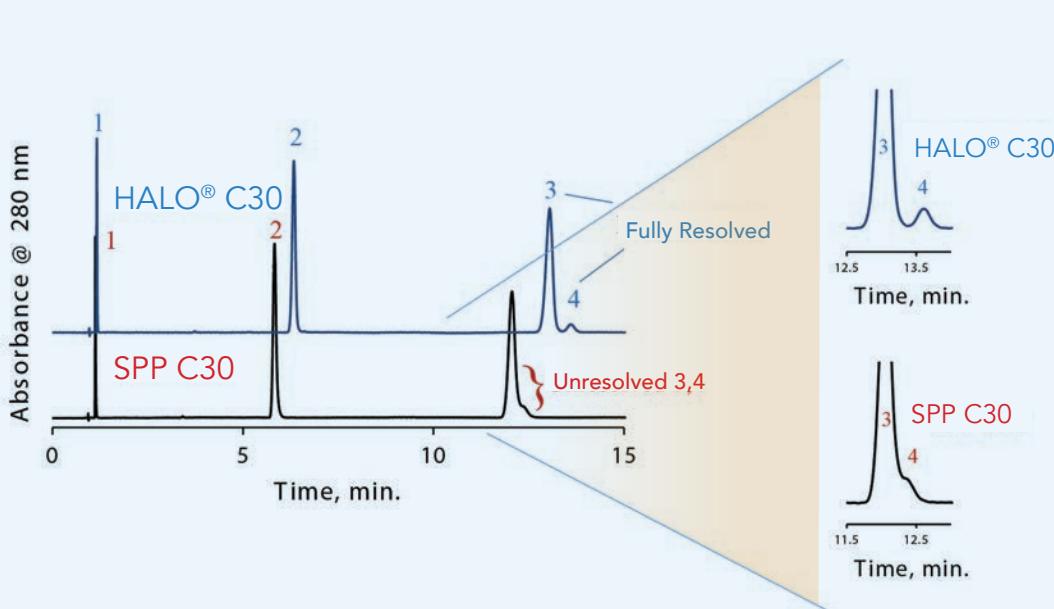
1. Retinyl acetate (A)
2. Delta tocopherol (E)
3. Ergocalciferol (D2)
4. Cholecalciferol (D3)
5. Alpha tocopherol (E)
6. DL-alpha-tocopherol acetate (E)
7. 2,3-trans-phylloquinone (K)
8. Retinyl palmitate (A)

### TEST CONDITIONS

Columns: HALO C30, RP-Amide,  
2.7  $\mu$ m, 4.6 x 150 mm  
Isocratic: 100% Methanol  
Flow Rate: 1.5 mL/min.  
Temperature: 30 °C  
Injection: 2  $\mu$ L  
Instrument: Nexera 062  
Detection: UV 280 nm, PDA

## COMPETITIVE ADVANTAGE

A HALO® C30 column shows increased retention and baseline resolution of vitamin K1 trans and cis isomers compared to a SPP C30 column. Since the cis isomer of K1 is biologically inactive, it is important to know how much of each isomer is present in vitamin enriched products.



### PEAK IDENTITIES

1. Menadione (K3)
2. Menaquinone 4 (K2)
3. 2,3-trans-phylloquinone (K1)
4. cis-phylloquinone (K1)

### TEST CONDITIONS

Columns: HALO 160 Å C30, 2.7  $\mu$ m, 4.6 x 150 mm; SPP C30, 2.6  $\mu$ m, 4.6 x 150 mm  
Mobile Phase A: Water  
Mobile Phase B: Methanol  
Isocratic: 95% B  
Flow Rate: 1.5 mL/min  
Temperature: 25 °C  
Injection Volume: 1  $\mu$ L  
Instrument: Shimadzu Nexera  
Detection: UV 280 nm, PDA

# SPECIFICATIONS

**Ligand:** Triacontyldimethyl  
**Particle Size:** 2.7 µm  
**Pore Size:** 160 Å

**USP Designation:** L62  
**Carbon Load:** 4.5 %  
**Surface Area:** 90 m<sup>2</sup>/g

**Endcapped:** Yes  
**Low pH Limit /Max T:** 2/60°C  
**High pH Limit/Max T:** 9/40°C

## PART NUMBERS

ANALYTICAL COLUMNS	
Dimensions: ID x Length (in mm)	Part Number
2.1 x 20	92112-230
2.1 x 30	92112-330
2.1 x 50	92112-430
2.1 x 75	92112-530
2.1 x 100	92112-630
2.1 x 150	92112-730
2.1 x 250	92112-930
3.0 x 20	92113-230
3.0 x 30	92113-330
3.0 x 50	92113-430
3.0 x 75	92113-530
3.0 x 100	92113-630
3.0 x 150	92113-730
3.0 x 250	92113-930
4.6 x 20	92114-230
4.6 x 30	92114-330
4.6 x 50	92114-430
4.6 x 75	92114-530
4.6 x 100	92114-630
4.6 x 150	92114-730
4.6 x 250	92114-930
10.0 x 50	92110-402
10.0 x 75	92110-502
10.0 x 100	92110-602
10.0 x 150	92110-702

CAPILLARY COLUMNS	
Dimensions: ID x Length (in mm)	Part Number
0.075 x 50	91219-402
0.075 x 100	91219-602
0.075 x 150	91219-702
0.1 x 50	91218-402
0.1 x 100	91218-602
0.1 x 150	91218-702
0.2 x 50	91217-402
0.2 x 100	91217-602
0.2 x 150	91217-702
0.3 x 50	91216-402
0.3 x 100	91216-602
0.3 x 150	91216-702
0.5 x 50	91215-402
0.5 x 100	91215-602
0.5 x 150	91215-702
1.0 x 30	92111-302
1.0 x 50	92111-402
1.0 x 75	92111-502
1.0 x 100	92111-602
1.0 x 150	92111-702

GUARD COLUMNS	
Guard columns, 3-pack	
Dimensions: ID x Length (in mm)	Part Number
2.1 x 5	92112-130
3.0 x 5	92113-130
4.6 x 5	92114-130
Guard Column Holder	94900-001

**INNOVATION YOU CAN TRUST – PERFORMANCE YOU CAN RELY ON**